Book of Abstracts

II International Congress of Young Marine Researchers

Málaga, Spain, 1-4 October 2019

UNIVERSIDAD DE MÁLAGA

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II International Congress of Young Marine Researchers

October 1st - 4th, 2019 - Málaga (Spain)

Foreword

We live a new era in the long history of the Earth System called the Anthropocene, a period characterized by a multitude of very rapid changes whose repercussions operate on a global scale, and which are a (direct or indirect) consequence of the pressures performed by just a single species, the human species.

The complex set of accelerated processes that characterize the Anthropocene include climate change, biodiversity loss, ocean acidification, eutrophication of coastal and continental waters, changes in land use, decreases in both quality and volume of freshwater, atmospheric pollution, or the introduction of emerging pollutants into the biosphere. All of them contribute decreasing the capacity of the ecosystems to provide goods and services to human societies, which consequently becomes seriously threatened in their survival and well-being.

In this context, it results absolutely essential to articulate solid responses acting on the drivers of change, proposing new political, social and economic formulas more compatible with the conservation of ecosystems, with social equality and with human well-being. These responses must emerge from robust scientific and technological knowledge, but also from a society with strong ethical principles, capable to provide all the necessary enthusiasm and efforts to achieve the necessary utopia of sustainable development.

This essential combination of excellence in research, innovation, ethical commitment, enthusiasm, effort and optimism converges on the profile of the young scientists who gathered at the II International Congress of Young Marine Researchers, organized by CEI·Mar and held at the University of Málaga from 1st to 4th October 2019. This was an international congress for hope, a forum to better understanding our marine environment, identifying the pressures, threats and impacts it suffers and the excellent ecosystem services it provides to our society. The congress was also a starting point to walk with firm and determined step towards the blue and sustainable development of our societies.

As a melting pot, this book gathers all the scientific communications presented during the congress, once reviewed and corrected by anonymous reviewers and by the Scientific Committee. The final format of the book as an edited and published work has required a remarkable additional effort from the editors, who have worked very hard, with generosity, inspiration, commitment and a lot of vocation to transfer knowledge. To all of them, I want to thank the excellent work done, which has crystallized in this excellent book, created by and for young researchers of the sea.

Málaga, 31st October 2019

Enrique Moreno-Ostos
CEIMAR coordinator at University of Málaga (Spain)

Presentation

Research, development and generation of knowledge in the marine-maritime field are essential strengths for our natural and geographical environment. Proof of this is the existence of different public research organisations specialised in such singular areas as marine sciences, nautical sciences, naval engineering and underwater archaeology, among others.

Given the diversity of research organisations in this field that support the training of Young Researchers (JIs), the organisation of a scientific congress dedicated to JIs was considered in order to create a meeting space where they can present their research that is being developed in different organisations and R+D+i marine centers.

The main objective of the II Congress of Young Marine Researchers, framed in the International Campus of Excellence in Marine Science (CEI·Mar), is the meeting between JIs that are developing their research work in the marine field. The objectives set by the II Congress of Young Marine Researchers are:

- To promote and dynamize the presentation of scientific works carried out by the new generations of researchers in the marine field.
- To promote interdisciplinarity among JIs who carry out their work in different public and private research centers.
- To establish cooperation between JIs: to know and to boost synergies in marine research.

Why to attend?

Although this congress is an open forum where any kind of public that is interested in this topic can attend, the event will be especially dedicated to JIs who carry out their research work in the CEI·Mar field. This is a congress organized by and for JIs, where the participation of them will allow to answer questions like: Who can be interested in my work? What I do, what it is for? What comes after the PhD?

In this way, a point of connection between the different research groups of these centers is intended to be created, where the JIs themselves expose their areas of work, as well as their concerns and difficulties at this stage. With this meeting of JIs, the diffusion and communication of information between the different lines of research carried out in our territory will be increased, promoting future collaborations and projects with a high multidisciplinary value.

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Plenary lectures

Biodiversity conservation and sustainable development: The necessary utopia

Jaime Rodríguez Martínez[1]

[1] Department of Ecology, Faculty of Sciences, University of Málaga, Málaga 29071, Spain

Abstract

Human activities have led the Earth to a new state, the Anthropocene, but the biosphere is finite in relation to both resource supply and waste assimilation. One of the more relevant planetary limits already surpassed is the loss of biodiversity (genes, species, ecosystems), the natural capital that contributes in an essential way to the human well-being. Also, in addition to causing environmental degradation, the dominant model of economic growth favours social inequality. All this represents a challenge to reaching sustainable development, which can be reduced to a utopian concept in spite of practical initiatives like United Nations Sustainable Development Goals.

The close relation between biodiversity conservation and sustainable development was in the origin of the Convention on Biological Diversity (CBD). It acknowledges that biodiversity is a resource for humans that should be used in a sustainable and equitable way. On one side, this anthropocentric approach to biodiversity conservation seems to be opposed to an ethical approach (the so called "deep ecology") that defend biodiversity "preservation" and the intrinsic value of species simply because they exist. On the other side, the consideration of an instrumental value to biodiversity within the CBD requires the identification of sustainability criteria. This would be consistent with the proposals of "ecological economics" but not with the strictly monetary approach of "environmental economy".

In the context of a limiting biosphere, "sustainable development" has been considered as an oxymoron derived from simply replacing the term "growth" by the term "development". From a less pessimistic point of view, "development" goes beyond the simple economic growth and "sustainable development" is identified as the three-dimensional space where the axes "economic growth", "environment" and "human well-being" have positive values. At this moment and at the global scale, this space remains a necessary utopia.

Managing our seas: Marine spatial planning in Spain. The role of the science.

Javier García Sanabria[1]

[1] University of Cádiz, Department of Geography.

Abstract

The relatively slow progress in the management of the marine territory contrasts with the extraordinary and worrying increase in the capacity of the human being to put pressure on marine ecosystems. It is worth remembering that more than a third of the world's population lives in coastal areas and small islands, and with it most of the uses and activities. Indeed, some of the most dynamic and consolidated economic activities (tourism, aquaculture, maritime trade), or emerging (marine renewable energies, offshore oil extraction), have in these areas their most recurrent action scenario. In this sense, Marine spatial planning, implemented in the European marine areas according to the Directive 2014/89/EU, will allow to develop uses and activities in a sustainable way, including not only economic but also environmental terms. In the case of Spain, the "Marine Spatial Planning" Directive has been transposed to national level by the Royal Decree 363/2017, establishing a general framework for the management of maritime territory. According to this, the Spanish marine plans must be finished and published by march, 2021. However, the planning process is still at a very beginning stage in Spain, and there are still many questions to be resolved: what is the ultimate goal to achieve in the management of the Spanish marine environment? What activities are intended to enhance? What is the desired scenario that we want to pursue?
How will the different actors participate in marine management? In what way will the scientific institutions, which raise the information, coordinate with the institutions responsible for making decisions in marine management? In this talk, the MSP process in Spain will be introduced, highlighting the opportunities and threats that can arise, but also highlighting the role that (in the opinion of the author) the science should play in the marine planning process.

Environmental control of fish population cycles in climate-to-fish ocean models

José Carlos Sánchez Garrido[1]

[1] Physical Oceanographic Group, Faculty of Sciences, University of Málaga, Málaga 29071, Spain

Abstract

Small pelagic fish represent up to 50% of the total landing of marine species (Koné et al.) and provide the forage for multiple predators, which include a broad variety of larger fish, sea birds and mammals. Situated near the base of the food web, small pelagic fish represent a major linkage between the lower and upper trophic levels and thereby play a key role in sustaining the whole ecosystem. These facts, along with their great commercial value (e.g., sardine and anchovy) and strong sensitivity to environmental changes, as reflected in landing records, make it particularly important and necessary to better understand their population dynamics, especially in response to the present scenario of climate change and overfishing.
End-to-end ecosystem models, combining models of ocean circulation and physics, lower trophic level (nutrient-phytoplankton-zooplankton) and an upper trophic level including fish (usually based on individuals; chain also referred to as climate-to-fish model), are comprehensive numerical tools designed to address these questions. Harvest is sometimes represented in these modes too as a dynamic component of the ecosystem. As computing power is ever increasing, end-to-end models are gaining in complexity and realism. Among other features, state-of-the-art models and modern parallel computing techniques now allow for feedback between all components of the ecosystem (fully coupled models), run over the same three-dimensional computational grid, and afford multidecadal-scale eddy-resolving simulations of large marine ecosystem (Rose et al., 2015). In this talk, I will show how a climate-to-fish model can be used to gain insights into the population dynamics of anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) in the Canary Current upwelling ecosystem. I will present analysis techniques to unravel the link between climate, environmental (i.e., biochemistry and physical) and the simulated biological variability. I will also compare the results with previous modelling efforts made in the California Current (Fiechter et al., 2015), highlighting the physical and biological processes giving rise to qualitatively different variability of anchovy and sardine in these two major eastern boundary upwelling ecosystems.

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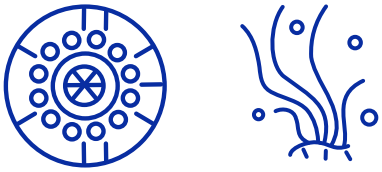
PART I

FLASH-TALKS

Session 1

The Sea Knowledge

Global change



Seasonal shifts in thermal resilience of multi-celled early-developmental stages of *Fucus guiryi*: Maternal acclimation *vs.* chronic stress

Raquel Sánchez de Pedro*[1] , Andrea Nieves Fernández[1] , María Jesús García-Sánchez[1] , Antonio Flores-Moya[1] , Elena Bañares-España[1]

*Corresponding author: rsdpc@uma.es
[1] Universidad de Málaga, Departamento de Botánica y Fisiología Vegetal. Facultad de Ciencias. Campus de Teatinos sn. 29010 Málaga, Spain

Keywords: Acclimation, Global change, Intertidal, Seasonality, Seaweed

Introduction

Fucus guiryi Zardi, Nicastro, E.S.Serrão & G.A.Pearson is an amphiatlantic fucacean species, distributed from the Canary islands and Moroccan coasts to the British isles. In the past decades, its southernmost and peripheral populations have experienced pronounced declines, undergone morphological changes and became extinct at past locations (Melero-Jiménez et al., 2017; Riera et al., 2015), as a result of ongoing global change. A bottleneck in the persistence of isolated seaweed populations is the recruitment success, due to the lack of ecological connectivity among populations and the low dispersal of gametes during external fertilization. Early-developmental stages are highly vulnerable to thermal, osmotic and UV stress, which in conjunction with other anthropogenic stressors, can undermine their ability to thrive under sub-optimal conditions (Coelho et al., 2000). Nevertheless, maternal acclimation of parental thalli of fucaceans can confer warm-hardiness to recruits of some fucacean juveniles (Li and Brawley, 2004), and in turn, might alter their thermal resilience across seasons.
In this study, we aim to test whether maternal acclimation and/or chronic stress modulates the seasonal thermal resilience of embryos of *F. guiryi* from the Strait of Gibraltar, by analyzing the seasonal trends in their growth, survival, development and physiological fitness, in relation to actual thermal conditions. Present maximum temperature in this region ranges from 23 °C (benthic) to 27 °C (air), but may rise up to 24.6-27.8 °C by 2050 (RCP 8.5).

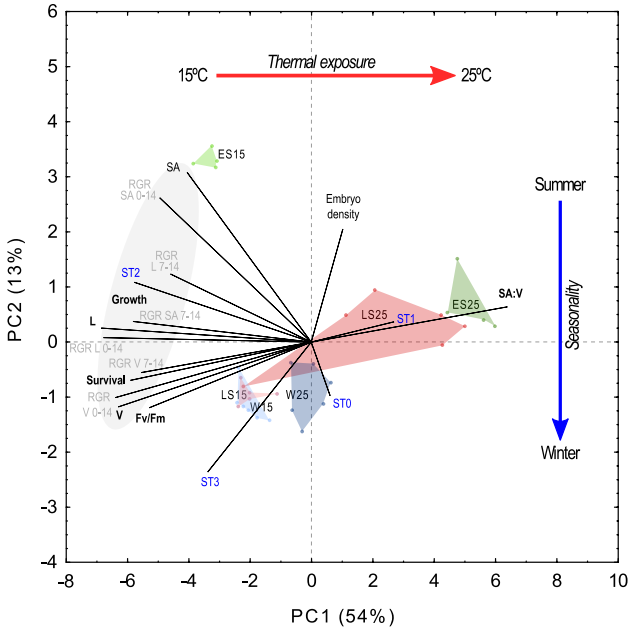
Material and methods

In early-summer (July'18), late-summer (September'18) and winter (March'19), mature receptacles (n=60) of the monoecious *F. guiryi* were sampled at the intermediate intertidal zone of Tarifa (Cádiz, Spain) from 12 individuals distanced more than 1 m from each other. Within this population *F. guiryi* produces fertile gametes across the year at this location (iteroparous). Cut-off receptacles were randomly assigned to experimental stocks (n=6). Gamete isolation protocol was specifically optimised for this species after trials of several published protocols for fucaceans: Air exposure for 6 days at 20°C and darkness; gamete release induction by osmotic and cold-shock (2 cycles) by spiking 3.5 ml of sterile GF-C filtered natural seawater. Only zygotes from the second extraction were harvested due to its 5 to 10 times higher cell release (pers. obs.). Zygotes from each stock were inoculated in 6 wells of a multi-well plate (24 wells), and 6 epoxy disks (Lamote et al., 2007) that were also placed inside of it, all of them filled with 2 mL of sterile natural seawater, salinity 36 psu. Embryos were acclimated to the temperature treatments once

settled and germinated overnight at 20°C, and then, grown for two weeks in a controlled culture chamber at 150 μmol photons m^{-2} s^{-1}, L:D 12:12 under control (15°C) and warming conditions (25°C).
Growth, survival and developmental stages were calculated from photomicrographs (n=3/ replicate/ week) with a Moticam 10.0 Mp digital camera coupled to an inverted microscope. Optimum quantum yield, as a proxy for physiological fitness, was measured by PAM-fluorometry onto the epoxy disks as described by Li and Brawley (2004). Reference values for fucacean embryos are usually below those of adult plants (Lamote et al., 2007).
Physiological responses obtained after 2-weeks or between 7-14 days (relative growth rates, RGR) were analysed across seasons and temperature levels in a principal component analysis (PCA) with scaled variables in STATISTICA 7. This multivariate method allows to identify underlying relationships and latent trends among the variables and the data.

Results and discussion

Multivariate analysis allowed to identify 5 groups among the seasons and temperature levels, where responses of late summer and winter embryos at 15°C overlapped (Figure 1). The first three ordination axes yielded 80% of the total variance. Principal component one (PC1) summarised the variables more influenced by temperature, while principal component two (PC2) reflected the seasonality across the data cases and variables (Figure 1). Survival and relative growth rates (RGRs), embryo length and volume, and optimum quantum yield had a major contribution to PC1, with absolute factor loadings above 0.70. This indicates that physiological performance of *F. guiryi* embryos is hampered under warming conditions (Figure 1).



	PC1	PC2	PC3
Percentage of variance	54%	13%	13%
Cumulative percentage	54%	67%	80%
Variable	PC1	PC2	PC3
Loading factors			
Length	**-0.97**	0.06	-0.03
RGR L (0-14)	**-0.96**	0.02	0.04
SA:V	**0.90**	0.16	0.28
RGR V (0-14)	**-0.90**	-0.26	0.07
Volume	**-0.89**	-0.30	0.10
Survival	**-0.83**	-0.18	0.31
RGR SA (7-14)	**-0.82**	0.10	-0.24
ST2	**-0.81**	0.28	0.31
RGR V (7-14)	**-0.78**	-0.14	-0.21
Fv/Fm	**-0.75**	-0.30	0.14
RGR SA (0-14)	**-0.70**	**0.67**	0.18
RGR L (7-14)	**-0.65**	0.31	0.14
Surface area	-0.57	**0.78**	0.10
ST3	-0.48	**-0.60**	-0.37
ST1	0.38	0.09	**0.74**
Embryo density	0.15	**0.52**	**-0.62**
ST0	0.09	-0.25	**0.78**

Figure 1: PCA of the physiological responses of embryos of *F. guiryi* at three seasons and two experimental temperatures. Seasons are represented by color polygons (green: early summer; red: late summer; blue: winter) and temperatures by color opacity (light: 15°C; darker: 25°C), with season × temperature as study cases. Relative growth rates (RGR) are in light grey summarised as "Growth" within the grey ellipse. Developmental stages are highlighted in blue letters (ST0: Non-germinated; ST1: primary rhizoid; ST2: Single rhizoid with apical hairs; ST3: Multiple rhizoids and apical hairs). Loading factors and percentage of variance of the three first PCA are summarised in the table.

Surface area (SA:V) quotient directly increased with temperature (max. value at ES-25°C, 96 mm^{-1}, factor loading 0.9, 9% of total variance). This might be related to an increase of cellular membranes fluidity. Also, embryos grown at warming conditions tended to present multiple rhizoids (stage 3), whilst predominantly showing the primary one at 15°C (stage 2).

Data clusters were clearly differentiated among seasons along the PC2 axis (Figure 1). Seasonality had a greater influence on embryo surface area (SA) and their derived RGRs. Early-summer embryos presented the highest SA values (0.085±0.006 mm^2) and growth rates (RGR L 0-14, 6.1±0.2 % d^{-1}) obtained at 15°C across seasons. This time of the year coincides with the growing season of adult thalli from the same population, which could shed evidence on the influence of parental thalli on embryo growth across seasons, which is in accordance with Li and Brawley (2004). Two alternative hypotheses might explain this: on the one hand, higher accumulation of storage compounds and nutrients might sustain a higher growth peak under control temperature (15°C); on the other hand, environmental cues might modulate the response of both adults and embryos at the same time. Winter embryos had a SA value 4-times lower than in early-summer and exhibited the greatest proportion of embryos with multiple rhizoids (27±15% at stage 3, r=-0.60, 16% of the variance in PC2).

Thermal resilience is understood as either resistance or recovery. In this case, shorter Euclidean distances in the PCA analysis from 15 to 25°C within each season would indicate an increase in thermal resistance (Euclidean distances: ES=9; LS=5; W=2, Figure 1. From early to late summer, embryos became more resilient, suggesting that summer maternal acclimation throughout summer confers warm-hardiness to the new recruits of this species. Actually, 25°C exerted disruptive but non-lethal stress within that experiment (recovered at 15°C after the experiment, pers. obs.) whereas early summer embryos did not survive more than 3 weeks.

Furthermore, from late-summer to winter, thermal resilience also kept increasing, reducing the differences in the physiological responses between temperatures (Figure 1). In addition, late-summer and winter embryos had similar responses at 15°C, whereas under warming conditions, winter ones performed better. Since cold acclimation took place in the field prior to that experiment, thermal resilience of winter embryos should be attributed to the absence of exposure of parental thalli to stress. This hypothesis is also supported by the reduced ability of *F. guiryi* from sheltered upper intertidal microhabitats to release viable gametes and success at fertilization and recruitment (pers. obs.).

The findings suggest that the two hypotheses initially posed are not mutually exclusive, since both maternal effects and chronic thermal stress can modulate the seasonal thermal resilience of embryos of *F. guiryi*. Early summer embryos were the most heat-sensitive, with 75% and 50% lower growth and survival rates, respectively. Enhanced physiological performance of late summer embryos suggested a warm-hardiness process due to maternal acclimation, as for other fucaceae (Li and Brawley, 2004), while also reflecting a detrimental effect of summer-heat accumulation when compared to the winter responses. Unexpectedly, winter embryos attained the greatest thermal resilience, showing similar survival and physiological responses at both temperatures, despite lower development at 25°C.

In the near future, embryos of *F. guiryi* might cope with projected warmer winters but strive at high temperatures, as during the onset of the warming season and chronic summer heat stress, or at microhabitats where thermal conditions are harsher (ie. outside or under reduced canopies of parental thalli). These results also help understand the current decline of peripheral populations of *F. guiryi* (Melero-Jiménez et al., 2017; Riera et al., 2015), since they are closer to their absolute thermal threshold. For instance, the SST gradient from the Strait of Gibraltar towards the Mediterranean, and the ongoing rise in the frequency and intensity of marine heatwaves might explain the disappearance of *F. guiryi* from its easternmost last refugia along the north coast of the Alboran sea (Melero-Jiménez et al., 2017).

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Temporal patterns of bleaching in anemones from temperate seas: Effect of ocean warming

Ana María Meléndez-Pérez*[1] , Bárbara Úbeda[2] , Ana Bartual[2] , María Laura Martín-Díaz[3]

*Corresponding author: ana.melendezperez@alum.uca.es

[1] Basic Sciences Department, Faculty of Engineering, Simon Bolivar University, 080002, Barranquilla, Colombia.

[2] Biology Department, Institute of Marine Research (INMAR), International Campus of Excellence of the Sea (CEI·MAR), Faculty of Marine and Environmental Sciences, University of Cadiz, 11510, Puerto Real, Cadiz, Spain.

[3] Physical Chemical Department, Institute of Marine Research (INMAR), International Campus of Excellence of the Sea (CEI·MAR), Faculty of Marine and Environmental Sciences, University of Cadiz, 11510, Puerto Real, Cadiz, Spain.

Keywords: Anemone, Bleaching, Climate change, Fluorescent protein, Zooxanthellae

Abstract

Anthropogenic stressors, such as climate changes, are driving shifts in the abiotic features of marine ecosystems. Such changes are occurring much faster than adaptive capacity of marine organisms (Bopp et al., 2013). High sea surface temperatures are known to disrupt the symbiosis between scleractinian corals and endosymbiotic dinoflagellates of the genus *Symbiodinium*. Not only in reef building corals, but also in some symbiotic sea anemones that host *Symbiodinium* sp., it has been observed disruption of symbiosis due to temperature increase. The loss of the dinoflagellates (known as zooxantellae) and/or their photosynthetic pigments from the host, results in a pale or whitened appearance that is called bleaching. The majority of mass bleaching events have been reported in tropical regions after extreme warming events (Pockley, 2000). Bleaching of anthozoans has received considerable attention, however results remain inconclusive and the process is still unkown. To date, there is only scant information available about bleaching of anthozoans in temperate seas, and after a real long term temperature increase. Host anemones tend to be long lived species with low natural mortality and recruitments rates, inhabiting temperate seas, so bleaching, caused by different stress agents as temperature, might pose a serious threat to their abundance and consequently to the structure of the ecosystem. Elucidating bleaching process in the sea anemone *Anemonia* sp., widely spread in Mediterranean Sea and Atlantic Ocean, would help to the development of early warning tools to monitor marine ecosystems affected by climate change drivers. This study addressed the analysis of the bleaching process resulting from a temperature increase event simulated in the laboratory. Specimens of *Anemonia* sp. from the Mediterranean Sea and Atlantic Ocean were long-term exposed to increasing temperature in order to simulate a water warming event (27°C) and maintained at this temperature during 23 days (552 hours). Bleaching percentage in sea anemones was determined over time as well as zooxanthellae, pigments and fluorescent proteins contents. Results showed the capacity of adaptation to temperature increase of specimens from the Mediterranean Sea, being observed bleaching event in specimens from the Atlantic Ocean. Moreover, the zooxantellae density was reduced over time and this reduction was negatively correlated with an increase of protective pigments and fluorescent proteins, showing their possible involvement in anemone protection and photosynthesis enhancement. For the first time, different zooxanthella densities were observed in two different anemones tissues, the column and the tentacles. Effective management of the health

state of marine environments affected by climate change drivers, requires an early detection or biomonitoring stress markers. The study of the environmental risk caused by water temperature increase, using a suitable bioindicator species, as sea anemone, might help in the understanding of the capacity of this organism to cope with thermal stress (bleaching process), and the finding of suitable markers for monitoring temperate seas.

Acknowledgments

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How ocean acidification and global warming affect Mediterranean jellyfish populations?

Angélica Enrique-Navarro*[1] , Laura Prieto [1] , Emma Huertas[1]

*Corresponding author: angelica.enrique@csic.es
[1] ICMAN-CSIC

Keywords: Jellyfish, Ocean acidification, Global warming, Mediterranean Sea, Polyps

Abstract

The world's oceans are becoming warmer and more acidic as a consequence of climate change and the increasing uptake of atmospheric CO_2. However, little is known about the long term impact of predicted future conditions on marine invertebrates, particularly on their early developmental stages. In this work, the interactive effect of rising temperature and ocean acidification on the physiology of Mediterranean jellyfishes polyps was examined to determine future responses of population dynamics in the Mediterranean Sea. Organisms were then exposed to different temperature and pH conditions to assess the effect of experimental conditions on life cycle. Experiment 1 examined polyp survival and asexual reproduction over winter by comparing survival rates under current and future winter temperature and pH conditions (ca. 2100) predicted on the RCP8.5 scenario (Stocker et al., 2013). The levels tested in experiment 1 were 18°C (average winter temperature in the Mediterranean Sea) combined with the current and future pCO_2 conditions (400 ppm and 935 ppm respectively). Experiment 2 was aimed to identify the mechanisms that trigger strobilation. Asexual reproduction, rates of survival, metamorphosis of polyps, formation of new medusae by strobilation (ephyra) and the formation of statoliths in newly metamorphosed ephyrae under current and future (ca. 2100) summer temperature and pH conditions, based on the RCP8.5 scenario (Stocker et al., 2013). Responses at current summer conditions of 24°C and a 400 ppm pCO_2 were compared with those obtained for predicted future summer conditions of 30°C and and 935 ppm pCO_2. Our results show that Mediterranean scyphozoan jellyfish polyps are quite tolerant to future pH and temperature conditions in the short term, surviving and reproducing asexually. However, these organisms are unlikely to thrive in the long term because acidification and temperature may affect statoliths formation and hence, the development and fitness of the ephyra. Future research on the behavior of ephyrae and their statoliths is nedeed to understand how ocean acidification and temperature affect jellyfish population survival in the Mediterranean Sea.

Acknowledgments

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El Niño as a predictor of Round sardinella abundance in northwest Africa

Jorge López-Parages[1] , Pierre-Amaël Auger[2] , Belén Rodríguez-Fonseca[3] , Noel Keenlyside[4] , Carlo Gaetan[5] , Angelo Rubino[5] , Maeregu Wolves Arisido[5] , Timothée Brochier[6]

*Corresponding author: jlopez@cerfacs.fr
[1] CERFACS/CNRS, Climate Modelling and Global Change Team, Toulouse, France
[2] Instituto Milenio de Oceanografía and Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile
[3] Dpto de Física de la Tierra y Astrofísica, UCM-IGEO, Complutense University of Madrid, Spain
[4] Bjerknes Centre for Climate Research, Univ. of Bergen, Norway
[5] Dpto di Scienze Ambientali, Informatica e Statistica, Ca Foscari University of Venice, Italy
[6] Institut de Recherche pour le Développement (IRD), UMMISCO, Sorbonne Université, Univertisé Cheikh Anta Diop, Dakar, Senegal

Keywords: ENSO, Round sardinella, Atmospheric teleconnection, Ocean and atmospheric dynamics

Introduction

The El Niño Southern Oscillation (ENSO) produces global marine environment conditions that can cause changes in abundance and distribution of distant fish populations worldwide. Understanding mechanisms acting locally on fish population dynamics is crucial to develop forecast skill useful for fisheries management.
This issue could be of major socio-economic relevance. For example in the northwest African coast, where the small pelagic fishes and, particularly, the round sardinella or *Sardinella aurita*, is a key species for food security (Failler, 2014). In this study we address the role played by ENSO on the round sardinella abundance in the southern flank of the Canary Current Upwelling System (CCUS) where this species is the most abundant.

Methods and Results

Determining the signature of global climate modes like ENSO on round sardinella abundance along the CCUS is complicated mainly due to: 1) the scarcity of abundance data, even with state of the art observational capabilities, and 2) the difficulty of distinguishing environmental influences (i.e., not directly related to changes in human activities) and human-related influences. A combined physical-biogeochemical framework (ROMS-PISCES; see Auger et al. (2016) for details) is used here to understand the climate influence on the hydrology, plankton productivity and carbon biomass in the study area. This information is used to simulate the round sardinella spatio-temporal variability with the evolutionary individual-based model Evol-Deb (Brochier et al., 2018). This approach, usually denoted as end-to-end (climate-to-fish) modeling, is having an increasing interest during the last years. The main reason is that it provides a comprehensive modeling framework where the underlying mechanisms connecting climate and marine ecosystems can be analyzed.
A robust anomalous abundance of round sardinella biomass is identified in winter, off the Cape Blanc region and the Saharan coast (Figure 1; top-left panel), in response to El Niño conditions (Figure 1; bottom panel). The resultant anomalous pattern is an alteration of the normal migration

between the Saharan and the Mauritanian waters. It is primarily explained by the modulating role that El Niño exerts on the currents off Cape Blanc, modifying therefore the normal migration of round sardinella in the search of acceptable temperature conditions. This climate signature can be potentially predicted up to six months ahead based on El Niño conditions in the Pacific.

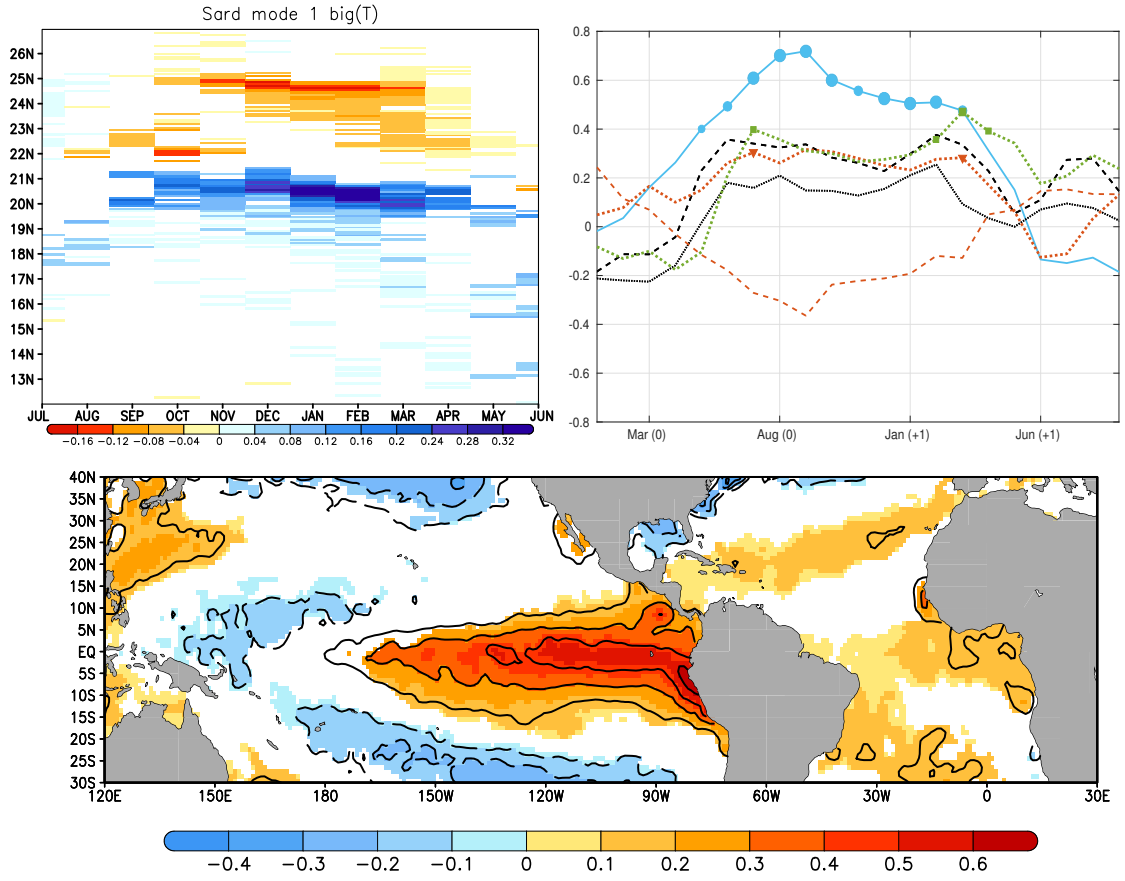

Figure 1: In the top-left panel the leading EOF of the anomalous round sardinella abundance (calculated from 19°N to 26°N and for the JFM season; units in tons m^{-2} per std in the corresponding Principal Component). In the bottom panel the regression map on the anomalous SSTs (units in degrees per std in the aforementioned Principal Component). Finally, in the top-right panel, the linear correlation between the Niño3 index at different lags and: the sardinella mode (blue solid line), the meridional current off Cape Blanc in Nov-Dec (dashed brown line) and March (dotted brown line), the water temperature off Cape Blanc in March (greeen line). The correlation between the leading sardinella mode and the zonal (meridional) windstress off the northwest African coast is also included with a black dashed (dotted) line.

Final discussion

The present study provides, for the first time, a consistent mechanism explaining how a remote climatic phenomenon (El Niño, understood as the ENSO warm phases) is able to alter the abundance of round sardinella (*Sardinella aurita*) in the narrow coastal band along northwest Africa. In contrast to the major factor that food availability plays for determining sardine and anchovy abundance in the northern part of the CCUS (Sánchez-Garrido et al., 2019), our experiments indicate that the alteration of the intake of the nutrient-rich water by El Niño seems to play a minor role for our El Niño-round sardinella teleconnection. This is consistent with previous works which suggest that the currents and the water temperature are the main

environmental variables (or limiting factors) influencing the round sardinella variability in the southern part of this upwelling system (Bacha et al., 2017).
One of the main potential societal benefits of this work is that it provides the basis for the development of a future seasonal forecasting tool of round sardinella latitudinal distribution along northwest Africa from El Niño-related SSTs. In particular, high lagged correlations between our round sardinella mode and the Niño3 index indicate the potential for skillful prediction (Figure 1; top-right panel). This and other related issues should be further analyzed in future works.

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Response of N_2-fixing bacteria associated with *Posidonia oceanica* to ocean acidification

Víctor Fernández Juárez*[1] , Elisabet Pol Pol[1] , Aida Frank Comas[1] , Maria Guadalupe Gil[1] , Xabier Alforja López[1] , Antoni Bennasar Figueras[1] , Nona Sheila Agawin Romualdo[1]

*Corresponding author: victor.fernandez@uib.es
[1] Área de Ecología, Departamento de Biología, Facultad de Ciencias, Universitat de les Illes Balears. Palma (Balearic Islands)

Keywords: *Posidonia oceanica*, Acidification, N_2 fixers, CO_2, Mediterranean Sea

Introduction

Posidonia oceanica is an endemic seagrass with key ecological functions in the Mediterranean Sea; high primary productivity, as carbon sink, as habitat and nursery for a variety of micro- and macro-organisms, as sediment stabilizers, and as an important site for biogeochemical processes (*e.g.* nitrogen cycles) (Campagne et al., 2015; Agawin et al., 2016). This plant harbours an incredible variety of N_2-fixing bacteria that are associated in epiphytic or endophytic form that potentially could supply the entire demand of nitrogen to the plant itself and rest of organisms associated with (Agawin et al., 2014). Healthiness of the N_2-fixing population is essential to maintain these *P. oceanica* meadows and their ecological functions. Diazotrophs, or N_2-fixing bacteria are subjected to many environmental factors that could limit their growth and their N_2 fixation rates. One of the big current threats is the Climate Change, that is due to the increase of the dioxide of carbon (CO_2) in the atmosphere. CO_2 is absorbed by the oceans, increasing the dissolved inorganic carbon (DIC), and part of it could be removed by the photosynthesis, and a clear example is *P. oceanica* that could actuate as a buffer for ocean acidification, thanks to its big capacity to fix carbon. There is CO_2 that is not removed, and it is transformed to carbonic acid (H_2CO_3), and this to bicarbonate (HCO_3^-) whose dissociation release protons (H^+) and carbonate (CO_3^{2-}). The consequence is the graduate decline of the pH. It is documented that in the Mediterranean Sea pH is decreasing -0.0044 ± 0.00006 annually (Flecha et al., 2015) and this could have many effects at macro and micro scale level. The effect of this decline of pH in N_2-fixing bacteria is unknown, and nothing is documented about the effects in these bacteria and the consequences that could have in *P. oceanica*.

Materials and methods

This report is the first part of a big study in which we are studying the effect of ocean acidification in N_2-fixing bacteria associated with *P. oceanica*. Here we are reporting the effect of the decline of pH in diazotrophs, without taking into account the increase of CO_2 amount. We tested a range between pH 4-8 in two autotrophic bacteria (*Halothece* sp. PCC 7418 and *Fischerella muscicola* PCC 73103) and four heterotrophic bacteria (*Pseudomonas azotifigens* DSM 17556T, *Halomonas* sp., *Marinobacterium litoralle* DSM 23545 and a natural heterotrophic community from rasp directly the leaves) in different conditions of nutrient availability (changing inorganic phosphorous [PO_4^{3-}] and nitrogen [NO_3^-], and iron [Fe]). We estimated changes in cell viability and abundance, measuring growth by cytometry analysis, nutrient uptake (*e.g* P-uptake), metabolic activity and oxidative stress. N_2 fixation by acetylene reduction assay (ARA) by gas chromatography (GC) was performed. Two selected bacteria, one autotrophic (*Halothece* sp. PCC 7418) and one

heterotrophic (*Halomonas* sp.) were selected and growth in the range of pH between 6.5-8 in two conditions, low PO_4^{3-} plus low Fe levels, and low PO_4^{3-} plus high Fe levels. The main goal was to study cell abundance and the Alkaline Phosphatase Activity (APA).

Results and discussion

Taking together all the results, growth was clearly affected by the pH, and by nutrient availability. Under optimal nutrient amount, generally, growth was higher in pH 7-8. However, under nutrient limitation, generally and with exceptions, growth was higher in lower pH (5-7). It is important to remark that every N_2-fixing bacterium had an optimal pH, indicating that changes in pH could select N_2-fixing population, suggesting that variation of pH could affect to *P. oceanica* microbiota. Oxidative stress and metabolic activity were increased under higher pH. However, N_2 fixation rate was barely affected by changes in pH. Otherwise, APA, especially in the cyanobacterium (*Halothece* sp. PCC 7418) was dramatically affected only changing 1.5 units of pH, showing the pH dependence of Alkaline Phosphatase (APase) (in the heterotrophic bacterium minimum changes were detected). Higher pH displayed a saturation curve of APase with higher V_{max}, indicating that a decline in pH could affect P-acquisition mechanisms, especially in the cyanobacterial population. With the goal of comparing the above APA results with the APA of the plant itself (in leaves, rhizome and root), *P. oceanica* was maintained in different aquariums under different CO_2 concentrations (400 ppm and 1200 ppm). Results showed that a decline of pH not necessarily affected APA, and even in some cases APA was enhanced. These results matched with those obtained in the heterotrophic bacteria in which low pH led to higher APA, suggesting that pH could affected P-mechanisms in different ways depending on the part of the plant and depending on the autotrophic or heterotrophic population. In short, here we presented the first insights of the effect of the decline of pH in N_2-fixing bacteria associated with *P. oceanica*, showing how growth, biochemical parameters and N_2-fixation can be affected, especially APA. We suggest that changes in pH could select N_2-fixing bacteria, and their effect in the plant itself should be deeply studied with further studies to shed light and evaluate the changes that *P. oceanica*populations can suffer by ocean acidification.

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Multidecadal variability of tropical cyclones in the North Atlantic

Carmen Ferrero Martín*[1]

*Corresponding author: ferrerocarmen0@gmail.com
[1] University of Cádiz

Keywords: Tropical cyclones, North Atlantic, Atlantic Multidecal Oscillation, Hurricane

Introduction

Tropical cyclones (TC) are meteorological phenomena with great influence on the lives of human beings, and are the cause of many catastrophes in coastal spaces around the world. In order to improve their capacity for anticipation, response and recovery, humans have always had the need to determine the causes found in these phenomena, and thus be able to reduce material and human losses. In addition, cyclones have become a mechanism in the global atmospheric circulation, as well as in a balance of temperature, heat displacement and humid air in the middle and polar latitudes. The variability of the North Atlantic tropical cyclones is linked to changes in sea surface temperature (SST) in this basin (Emanuel, 2005; Webster et al., 2005). Under conditions of global warming, it is imperative to anticipate what tropical cyclones will look like due to climate change. For this purpose, it is necessary to assess what factors determine its multidecadal variability.

Methodology

To improve the quality and accuracy of the analysis, considering the accuracy of the data, the time period was delimited between 1950 and 2017. The historical record has been separated into “warm" and “cold" periods, defined from the anomalies of the SST, which are determined by the Atlantic Multidecadal Oscillation (AMO) index. Since the length of the warm and cold time series is different, the results are normalized to be able to compare them. The database used HURDAT2 has cyclone records by different institutions and at different times of its trajectory. This made their work difficult, so they were processed by codes made in FORTRAN language, which allows selecting the first and last record of each cyclone, which allows us to map the cyclogenesis (point of generation of the cyclone) and cyclolysis (point of final dissipation of the same). In addition, they allow the incorporation of data in a 2° x 2° grid, which facilitated the application of spatial and temporal analysis techniques, such as the study of the trend and the correlation with the AMO.

Results and discussion

In the North Atlantic Ocean (NATL), the AMO is seen as a cyclical variation of large-scale oceanic and atmospheric circulation, which is identified with a consistent pattern of variability with a period of 20 to 40 years with phases of positive anomalies SST and negative (Figure 1). The activity of tropical cyclones has significant impacts due to weather patterns such as the Atlantic Multidecadal Oscillation (Goldenberg et al., 2001; Klotzbach and Gray, 2008). The cyclonic and AMO energy indices have a strong relationship, where warm phases coincide with

an increase in cyclonic activity and vice versa for cold. Observing the quadratic adjustment of the accumulated cyclonic energy index (ACE), it is determined that at the present time and after passing through a period with a minimum of activity, we would find ourselves in a more dynamic phase. These fluctuations of a natural nature cause difficulties in attributing changes in cyclonic activity to global warming when the length of the available time series is not much greater than the period of the oscillation.

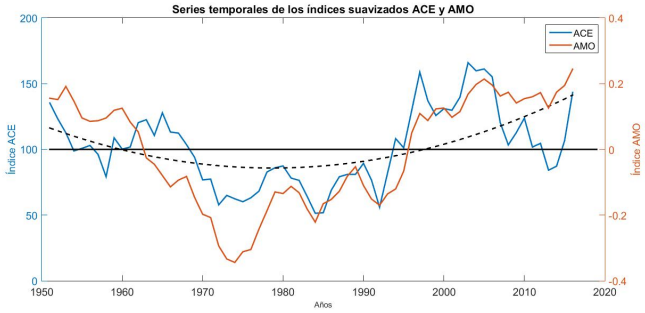

Figure 1: Time series of the ACE and AMO indices smoothed with a moving average with a 5 year interval (ACE, blue line, AMO, orange line). In addition, the quadratic setting of the time series of the ACE index (dotted line) is shown.

Frequency

Table 1 shows that the number of tropical cyclones and hurricanes is greater in the warm phase. This increase is much more pronounced if we take into consideration the major hurricanes (MH), that is, category 3 or higher on the Saffir-Simpson scale.

Table 1: Average ACE index and frequency of tropical cyclones, hurricanes and major hurricanes for each phase. In addition to the classification to which I arrive each season and the ratio.

Average	Tropical cyclones	Hurricane	Major hurricane	ACE	Classification
Cold phase	9.50	5.31	1.75	79.31	Below normal
Warm phase	11.42	5.95	2.79	123.86	Above normal
Ratio	1.20	1.12	1.59	1.56	

Intensity

In the maps of the trajectory density of the major hurricanes it is evident that in the warm (positive) phase of the AMO there is a greater number of MH in the NATL compared to the cold (negative) (Figure 2). This difference is seen particularly clearly in the Caribbean Sea.

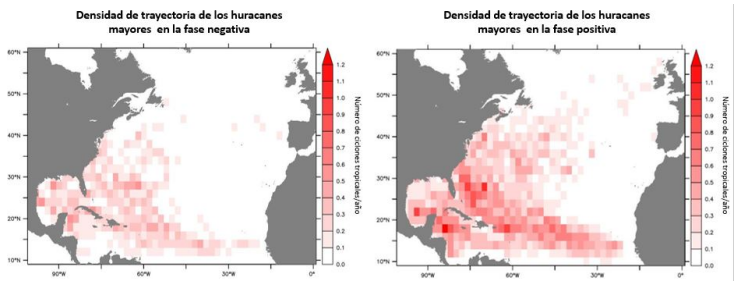

Figure 2: Density of the number of major hurricanes in a 2 x 2 mesh for the positive and negative phase of the AMO.

Cyclogenesis

The most striking differences in the location of the regions of cyclogenesis are that in the cold phase less TC originates in the western Caribbean and nearby Atlantic, but instead they form more in the eastern United States (Figure 3). While for the warm phase they are developed more in the Caribbean and in the Main Developed Region (MDR), especially in the areas closest to Africa and the equator. The increase in the duration of the hurricanes is linked to the positive phase of the AMO, with a correlation coefficient of 0.62. This is physically compatible with the fact that hurricanes form more towards the east and towards the equator because, when the cyclogenesis occurs more to the southeast, it moves away from the American continent and causes the storms to last longer before touching Earth or curl to the north around the western edge of the subtropical wedge and towards the hostile environment of the middle latitudes. If one takes into account the fact that longer TCs tend to reach higher intensities, it can be deduced that, during warm periods, these cyclones will register greater intensity and duration.

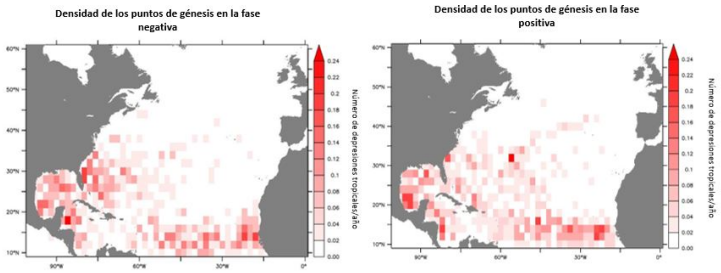

Figure 3: Density of the number of genesis points (defined when they reach the intensity of tropical depression) in a 2 x 2 mesh for the positive and negative phase of the AMO.

Cyclolysis

We proceed to study how AMO affects deviations of trajectories towards the Northeast in tropical cyclones. Positive correlations are obtained for both longitude and latitude with TCs and major hurricanes. Therefore, the TCs arrive more to the northeast in the positive phase. although the correlation is lower than expected for the major hurricanes because the point cloud of these is more horizontal than that of tropical cyclones, since these are confined to the longitudinal coverage of the isobars of the North Atlantic subtropical wedge. Likewise, an analysis of the trends is made determining that there is a small tendency for tropical cyclones to reach more to the east, while the latitude decreases. This shift towards positive lengths is further intensified in major hurricanes.

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Vulnerability Assessment applied on case studies from Western and Eastern Mediterranean Small-scale and Industrial Fishing (NAFO area)

Mauro Gómez Murciano[1]

*Corresponding author: mgm129@alu.ua.es
[1] Department of Marine Science and Applied Biology, Alicante University

Keywords: Adaptive Capacity, Climate Change, Fishermen, Vulnerability Assessment

Abstract

Together with pollution and overfishing, Climate Change (CC) is the greatest environmental threat facing the fishing industry. CC is impacting and will continue to impact marine fisheries. These impacts generate a series of mishaps for the fishing activity at the biological, economic and social levels. For these reasons, the aim of this study was to assess the various threats to fisheries, focusing on CC. To perform this task, Vulnerability Assessment (VA) was the methodology employed. Vulnerability (V) has three components: 1) Exposure (E), 2) Sensitivity (S) and 3) Adaptive Capacity (AC). V was measured through surveys that were carried out among fishermen from the small scale-fishing (SSF) fleets of Castelló (Spain) and Aegean Sea (Turkey) and one case of industrial fishing in NAFO area, between 2018 and 2019. Survey results show fishermen's perception of CC. Firstly, biggest threat to fisheries these days is generational succession. Furthermore, the problems arising from CC that most affect fishermen are the increase in temperature and the greater presence of storms. On the other hand, Turkish fishermen face another threat that is not equally present in Spain, namely invasive species (lessepsian migrations). In order to mitigate the effects of CC on fisheries, this study proposes a series of adaptation measures that will help to mitigate CC in addition to improving measures that are already in place. Finally, literature was reviewed to identify examples of current and recommended adaptations in the fishery sector around the world.

Introduction

In the Anthropocene there are a great variety and diversity of threats that make fishing a vulnerable activity. For example: overfishing, habitat destruction, human interactions with marine ecosystems, marine pollution, invasive species and CC. Global CC is affecting the Earth's atmospheric and oceanic system interacts in many ways with global biogeographic changes arising from marine species translocations. Rising temperatures are aiding the establishment of foreign species into the Mediterranean. These species are mostly introduced via the Suez Canal and their successful establishment is believed to have been assisted in recent years by CC (Ünal et al., 2015). CC is challenging the effectiveness of contemporary fishery management and gives rise to significant additional uncertainties and threats to fishers and to the fishing industry (Poulain et al., 2018). Many economies and people are dependent on fisheries (Brugère and De Young, 2015).

Vulnerability Assessment

Vulnerability is the propensity or predisposition to be adversely affected. There are several different components to examining vulnerability of the Mediterranean fishing population in the face of CC (Cinner et al., 2012). These components usually measure: 1) Exposure (E), 2) Sensitivity (S) and 3) Adaptive Capacity (AC) (Adger, 2006) There are no independent measures of E, S or AC, so their interpretation depends on the scale of the analysis, the particular sector considered and the availability of data. Vulnerability, in the context of social and environmental change, is defined as the state of susceptibility to harm from perturbations (Adger, 2006). Knowledge about how vulnerable a system is, and the specific conditions that make it vulnerable, can help to provide a foundation for developing key actions that minimize the impacts of environmental change on people.

Acknowledgments

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Carbon fluxes variability in oligotrophic Mediterranean systems

Natalia González-Benítez*[1] , Lara S. García-Corral[1] , Xosé Anxelu G. Morán[2] , Jack B. Middelburg[3] , Marie Dominique Pizay[4] , Jean-Pierre Gattuso[4]

*Corresponding author: natalia.gonzalez@urjc.es

[1] Department of Biology, Geology, Phyisics and Inorganic Chemestry, King Juan Carlos University. C/ Tulipán s/n, 28933 Móstoles, Madrid, Spain

[2] King Abdullah University of Science and Technology (KAUST), Red Sea Research Center, Biological and Environmental Sciences and Engineering Division, 23955-6900 Thuwal, Saudi Arabia

[3] Department of Earth Sciences, Utrecht University, Princetonlaan 8A, 3584 CB Utrecht, The Netherlands.

[4] Sorbonne Université, CNRS, Laboratoire d'Océanographie de Villefranche, 181 chemin du Lazaret, F-06230 Villefranche-sur-mer, France

Keywords: Bacterial respiration, Bacterial production, Oligotrophic bays, Organic carbon metabolism, Northwestern Mediterranean

Abstract

The effect of temporal and spatial variability on the carbon fluxes coupling between heterotrophic bacterioplankton and phytoplankton was studied in two coastal oligotrophic sites (NW Mediterranean). Phytoplankton and bacterial production rates were measured under natural conditions using different methods (^{14}C-particulate and dissolved primary production, ^{3}H-leucine bacterial production, light and dark changes in dissolved O_2). In the Bay of Villefranche, temporal variability was studied and revealed net heterotrophy during summer and autumn and net autotrophy during winter and spring. The Bay of Palma showed autotrophic areas at the western stations and heterotrophy at the eastern stations. The bioavailability of dissolved organic carbon (DOC) plays a key role on bacterial metabolism. Temperature increased DOC concentration and therefore, bacterial growth efficiency (BGE), only under autotrophic conditions. Total primary production was potentially able to sustain bacterial carbon demand (BCD) in autotrophic periods, whereas it did not in heterotrophic periods. On average, bacterial respiration (BR) represented 62 % of the total community respiration. BGE values were significantly higher in heterotrophic systems compared to those autotrophic. The results obtained in these oligotrophic bays suggested that shifts in ecosystem trophic status reflect the response of bacterial carbon processing (i.e. BGE) to the bioavailability of dissolved organic carbon and temperature.

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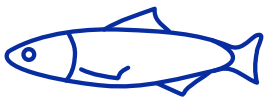
Session 2

The Sea Knowledge

Fishing resources



Ecology of the stalked barnacle, *Pollicipes pollicipes* (Gmelin, 1970): reproduction, recruitment and relation with the environmental conditions

Salvador Román*[1] , Carlota Muñiz[1] , Alba Aguión[2] , José A. Santiago[3] , Pablo Seoane[4] , Berta Barreiro[5] , Elsa Vázquez[1] , Gonzalo Macho[1]

*Corresponding author: salvador.roman.del.valle@gmail.com
[1] Dpto Ecoloxía e Bioloxía Animal, Facultade de Ciencias do Mar, Universidade de Vigo, 36310 Vigo, Spain
[2] Future Oceans Lab, Universidade de Vigo, 36310 Vigo, Spain
[3] Cofradía de Pescadores La Anunciada de Baiona, 36300 Baiona, Spain
[4] Cofradía de Pescadores de A Coruña, 15006 A Coruña, Spain
[5] Cofradía de Pescadores San José de Cangas, 36940 Cangas, Spain

Keywords: Barnacles, *Pollicipes pollicipes*, Reproduction, Recruitment, Environmental conditions

Introduction

The barnacle *Pollicipes pollicipes* (Gmelin, 1970), commonly known as stalked barnacle is a marine sessile crustacean (Class Hexanauplia, Subclass Thecostraca, Infraclase Cirripedia, Order Scalpelliformes, Family Pollicipedidae) that lives adhered to rocks or other artificial hard substrata of intertidal shores. Its distribution extends along the East Atlantic coast from French Brittany to the coast of Senegal, including the Canary Islands (Fernandes et al., 2010). *P. pollicipes* is an economically important resource in fisheries in Spain and specifically in Galicia, where in 2018 the price ranged between 17.9 and 97.1 euros Kg^{-1} in the localities addressed in the present study (Xunta de Galicia, 2019). Further knowledge on the ecology of the organism is required to improve the management of the fishery.

Materials and methods

Reproduction and recruitment of the stalked barnacle (*Pollicipes pollicipes*), were studied in three sites along the Atlantic coast of Galicia (Figure 1). Adult individuals (rostro-carinal length, RC $>$ 15 mm), were sampled monthly between August 2017 and February 2019. For each month and site, the reproductive stage of 40 individuals was determined assessing the presence of egg masses and their maturity status. Recruitment rates were obtained counting the presence of recruits (RC $<$ 2 mm) in the peduncle of 20 adult individuals. The relationship of reproductive state and environmental conditions was studied for a set of environmental predictors including sea surface temperature (SST), mean, maximum and minimum air temperatures, and upwelling index (UI). Environmental conditions were averaged during the period of gonadal development (25 days). Reproductive state in each site was modelled against the set of environmental predictors through Multiple Linear Regression (MLR), and the best model was selected using the Akaike Information Criterion. The same analyses were carried out for recruitment, considering three size clases of recruits.

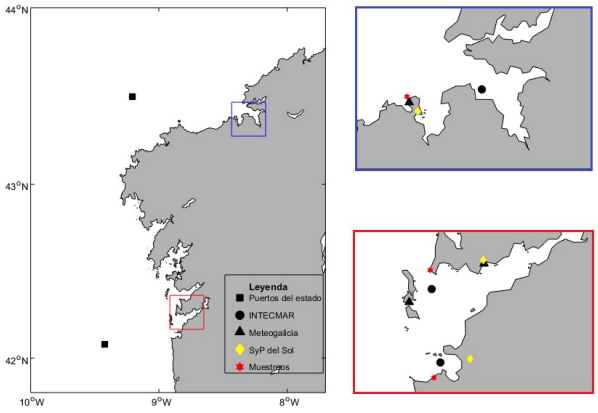


Figure 1: Study area specifying the location where the biological data were obtained (indicated in the map as "Muestreo") and environmental data (Buoy of "Puertos del Estado", INTECMAR Buoys, location of the METEOGALICIA stations and the "SyP del sol" stations).

Results and discussion

A Coruña and Cangas showed a similar reproductive pattern throughout the year, with higher percentages of adults carrying egg masses in Cangas (Figure 2). In both sites, more than 50% of the individuals were carrying egg masses from the end of spring until autumn, with a peak during summer. In Baiona, reproduction was continuous throughout the year, showing more than 25% of the individuals with brooding activity during winter months (Figure 2). The number of broods per year was close to five in A Coruña and Cangas, and close to eight in Baiona, showing the highest values recorded to this date, despite the time series being incomplete. Model results showed that the best predictors of reproductive state for the three sites were minimum air temperature and upwelling index (MLR, $p < 0.001$).

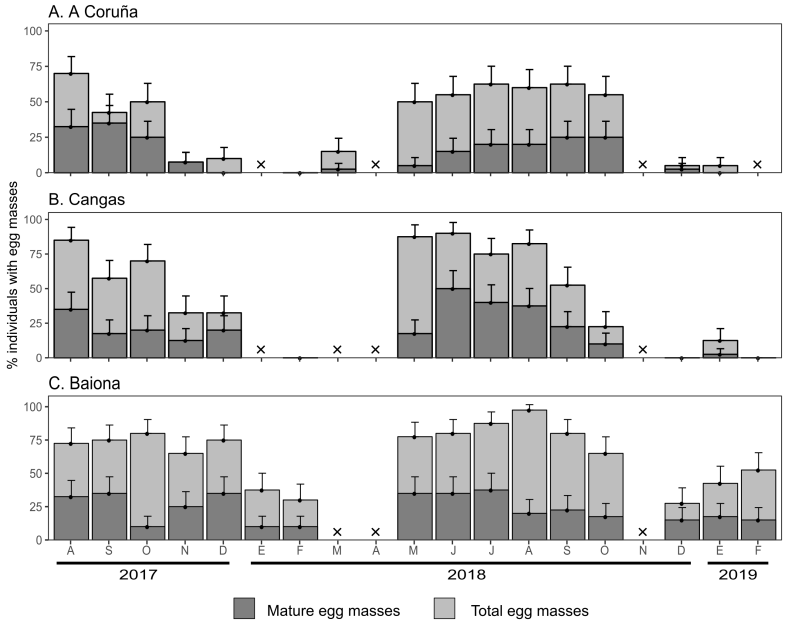


Figure 2: Percentage of adult individuals in reproduction differentiating individuals that carry mature egg masses. The error bars show the confidence interval at 90%. The x represent the months in which no sample was collected becouse the bad conditions of the sea.

Two recruitment periods appeared in the three sites during the period of study, extending from August until February, with maximum recruitment rates in September (Figure 3). Comparison of recruitment among sites and periods showed that recruitment during the first period studied in Baiona was significantly lower than on the other sites and periods (ANOVA, $p < 0.05$). MLR showed significant results only for Cangas, where the selected model showed a positive relationship with maximum air temperatures and a negative relationship with SST (MLR, $p < 0.05$).

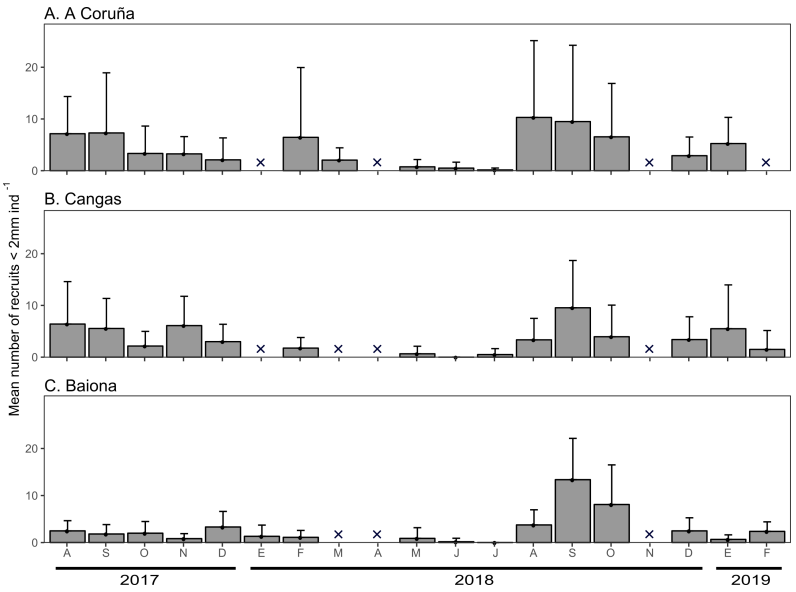


Figure 3: Time series of total recruitment (RC < 2 mm) in the three localities studied. The error bars show standard deviation. The x represent the months in which no sample was collected becouse the bad conditions of the sea.

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Connectivity patterns of the blackspot seabream (*Pagellus bogaraveo*) in the Alboran Sea

Irene Nadal Arizo*[1] , Simone Sammartino[1] , José Carlos Sánchez Garrido [1] , Jesús García Lafuente[1]

*Corresponding author: irenenadal@ctima.uma.es
[1] Physical Oceanography Group, University of Malaga

Keywords: Blackspot seabream, Hydrodynamic connectivity, Lagrangian algorithm, Tidal dynamics, Alboran Sea

Introduction

Several researchers have reported that the Blackspot seabream (*Pagellus bogaraveo*) have been subjected to an excessive exploitation over the years (Gil Herrera, 2006). Experts in fishery ecology agree that for optimizing the fisheries, as well as curbing the over-exploitation, is necessary to study the population dynamics and the connectivity of the species according to the circulation patterns and the oceanographic variability (Cowen and Sponaugle, 2009). The objective of the present work is to analyse the blackspot seabream connectivity with a hydrodynamic numerical model, using early life stage (ELS) blackspot seabream virtual particles as passive tracers advected by simulated currents.

The numerical model

The model used to carry out the numerical simulations is the MIT general circulation model (Marshall et al., 1997), widely employed to describe successfully the complex dynamics of the study region. The model domain spans from the Gulf of Cadiz to the Alboran Sea eastern edge throughout a curvilinear horizontal grid, with variable horizontal resolution, maximum at the Strait of Gibraltar (SoG hereinafter), and uneven vertical discretization (Figure 1). The model was forced with different meteorological and oceanographic datasets at its surface and lateral open boundaries.

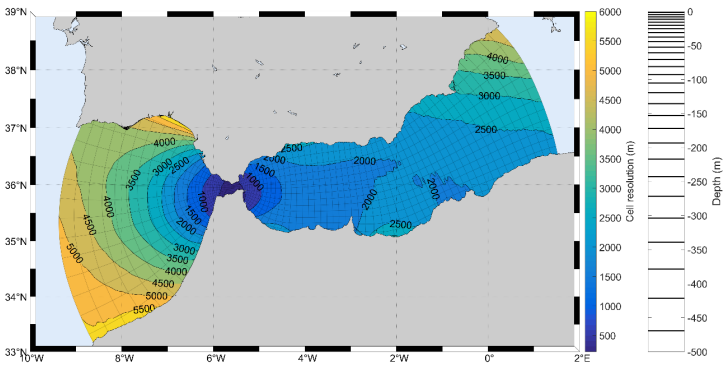

Figure 1: Model domain, with horizontal (one every 7 cells has been displayed for clearness) and vertical grid (up to 500m depth).

The model runs in the PICASSO cluster of the Supercomputing and Bioinnovation Center of the University of Málaga, and simulates a total of 6 month hindcast, recording the zonal and meridional components of the horizontal velocity every 30 minutes. Due that the aim of the model is to reproduce the blackspot seabream dynamics in the ELS phase, the simulated hindcast corresponds with the most active spawning stage, from December 2004 to April 2005. To assess the dynamic interaction of ELS blackspot seabream with marine environment, a lagrangian particle tracking (LPT) algorithm was used. To prove the representativeness and reliability of the model, several sensitivity analyses according to the spatial and the temporal variability are needed. First, to see the particles behaviour according to the release area, three starting areas were defined within the SoG: Tanger, TangerMED and Tarifa (Figure 2). On the other hand, ten possible destination areas were defined: Cadiz, Estepona, Malaga, Roquetas, Carboneras, Oran, Melilla, Alhucemas, Tetuan and Arcila (Figure 2). Secondly, to see the particles behaviour at several vertical levels, five release depths were considered, at 1m, 12m, 25m, 52m and 81m. Third, with the aim of estimating the effect of tides on the ELS dispersal patterns, an array of LPT simulations have been started under a set of eight tide combinations of the different conditions depending on the tidal phase [(H) High, (L) Low, (F) Flood, (E) Ebb tide] and the tide strength [(S) Spring, (N) Neap tide]. Finally, a total of four replicas for each of the aforementioned condition, were implemented throughout the first two months of 2005. Simulations results in (4 tide phase x 2 tide strength x 4 replicas x 5 depths) 160 runs per release areas, each of 60 days long, according a total of 480 runs.

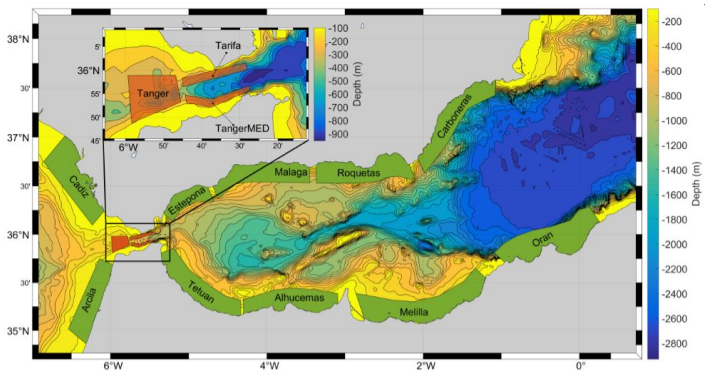

Figure 2: Three release areas (Tarifa, Tanger and TangerMED) and ten adjacent connectivity boxes (Cadiz, Estepona, Malaga, Roquetas, Carboneras, Oran, Melilla, Alhucemas, Tetuan and Arcila).

Results

Because of the high energetic dynamics and variability of the study area, and the strong current acting in there, a fixed time metric (percentage of particles released in box a and found in box b, at specific time t) was not convenient to use. In turn, the maximum percentages of particles observed in all the connectivity boxes, and the corresponding times have been considered. Table 1 shows the averaged ELS percentages (over the four replicas) as well as the coefficient of variation for each release area. After analyzing the maximum percentages of particles observed in all the connectivity boxes (Table 1), the most remarkable dynamic relative that can be concluded is that fortnightly modulation (spring tide-neap tide) has a greater effect on the connectivity patterns and horizontal dispersion than the tidal phase. Within the tide modulation, the neap tides generally resulted in a lower dispersion and more uniform velocities, while spring tides resulted in higher velocities and higher dispersion.

Table 1: Mean ELS percentages and the corresponding coefficient of variation for Tarifa, Tanger and TangerMED release areas.

Connectivity box		Averaged connectivity (%)		
		Tarifa	Tanger	TangerMED
Northern limit	Cadiz	0.10 ± 0.00	0.03 ± 0.00	0.00 ± 0.00
	Estepona	37.91± 0.67	9.56 ± 0.63	8.38 ± 1.40
	Malaga	45.27 ± 0.26	14.85 ± 0.49	11.25 ± 1.06
	Roquetas	16.56 ± 0.80	5.45 ± 0.56	4.56 ± 0.98
	Carboneras	6.46 ± 0.52	1.96 ± 1.10	2.06 ± 0.87
Southern limit	Arcila	0.00 ± 0.00	1.47 ± 0.52	0.01 ± 0.00
	Tetuan	8.45 ± 0.87	32.60 ± 0.42	44.91 ± 0.48
	Alhucemas	8.36 ± 0.68	13.75 ± 1.04	17.33 ± 0.97
	Melilla	6.20 ± 1.14	3.42 ± 0.52	3.75 ± 1.11
	Oran	13.69 ± 0.48	6.48 ± 0.21	4.66 ± 0.51

Moreover, it was observed that the highest values of average connectivity for the particles released from Tarifa were found in the northern limit of the Alboran Sea, whilst the highest values for the particles released from Tanger and TangerMED areas were found in the southern limit, which suggested a zonal (west-east) predominance connectivity instead of meridional (north-south) as a consequence of the stable surface current (Atlantic Jet, AJ hereinafter) intruding from the SoG. On the other hand, the lowest percentages were registered at Cadiz and Arcila boxes for the three release areas, which supports the idea that the AJ advect the released particles predominantly eastwards, in the Mediterranean basin, while only a small percentage of particles leap backward to the Atlantic Ocean. Concerning the discrepancy between the release areas, it was observed that the differences between Tarifa and Tanger were considerably higher than between Tanger and TangerMED. This confirmed the fact that the main transport is zonal instead of meridional, and that the AJ acts as a barrier for the connectivity patterns. Furthermore, it is known that the central part of the SoG, where the Tanger box is located, is more dynamically biased to the south and more isolated from the north mainly due to geostrophic balance (Albérolaa et al., 1995). The variability according to the depth level showed a higher energy and, thus, higher percentages on the shallower levels. Finally, after the four repeated simulations of the same tidal conditions, it could be observed that the internal variability (intra-class) between them was relatively homogenous and lower than the estimated external variability (inter-class), i.e., the simulations of different tidal conditions. Even so, since the different forcings applied to the model are based in real data and not on climatologies, it is expected that the intra-class simulations, still being very similar, vary slightly.

Acknowledgments

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Volatility of blackspot seabream (*Pagellus bogaraveo*) landings in the Strait of Gibraltar

Víctor Sanz-Fernández*[1] , Juan Carlos Gutiérrez-Estrada[1]

*Corresponding author: victor.sanz@dcaf.uhu.es
[1] Dpto. de Ciencias Agroforestales, Escuela Técnica Superior de Ingeniería, Universidad de Huelva, 21007 Huelva, Spain

Keywords: Uncertainty analysis, GARCH models, Coastal Fishery, Artisanal Fishery, Catch time series

Introduction

Regarding the exploitation of marine resources, overexploitation and environmental variability are key factors that endanger the sustainability of the fisheries worldwide. In fisheries data series, volatility, changes in trend or variability of something with respect to its average over a certain period of time or changes in the variance / deviation (σ) in the past is caused, among other factors, by natural and management-related factors, such as changes in environmental conditions, fishing methods, levels of illegal fishing, data limitations and human behaviour. Evidently, volatility is difficult to analyse and predict and has to be contained in the variation of the series itself (Agnew et al., 2009; Fulton et al., 2011). Bollerslev (1986) developed a family of models known as generalized autoregressive conditional heteroscedasticity (GARCH) models that are able to quantify the volatility associated with a series. The use of this type of model is relatively widespread in different fields, as for example, finance and economics. In contrast, their potential application to fishery data series has been overlooked to date, such models having been used in studies for analysing the variability associated with fisheries and aquaculture products. In this study, we used a univariate modelling (a wide range of GARCH (p,q) models; EWMA, APARCH, IGARCH, sGARCH, csGARCH, FIGARCH, fGARCH) to study the time series of the catches of blackspot seabream (*Pagellus bogaraveo*) between 1983 and 2016 in the Strait of Gibraltar. The primary objective was to assess the effect of variation in the patterns of volatility associated with changes in the series over time.

Material and methods

For building the GARCH models, a function was developed that executed all the possible combinations and selected the best model as indicated by the lowest AIC, varying the parameters (p,q) of the models in the range. Having modelled the variance, an autoregressive moving average model was incorporated into the GARCH model, in order to consider the effects of the mean, yielding an ARMA (p,q) + GARCH (p,q) model.

Results and discussion

The blackspot seabream fishery started in 1983 with two peaks in June 1997 (144.6 tonnes) and June 1999 (138.9 tonnes) (Figure 1). The catch data series of blackspot seabream was characterised by a marked seasonal component. The months of April, May, June, July and August accounted for 53.80% of the catches followed October, November and December which accounted for 25.06%.

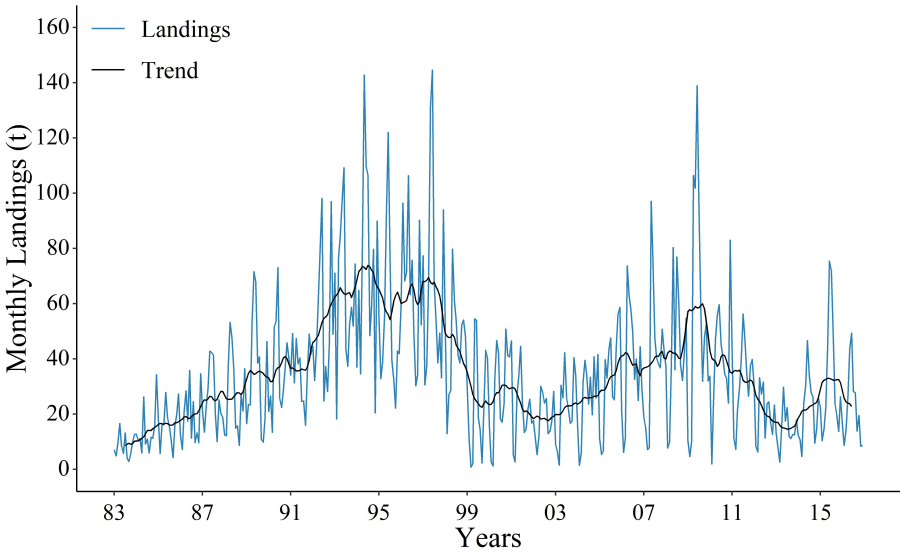

Figure 1: Time series (1983-2016) of blackspot seabream landings considering in blue and in black the trend. The trend is the result of application of the moving average filter, order 12.

The estimated volatility for the best ARMA and GARCH (p,q) models had a mean variation of between 22 and 24 tonnes between 1983 and 2016 (Figure 2).

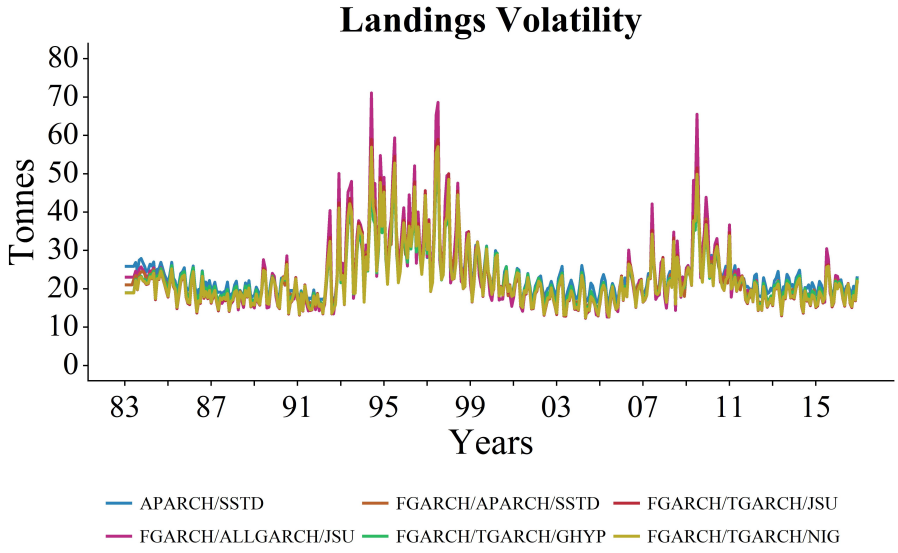

Figure 2: Time series of landings volatility or deviation (σ) estimated (τ) by the best models of the ARMA + GARCH family, with their corresponding probability distributions. SSTD = Skew Student-t, JSU = Johnson's Reparametrized SU, GHYP = Generalized Hyperbolic, NIG = Normal Inverse Gaussian.

Finally, all the models indicated that volatility did not have a trend and again indicated two clearly marked cycles, the first one from 1993 to 1999 and the second from 2008 to 2012, which could be related to both climate and management-related factors. This new way of analysis with a wide range of GARCH models would allow fisheries managers to integrate in decision making the importance of volatility or uncertainty in fisheries, and implement ecosystem-based fishery management.

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Fish and macroinvertebrate communities in the surf zone of sandy beaches on the Gulf of Cadiz coast (SW Spain): Seasonal, diel and environmental pattern variations

Carlos Rodríguez García*[1] , Manuel Gutiérrez Martínez[1] , Rubén Muñoz Lechuga[1] , Victor Sanz Fernández[2] , Remedios Cabrera Castro[3]

*Corresponding author: carlos.rodriguezgarcia@alum.uca.es

[1] Departamento de Biología, Facultad de Ciencias del Mar y Ambientales. Universidad de Cádiz. Avda. de la Universidad, 11510 Puerto Real, Cádiz, Spain. Campus de Excelencia Internacional del Mar (CEIMAR)

[2] Dpto. de Ciencias Agroforestales, Escuela Técnica Superior de Ingeniería, Campus de la Rábida, Universidad de Huelva, 21819 Palos de la Frontera, Huelva, Spain

[3] Instituto Universitario de Investigación Marina (INMAR). Campus de Excelencia Internacional del Mar (CEIMAR). Avda. de la Universidad, 11510 Puerto Real, Cádiz, Spain

Keywords: Gulf of Cadiz, Beach seine, Marine fishes, Marine invertebrates, Community composition

Introduction and objectives

Beaches have traditionally been high productivity habitats. The "surf zone" refers to the area between the coastline and the wave breaking zone (Suda et al., 2002). These areas are often used by the biological community as nurseries due to their high productivity, presence of refuge areas and food availability (Taal et al., 2017). The presence of individuals in these environments does not usually exceed one year, depending on the species. Different studies on the fish communities in surf zone beaches have been carried out in different parts of the world (Suda et al., 2002; Taal et al., 2017). However, in Spain there are few studies of this type, whilst on the east coast of the Gulf of Cadiz the published studies have focused on ichthyoplankton (Baldo and Drake, 2002) or the macroinfauna in sandy beaches (Muñoz-Lechuga et al., 2018). The starting hypothesis of this work was that the sampled beaches are used as nurseries by different species and variations occur in the community on a diel and seasonal basis. The objectives of this study were: (1) to evaluate the species of fish and macroinvertebrates that inhabit the surf zone of the beaches of the coast of the Gulf of Cadiz; (2) evaluate the daily and seasonal influence in the community of fish and macroinvertebrates; and (3) estimate the annual variation in the structure of the community to relate this variation to changes in the environmental variables studied.

Materials and methods

Monthly samplings were carried out from December 2016 to November 2017 during the periods of low tide in five beaches of the Gulf of Cadiz (Figure 1) through a beach seine net modified with measures of 10 m long, 1.5 m high with 5 x 5 mm mesh light, with a 5 m long snowflake in the center and, in addition, with leads in the bottom rope. The samples were taken perpendicular to the coastline, sampling twice a day, at dawn and dusk, with five replications per sample 25 m apart. The individuals were stored in frozen at -20 °C. In the laboratory, all of the fauna samples were counted, weighed, measured and identified to species level whenever possible. Abiotic

variables selected were: season, diel, wind, temperature, wave height (http://www.puertos.es/es-es/oceanografia/Paginas/portus.aspx) and moon phase; while the biological variables were: abundance, specific richness, species density and biomass. PERMANOVA, SIMPER, nMDS and canonical correspondence analysis (CCA) were the statistical analyses that were carried out using the R language for statistical computing.

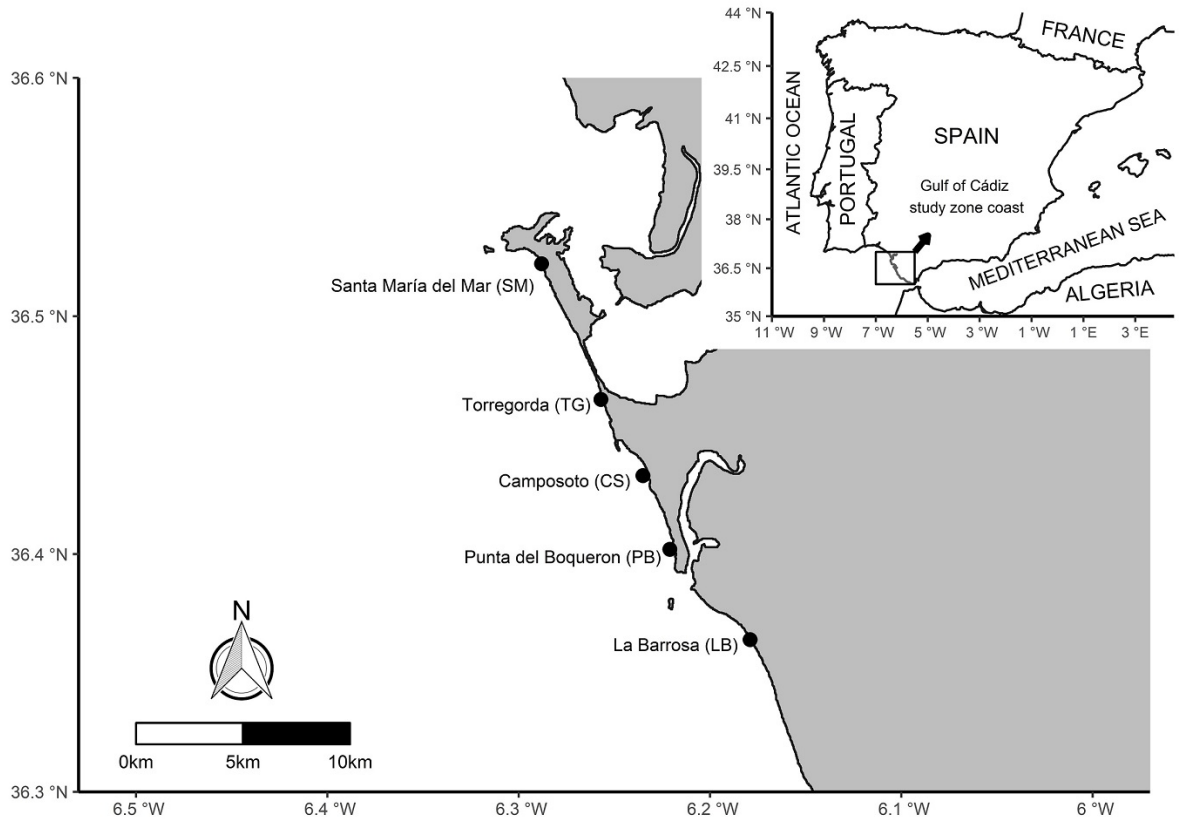

Figure 1: Location of the five beaches selected for sampling on the Gulf of Cádiz coast.

Results and discussion

In total 48 species were found, of which fish (23 species, 48%) and crustaceans (19 species, 40%) were the most abundant groups in comparison with mollusc (2 species, 4%), cnidaria (2 species, 4%), polychaeta (1 species, 2%) and chelicerata (1 specie, 2%). Season (mainly related to temperature), moon phase and diel were the variables that best explained the differences in the community between the beaches studied. The PERMANOVA test based on the analysis of the abiotic variables showed significant differences between seasons, but not between beaches. However, there were significant differences between diel and beaches with respect to the biological variables. Differences between all the beaches were observed through the SIMPER analysis, with coefficients of similarity of the community abundance less than 33%. The abundance and specific richness were significantly high in spring and summer. Season, diel and moon phase were the most influential components in the variation of the community (Figure 2). *Trachinotus ovatus* and *Crangon crangon* appear to be resident species in this habitat; in addition, *Trachinotus ovatus, Sardina pilchardus* and *Engraulis encrasicolus* use the surf zone as a breeding area after spawning. During the summer and autumn periods, high concentrations of *Carybdea marsupialis* were found. This species, which inhabits the Mediterranean and tropical Atlantic (Gueroun et al.,

2015), is invasive and was described for the first time in waters of the Atlantic Ocean NE in this work. Additional studies in the surf zone, with periodic monitoring, would serve to analyze the effects of other variables, not included in this study, allowing us to increase our knowledge about the species and significantly improve the ecological descriptors used to study environmental or anthropic changes.

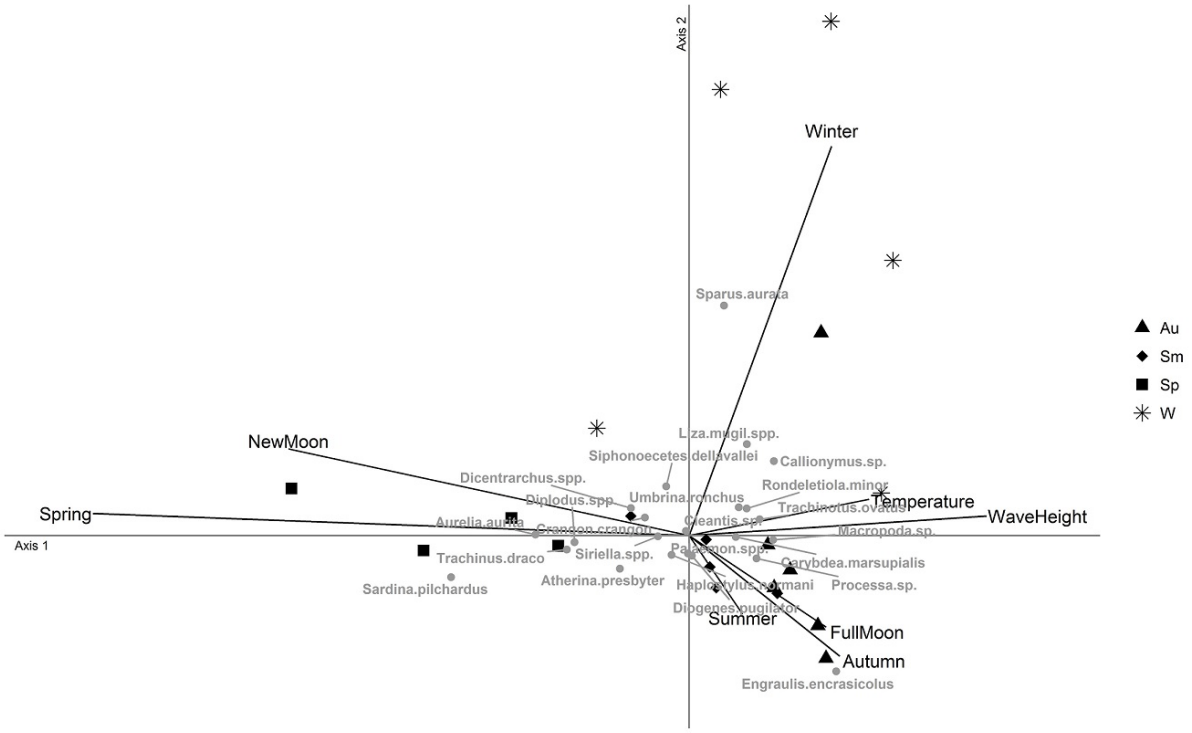

Figure 2: Distribution of species according to their correlation for different environmental variables: seasons, moon phase, temperature and wave height.

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Analysis on distribution and growth of the clam *Donax trunculus* (Linnaeus, 1758) on five beaches of the Cadiz coast

Sara Palma-Pedraza*[1] , Carlos Rodríguez-Garcia[2] , Remedios Cabrera-Castro[3]

*Corresponding author: sarappalma@gmail.com

[1] Estudiante de Máster Universitario "Acuicultura y Pesca". Departamento de Biología, Facultad de Ciencias del Mar y Ambientales. Universidad de Cádiz. Avda. de la Universidad, 11510 Puerto Real, Cádiz, Spain

[2] Departamento de Biología, Facultad de Ciencias del Mar y Ambientales. Universidad de Cádiz. Avda. de la Universidad, 11510 Puerto Real, Cádiz, Spain. Campus de Excelencia Internacional del Mar (CEIMAR)

[3] Instituto Universitario de Investigación Marina (INMAR). Campus de Excelencia Internacional del Mar (CEIMAR). Avda. de la Universidad, 11510 Puerto Real, Cádiz, Spain

Keywords: Cadiz coast, Age, Clam, *Donax trunculus*, Growth rings

Introduction

Benthic fauna is a key component in the marine ecosystem of energy flow for higher trophic levels, as well as for detrital decomposition. The clam (*Donax trunculus*) is a benthic marine invertebrate which live buried in the sand. The changes in its distribution are associated with the bathymetric, texture and organic matter content of the sediment among others. Those which live in the Atlantic have a slower growth and a reproductive period less broad than Mediterranean (Salas, 1987). In Andalucia, *Donax trunculus* constitutes a great source of income and a means of subsistence, although their catches underwent a decline in 2016 and 2017 with differences of 50 Kg in the total catches compared to 2013, catches of illegal size being the main reason. It is therefore a species that is overfished in the Spanish southwest (Delgado et al., 2017). The environmental factors as temperature, salinity and feeding are the most influential on the population growth (Ramón et al., 1995). When the organisms are exposed to regular periodic changes, which activate and stop, alternately, the growth, the growth lines or bands are made. Also the short term variation can determinate the formations of these lines and so are used to estimate the age and growth rate (Cerrato, 2000). The main aims of this study were to analyze the distribution and growth patterns in the Cádiz coast, with the purpose of contributing a better knowledge for good and suitable management.

Materials and methods

Five important beaches of the Cadiz coast were sampled (Santa María del Mar, Torregorda, Camposoto, Punta del Boquerón y La Barrosa) during six months from February to July following the methodology of Cerrato (2000), in which 1439 individuals were caught with a modified manual track. The biometric analysis of the specimens was carried out measuring the width, height and thickness of the valves with precision of one millimeter using a vernier callipers, as well as total weight and weight of the valve and flesh using a weighing scales. The external growth rings were counted using a dissecting microscope. In addition, the measurements between the radiuses of the rings were included. The distance was measured from the umbo to each identified

rings in a straight line using an ocular micrometer, which afforded calculate Von Bertalanffy's non-stationary growth model, according to which the length of an individual at time t, responds to the equation:

$$L(t) = L^{\infty}(e^{-kt-t_0}) \quad (1)$$

The statistical analysis R-Studio program was used. Linear regressions were fitted for all morphometric relations (lengths and weights) observing very high correlation. The variable most used by the authors who focus their studies on the growth of this species is the length. For this reason and due to the good fit shown, it was the variable used in our growth studies.

Results and discussion

The specimens caught show lengths in an interval between 7.5 y 37.44 mm. The results show that there is an increase in the number of growth marks when the size of the organisms is larger. The biggest organisms were discovered on the beaches of Barrosa and Camposoto (Figure 1).

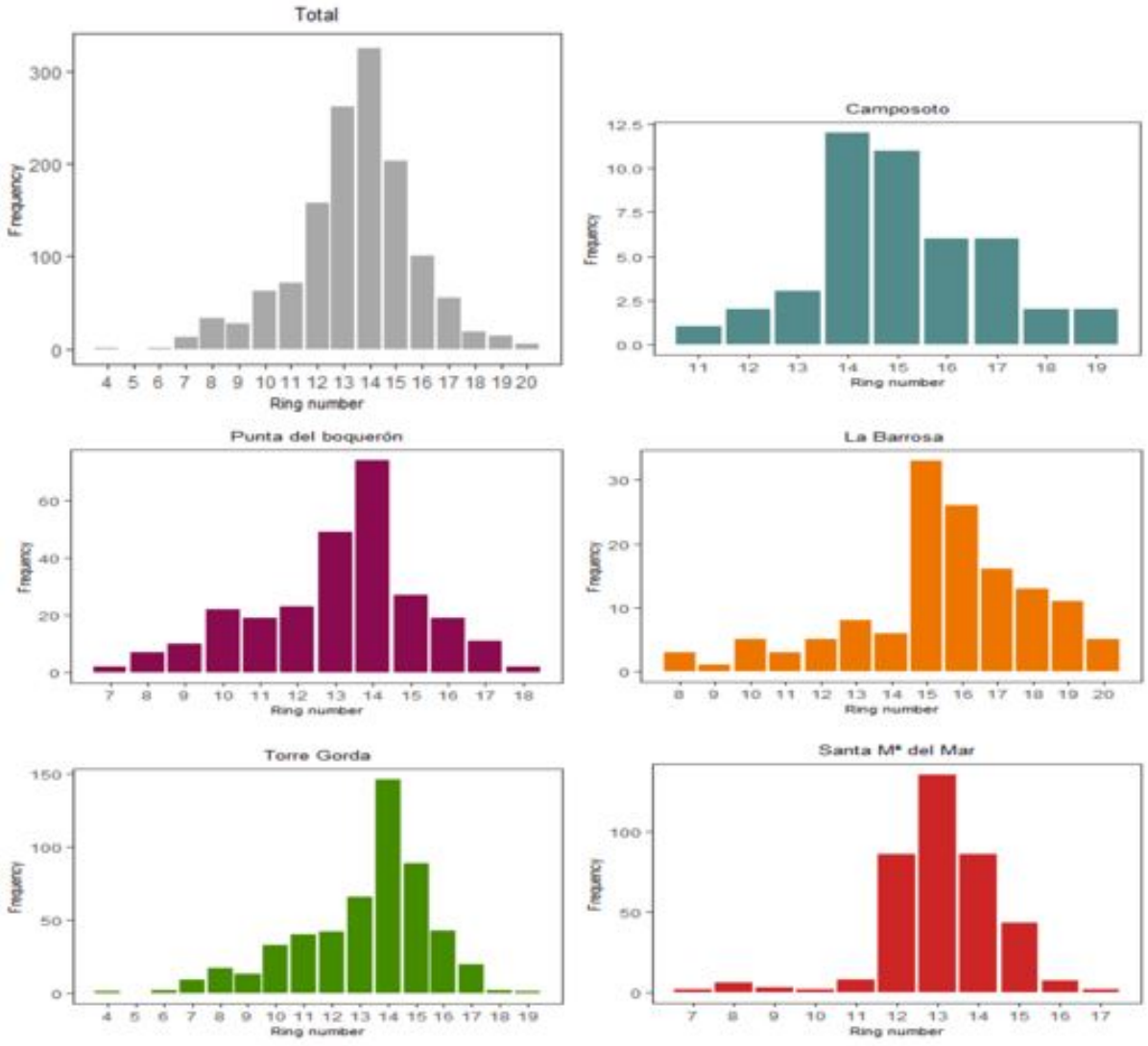

Figure 1: Frequency distributions by ring number for the combination of beaches (total) and for each beach.

A length was associated with a determinate ring number, being the most common ring observed 13 and 14 with an average length of 23.58 mm. In addition, it was observed that all specimens bigger than 25 mm presented a hyaline mark corresponding to a one year old (12 rings). Several authors suggest that the formation of this hyaline ring is connected with the spawning period (Ramón et al., 1995). The distance from the umbo to twelfth ring was measured with an average of 12.78mm. The length measured from umbo to each ring is shown in Table 1.

Table 1: Average of length to each growth ring measured from umbo. The length is expressed in mm. The ring number 12 identified as a ring of reproduction (equivalent to one year old) is indicated in red.

Distancia	Longitud radios (mm)	Distancia	Longitud radios (mm)
0-1	1.68	0-11	11.41
0-2	2.38	0-12	12.78
0-3	3.14	0-13	13.67
0-4	3.93	0-14	14.77
0-5	4.78	0-15	15.88
0-6	5.63	0-16	17.11
0-7	6.53	0-17	18.25
0-8	7.5	0-18	19.36
0-9	8.82	0-19	20.23
0-10	10.9	0-20	20.58

It was observed that the growth rings were closer at the end distal to the umbo, hence the increases in the final rings were smaller. The detailed examination and the identification of the ring of reproduction showed that for *D. trunculus* each ring could be approximately one month of growth, specifically one lunar cycle (28 days) and be influenced by tides because between the rings identified were found another four growth rings, which could be associated with short term growth, possibly weekly variations (Figure 2).The intertidal influence of these species and the predominance of lunar cycles could be reflected in the valves. This could lead to reconsidering, at least in intertidal species, the interpretation of the estimated time for each ring of growth.

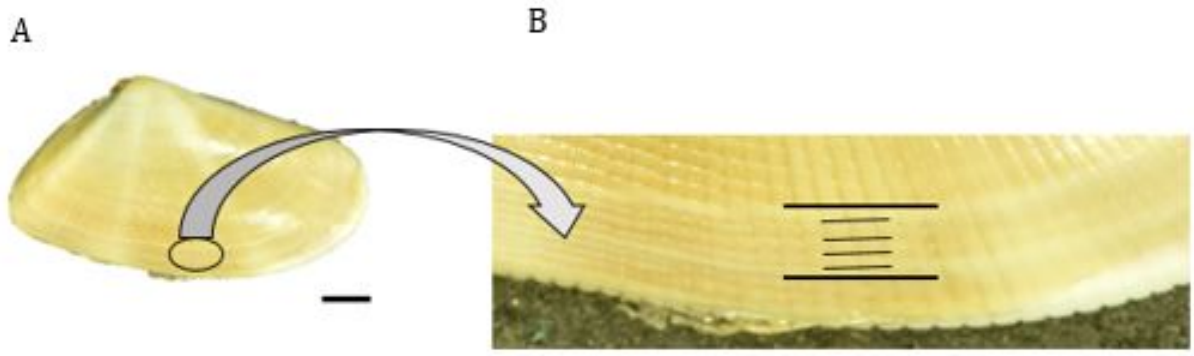

Figure 2: A) Rings of growth of *Donax trunculus*. B) Detail enlarged zone where growth rings are observed and another four rings. Scale bar=1mm.

The parameters of the V-B growth model were obtained from the Ford and Walford model. This model proposed by Von Bertalanffy is one of the most used model by other authors to describe seasonal growth, L^{∞} (46.86 mm), K (0.0327 mm) and t_0 (2.22) were the parameters

obtained with this growth model. The value of L^{∞} is agreed with other author with the same specimen and latitude (Delgado et al., 2017). The parameter L^{∞} obtained by other authors who studied the growth of *D. trunculus* in the Mediterranean are smaller than obtained to the Atlantic (Ramón et al., 1995; Muñoz-Lechuga et al., 2018). Several authors (Cerrato, 2000; Ramón et al., 1995) considered the growth of this species as seasonal, with dependence on variation in temperature, food availability or lunar and tidal rhythms among other things, although according to our study, the reading of rings seems to point to a monthly growth if we keep in mind the reproduction mark discovery in the specimens of more than 25 mm and the pressure that is put on the resource as well as the fine structure of the valve, which suggests that it is not a very long-lived species and the largest individuals studied are around two years old. It is necessary more studies for this specimen to help to better understand the growth of the species for an appropriate management of the resource, which ensure its sustainability in the medium and long term.

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Identification and evaluation of the status of elasmobranch species captured in the state of Ceará (Brazil)

Jessica Sarmiento Carbajal*[1] , Jose Belquior Gonçalves Neto[2] , Remedios Cabrera Castro[3] , Vicente Vieira Faria[2]

*Corresponding author: jesica.sarmientocarbajal@hotmail.com
[1] Departamento de Biología, Facultad de Ciencias del Mar y Ambientales. Universidad de Cádiz. Avda. de la Universidad, 11510 Puerto Real, Cádiz, Spain. Campus de Excelencia Internacional del Mar (CEIMAR)
[2] Universidade Federal do Ceará (Federal University of Ceará), Laboratorio de evolución y conservación de los vertebrados marinos, Campus do Pici, Fortaleza, Brasil
[3] Instituto Universitario de Investigación Marina (INMAR). Campus de Excelencia Internacional del Mar (CEIMAR). Avda. de la Universidad, 11510 Puerto Real, Cádiz, Spain

Keywords: Artisanal fishing, Bycatch, Conservation, Elasmobranch, Reporting

Introduction and objectives

The lack of fishery data for the capture of elasmobranchs, key elements in the regulation of some marine ecosystems (Camhi et al., 2008), puts at risk populations with an unknown current state of conservation, mainly in developing countries, making them susceptible to decline, as shown by world trends. The global statistics indicate that the number of species captured is much higher than that reported by official agencies (Cashion et al., 2019). Many countries continue to neglect the collection and publication of fisheries data on the capture of elasmobranchs that are often caught incidentally by the fisheries of other target species. The objectives of this study were: (1) to identify the species of elasmobranchs captured by the artisanal fleet of Ceará and to evaluate these species state of conservation; (2) to characterise the fleet that make landings in the port of Mucuripe (state of Ceará), as well as, evaluate the artisanal fishing in this port; (3) to study if elasmobranchs are commercialised as bycatch or as species of commercial interest; (4) to identify possible marketing channels for different species; and (5) to develop a strategy to involve the fishing sector itself in the conservation of threatened species.

Materials and methods

The identification of the species captured was carried out by means of observation and photographic compilation of the landings in the port of Mucuripe. The specimens were identified to the lowest possible taxonomic level and were assigned a conservation status according to the UICN (International Union for the Conservation of Nature). Through questionnaires made to fishermen, skippers and boat owners information was gathered that allowed us to advance in the characterisation of the artisanal fleet, the evaluation of this fishery and the existing marketing channels. Likewise, contact made with the fishermen through the interviews is intented to aide the development of the strategy involving the sector in the protection of the most vulnerable species.

Results and discussion

Of the species identified so far all have been classified as at risk, endangered or with insufficient data (Table 1).

Table 1: Classification of species landed according to their conservation status for IUCN; CR (Critically Endadgered), DD (Data Deficient), EN (Endangered), LC (Least Concern), NT (Near Threatened).

	GENUS	STATUS	SPECIES	STATUS
RAYS	*Aetobatus* sp.	EN /NT/VU	*Aetobatus narinari*	NT
	Hypanus sp.	DD/ LC	*Hypanus americanus*	DD
			Hypanus guttatus	DD
			Hypanus marianae	DD
	Pseudobatos sp.	CR/NT	*Pseudobatos horkelii*	CR
			Pseudobatos percellens	NT
	Rhinoptera sp.	EM/NT	*Rhinoptera brasiliensis*	EN
			Rhinoptera bonasus	NT
SHARKS	*Carcharhinus* sp.	LC/NT/VU	*Carcharhinus falciformis*	VU
			Carcharhinus Limbatus	NT
	Galeocerdo sp.	NT	*Galeocerdo Cuvier*	NT
	Ginglymostoma sp.	DD	*Ginglymostoma cirratum*	DD
	Negaprion sp.	NT/VU	*Negaprion brevirostris*	NT
	Mustelus sp.	NT/CR/LC/EN/DD	*Mustelus canis*	NT
			Mustelus fasciatus	CR
			Mustelus higmani	LC
			Mustelus norrisi	DD
			Mustelus schmitti	EN
	Rhizoprionodon sp.	DD/LC	*Rhizoprionodon Lalandii*	DD
			Rhizoprionodon porosus	LC
	Sphyrna sp.	EN/DD	*Sphyrna lewinii*	EN
			Sphyrna mokarran	EN
			Sphyrna zygaena	DD

Only two species were considered to be at low risk of extinction: *Rhizoprionodon porosus* and *Mustelus higmani* (Figure 1).

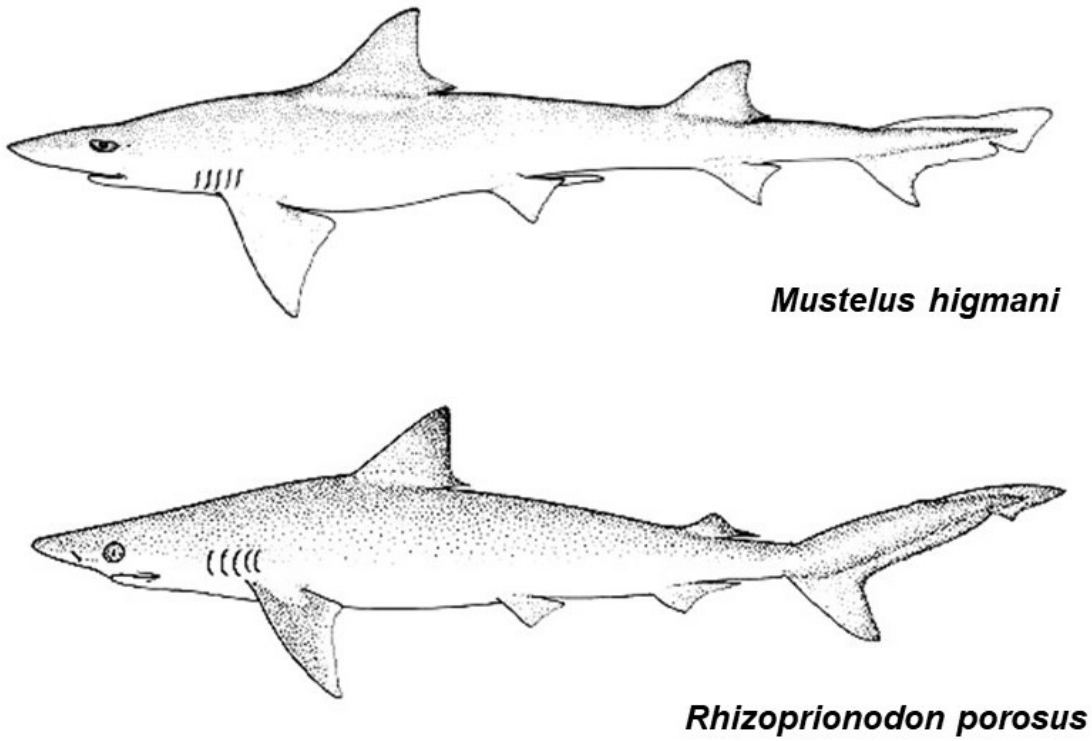

Figure 1: Species of sharks identified with low risk of extinction.

The completion of questionnaires by fishermen has made it possible to estimate the characteristics of the fishing gear used in their fishery and to obtain information on the form of fishing, the by-catches and marketing channels after their landing. The identification and evaluation of these species are fundamental for the sustainable management model to be implemented in this area with the involvement of the fishermen themselves.

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The beginnings of the tuna industry in Ecuador 1926-1956

Eduardo Pico-Lozano*[1]

*Corresponding author: eduardo.pico@uleam.edu.ec
[1] Universidad Laica Eloy Alfaro de Manabi; Universidad de Cadiz

Keywords: Manabí, Manta, Canned fish, Fishery, Tuna

The beginning of the story

At the beginning of the nineteenth century in Paris (1810), Nicholas Appert published the art of preserving, where he described the method of food preservation. On 13th May, 1830 the Republic of Ecuador is born, being called before Real Audiencia de Quito and denominated with the name Ecuador after the name used by French scientists, who were associated to the geodesic mission who came to explore the place of the Earth's equator (Alberts, 1947). But there would have to pass one hundred years and many civil, military and various confrontations for the first canned industry to arrive into the Ecuadorian territory.
In August 1926, the company "Santa Cruz", initiative of Norwegian residents, built the first canned company in Ecuador. It was located on Academy Bay, in southern Santa Cruz Island, in the Galapagos (Figure 1). Its first canned product was the red lobster, Panulirus penicillatus (Olivier, 1791). Facilities include a dock and installation of well water and freshwater pipes on the construction site. The spring was located on the shoreline of Pelican Bay; 400 meters away and the pipe was brought from Norway. However, due to the lack of fresh water and the explosion of the boiler, the company closed in November 1927 (Hoff, 1985).

Figure 1: New Year, 1927. Academy Bay at high tide. Leading to the factory is a rail track. The baking oven is hidden behind the factory. The open area is "The Square." Courtesy Jens Furunes.

In 1938, another failed attempt by the canned industry was recorded, for the same reason mentioned above, the lack of drinking water, located this time in a site called Cangrejo, currently General Villamil, Playas, in the province of Santa Elena (Alberts, 1947). Some 69 presidential

terms and a political environment very hostile to business development had passed in Ecuador from 1830 to 1947, but would come a new character of politics, President Carlos Julio Arosemena Tola, who spoke fluent English as Merchant and banker, then he would lead the country into a period of financial and political tranquility (Ayala Mora, 2008).

The most important year

It was the year of 1947 in Ecuador and specifically in the province of Manabí, when a thousand people were engaged in fishing, most of them had their own interests and employed family members. The wage earned depended on the ship owner and the value of the fish at the time of the sail (Alberts, 1947). Fishermen were 85% distributed along the coast and 15% along inland rivers and streams. It is estimated that approximately 10% of the population caught fish regularly for subsistence and the rest of the fishermen traded them (Alberts, 1947). Sport fishing was negligible, due to unfavourable weather conditions and remote distances from fishing spots along the coast. The temperature was too high from 10 a.m. to 2 p.m. to perform sport fishing activities (Alberts, 1947). The main fish species captured for local sale were corvinas, Cynoscion spp. Other common species such as the red snapper, snook, croaker and oysters found along the coast, and which are small in size and of high quality. There were also lobsters, shrimps and mollusks, as well as whale fishing and pearls, but the latter were not performed by Ecuadorian ships (Alberts, 1947). Almost all fishing vessels consisted of sail-propelled canoes on the coast and by oars in inland rivers. Few had engine-powered boats. On a common day one could see, during high tide, approximately 75 canoes gliding by the sea from Manta. There was only one motor boat suitable for fishing, named "Cecile" which had a storage capacity of 15 tons and operated on the outskirts of Guayaquil. It used diesel and was mainly dedicated to shark fishing (Ayala Mora, 2008). No production statistics or quantities of fish landed are available until the end of XX century. With regard to sharks, only livers were landed and commercialized, while the rest was disposed of as waste (Alberts, 1947).
Fish production in the inland waters of the coastal region and in the waters off the coast is unknown. However, in 1947 there are landing records of 150 metric tons per month, of which 100 metric tons were seafish and 50 metric tons river fish. Of this amounts, approximately 2 per cent of seafish were processed as dry-salted and transported to the sierra region for consumption; the rest was used as fresh fish. No fish was frozen, canned, preserved in vinegar, or processed smoked at that time. Imports consisted mainly of sardines from the United States. Imported salted fish was mainly from Peru (Alberts, 1947). The government had not issued fishing regulations for inland or coastal waters. The only rules issued were for fishing in the waters of the Galapagos Islands. There was no fisheries research in Ecuador at that time, and the government was only investigating the possibility of establishing a fertilizer plant in the vicinity of Machalilla on the west coast of Ecuador (Alberts, 1947).

The next years

In 1948 fishermen from the Ecuadorian city of Manta led by the American Mauricio Rankin pressured the government to ban all fishing with foreign-flagged vessels in the waters near the city. Rankin started industrial operations with what would be the start of the first company that would capture, process and export canned tuna. Rankin and his company would have the right to fish for tuna in Ecuadorian waters in exchange for establishing a canning factory in mainland Ecuador. However, due to the U.S. tariff on imported canned tuna, Rankin never established the factory. Instead, he had focused on sending fresh and frozen tuna directly to the U.S. market, where it was sold or packaged by local businesses (Epps, 2009). On April 25, 1949, entrepreneurs Mauricio Rankin and Francisco Roseney established in Manta, the Ecuadorian National Food Production Industry (Inepaca). They started with 2 shrimp vessels. Then the

powerful Californian fishing company Van Camp Sea Food acquired Inepaca to provide the raw material to its plants in California and Puerto Rico; during the first few years this company made large investments. From 1956 on, a tuna packaging plant was installed for the domestic and later the export markets. Since the fifties, more fisheries have been established in Manta, Posorja and other ports in the province of Guayas: La Portuguesa, Pesquera Jambelí, Packer Nacional, Business Industriales Real, etc. For decades the fisheries sector grew slowly, but from the 1990s their export volume became a significant share in ecuadorian exports. Today tuna companies such as Marbelize, Conservas Isabel, Tecopesca and others, provide work to thousands of manabites (Arosemena, 2017).

Acknowledgments

To Jose Alio Mingo, for his help in carrying out this document.

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Ethnography and fishing: popular culture surrounding tuna fishing with the traditional almadraba technique

Mercedes Soto Melgar*[1]

*Corresponding author: sotomelgar@ugr.es
[1] Universidad de Granada

Keywords: Almadraba, Traditional religiosity, Work songs, Proverbs, Folklore

Introduction

This paper deals with the traditions and popular customs that accompany tuna fishing with almadraba, an ancestral fishing technique that is still present today on the coasts of Cadiz. In a globalized world, where traditional crafts no longer have a place, glimpses of folklore can still be found, and this tuna trapping technique, typical of Cadiz, is a clear example of this. The traditional religiosity of the almadraberos – or almadraba fishermen –, the songs sung by their parents and grandparents, their proverbs, etc., are all clear proof of the survival of this culture into the 21st century.

Traditional religiosity, work songs and proverbs in the almadraba

In this research, religious beliefs such as that in the saint, the cross of Caravaca and the worship of Our Lady of Mount Carmel, patron saint of sailors, are reflected and explained. This section compiles the work songs sung in the past by fishermen as they coordinated to pull up the nets and which would also make the fishing task more bearable. These include genres such as "salomas" and "coplillas carceleras". We will focus on the saloma dedicated to pulling the net. The traditional wisdom found in proverbs, which faithfully reflects the knowledge that speakers have of the world around them, also has a place in the world of almadraba fishermen. Some of the sayings that have been documented are "to go for tuna and to see the duke", "to be thrown around like a tuna", "tuna is for the common people and grouper is for the knights", among others.

Conclusions

These beliefs, songs and paroemias, in spite of their antiquity, are still alive in the minds of the almadraba fishermen of Cadiz, as documented in the semi-directed interviews we conducted with the "ranas" and "copejadores" fishermen of the almadrabas of Tarifa, Zahara de los Atunes, Barbate and Conil de la Frontera.

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Reproductive biology of the striped red mullet, *Mullus surmuletus*, and implications for its management

Joaquim Tomàs-Ferrer*[1] , Ulla Fernandez-Arcaya[2] , Amàlia Grau[3] , Beatriz Guijarro[2] , Natalia González[2] , Enric Massutí[2]

*Corresponding author: xim.tomas.15@gmail.com
[1] Universitat de les Illes Balears
[2] Instituto Español de Oceanografía - Centre Oceanogràfic de Balears
[3] Laboratori d'Investigacions Marines i Aqüicultura

Keywords: Fecundity, Maturity, *Mullus surmuletus*, Reproduction

Introduction

Mullus surmuletus Linné, 1758 is one of the most important target species of Iberian Peninsula fisheries, including the Balearic Islands, where the official registers of annual catch vary between 100 and reaching maximums of 400 tones. However, in the last 7 years, annual catches have been decreasing and now they oscillate between 60 and 100 tonnes. Despite its economic relevance, some of its reproductive aspects are still unknown. The objective of the present study is to characterise the reproductive ecology of *M. surmuletus* including the reproductive cycle and strategy, fecundity, population distribution and size-at-maturity of this species. This study was based in the analysis of 1998 individuals captured with two different fishing gears, trammel and trawling, between 13 and 518 m depth (mostly at depths inferior to 100 m), covering an entire year of sampling. The populational structure of this species showed a marked bathymetric pattern, with the presence of larger individuals at shallower waters ($<$ 50 m depth), as well as a marked sex ratio segregation between shallow and deeper waters. Macroscopic and microscopic analyses of the gonads as well as Gonado Somatic Index showed a seasonal reproductive cycle with the presence of spawning females from March to May, while males spawn from January to May. The size-at-maturity was 14.4 cm for females and 12.2 cm for males. The size-frequency distribution of oocyte diameter showed an asynchronic ovarian organisation. The counting of mature oocytes in females that were just about to spawn showed a batch fecundity of, approximately, 74,500 $\pm$ 40,000 oocytes per female. *Mullus surmuletus* is an Actinopterygii fish, from the family Mullidae. It is distributed in areas of the Atlantic Ocean and the Mediterranean Sea. The red striped mullet is a demersal species with a bathymetrical range of 10 to 400 m depth, living on rocky or sandy bottoms or seagrass meadows (Reñones et al., 1995). *Mullus surmuletus* has a high commercial value and is an important resource for the Mediterranean demersal fisheries. This species is captured by two different fishing gears in the Balearic Islands: trawling and small-scale fisheries. *Mullus surmuletus* biology has been poorly studied. Despite its relevance, there is still an important gap related to the reproductive biology of the red striped mullet, especially in the Mediterranean Sea. The lack of information about the fecundity and reproductive strategy of this species, including the fact that there are no recent studies on relevant biological aspects as size-at-maturity, represents a great opportunity to make a current approach of those parameters to verify if they have varied during the last years, comparing them to older ones. The lack of currently gathered biological data on reproduction of *M. surmuletus* is an obstacle to conduct a sustainable stock assessment of this resource. The main goal of this study is to describe the reproductive biology of *M. surmuletus* in the Balearic Islands and to provide new and updated

scientific knowledge that can improve the management options for this species in the Balearic Islands.

Material and methods

A total of 1998 specimens of *M. surmuletus* were collected monthly for each fishery gear during the period from October 2017 to September 2018. A biological sampling was done, measuring and weighing individuals, then weighing its gonads and hepatopancreas. Sex was also defined. Gonads were examined macroscopically in order to determine its maturational stage. Gonads were fixed in formalin for histological sampling. 195 gonads were sampled in order to calculate the fecundity of this species by gravimetric method and other analysis, consisting on determine the microscopic gonadal maturation stage and measuring oocyte diameters. Data analysis was done in order to find significative differences in size-frequency distributions of captures (Kolmogorov-Smirnov tests) and in sex ratio (Chi-square tests).

Results and discussion

Individuals collected ranged between 10 and 34 cm. Small-scale captures showed a unimodal-like length-frequency distribution during all the seasons. On the other side, trawling captures showed a bimodal-like length-frequency distribution, although it was not so evident in summer. Recruitment of juveniles to adult stock has been observed to occur in November. Our results showed a marked bathymetric segregation with the presence of larger individuals as well as higher proportion of females at shallower depth where trammel fisheries (< 50 m) occur. Sex ratio of this species was 2.2 females for every male. Individuals captured in shallower waters are mainly females (sex ratio 6.1:1) whereas those captured in deeper waters (by trawl) present a more balanced sex ratio (1.6:1). Sex ratio varies for each length class of *M. surmuletus*. As the size increases, females become more predominant. This huge skew could be provoked by the fact that only a few males (in addition, the largest) were found in shallow waters, clearly predominated by females. Spawning capable females were observed from February to May, with maximums in March (24%) and April (25%), and actively spawning females from March to June, prevailing in March (72%), April (61%) and May (73%). Regressing females were observed from March to July, being most common in June (40%). The spawning pattern identified histologically in females and males seems to be corroborated by the seasonal variability in the GSI. HSI values follow similar pattern than GSI. The reproductive activity of *M. surmuletus* seems to occur during the end of winter and extending to the late spring (Pajuelo et al., 1997). Our results showed that the individuals captured by trawling, at deeper depths, showed higher of GSI than individuals captured by trammel at shallower waters, suggesting that this species might migrate to deeper ranges for spawning, although larger females and males are found in shallow waters. Despite this bias in size, larger females captured by trammel presented similar ratios of gonads development phases and oocyte development stages. An explanation for the resulting lower GSI values is that the gonads weight – female weight relation may be allometric. Size-frequency distribution of oocyte diameter showed the presence of oocytes from all the developmental stages during the spawning seasons on ovaries of actively spawning females without observing a bimodal ovarian organisation. These results suggest that this species present an asynchronous ovarian organisation (Murua et al., 2003). The different stages are a consequence of a continuous development process, any evident hiatus is observed between the immature and vitellogenic oocytes nor between vitellogenic and germinal vesicles migration stages oocytes. The estimated mean total length (L_{50}) at which 50% of females attain maturity was 14.36 cm. The L_{50} for males was 12.18 cm, which represents a decrease of 2.4 cm for females and 2.8 for males from previous published data of this species on the same study area. Above 17 cm, all individuals were mature. In females with hydrated oocytes, the number of developing oocytes (NDO), considered as those in vitellogenic stage or

superior was of 334,000 ± 76,000 oocytes. Batch fecundity, which is the number of migrating and hydrated oocytes, was 74,500 ± 40,000 oocytes. Relative number of developing oocytes (RNDO), which is the NDO per gram of female, was estimated to be 2,200 ± 370 and the relative batch fecundity was 450 ± 200 oocytes per gram of female for total and batch fecundities, respectively. NDO and batch fecundity increase potentially with the female body weight. This study provides new key data on aspects of reproductive biology of *M. surmuletus* in the Balearic Sea, where it constitutes a specific stock unit. The results of this work will contribute to the knowledge of the reproductive biology of *M. surmuletus* in the Balearic Sea and provide actualised information on several of its life parameters. This study corroborates previous studies' calculi of the sex ratio, confirming a female-male relation close to 2.2:1 (Morales-Nin, 1991). The results on this study show a bathymetric migration behaviour on this species. Seasonal variations in the size-frequency distribution are mainly due to the life cycle of the species. Smaller individuals are captured in autumn, when recruits join the adult population. This study also confirms that this species has a well delimitated spawning season that lasts from March to May, with a peak that oscillates between late April and early May. This species has confirmed to be an income breeder rather than a capital breeder, since it does not storage energy resources before reproduction to be used but it uses energy directly from food intake. Fecundity was calculated for first time in this species in the Western Mediterranean with results quite similar to the species *M. barbatus*, although it has slightly smaller sizes. More studies with larger sample sizes will be needed to be done in order to confirm or refute the results obtained. However, this data obtained is now available for the species' fishery management and the construction of stock assessment models. Results obtained in this study, together with the characteristics of the landing patterns observed in *M. surmuletus*, reveal the need of carry out a continuous scientific monitoring on this species. These results suggest that several parameters of reproductive biology of *M. surmuletus* are quite similar to the ones of the other Mediterranean species of *Mullus*, *M. barbatus* (Ferrer-Maza et al., 2015). In the Mediterranean, a minimum landing size of 11 cm has been stipulated by the European Commission in 1994. This value is still lower than all the L_{50} calculated in this study since 2001 and lower than the majority of L_{50} provided by many authors for Mediterranean populations. We thereby suggest an increase of the minimum landing size closer to the L_{50} calculated to this species, in order to ensure the *M. surmuletus* captures under the maximum sustainable yield, since the species is catalogued as overexploited with relative high biomass by FAO.

Acknowledgments

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Reproductive aspects of *Peprilus medius* (Peters, 1869) captured in the coasts of Manabí (Ecuador)

Kléver Mendoza Nieto*[1] , Milagrosa Casimiro Soriguer [2] , Maribel Carrera Fernandez[1] , Felix Figueroa Chavez[1]

*Corresponding author: klevernime@gmail.com
[1] Biology Laboratory, Faculty of Marine Sciences, Laica University "Eloy Alfaro" of Manabí. Av. Circunvalación, Manta-Ecuador.
[2] Department of Biology, School of Marine Sciences and Environment, University of Cádiz. 11510 Puerto Real, Cádiz-Spain.

Keywords: Size, Weight, Sexual maturity

Introduction

Peprilus medius (Peters 1869) is distributed from the Gulf of California, Mexico, to northern Chile, and the Galapagos Islands (Chirichigno and Cornejo, 2001; Galván-Magaña et al., 1993). The species represents an abundant fishery resource in the Ecuadorian continental shelf. *Peprilus medius* has costal benthic and pelagic habitat, and can penetrate into estuaries (Martinez-Ortiz, 2010). In Ecuador it is known locally as pámpano or gallinazo, and is an important resource whose destination is mainly for local consumption and export. This species is captured as an accompanying fishery for the artisanal fleet and the industrial purse seine fleet. At the local level, there are few studies on their reproductive biology and their catch is not regulated, therefore, the present study evaluates the most relevant aspects of its reproductive activity, in order to contribute to the management of this species in Ecuador.

Materials and methods

The samples were obtained monthly from April 2017 to May 2018, from the catch of the purse-seine fleet that lands in the vicinity of Manta. The specimens were transferred to the Biology Laboratory of the Faculty of Marine Sciences where morphometric analysis was performed. The length-weight relationship was estimated using the allometry equation (Ricker, 1975), the size of sexual maturity was determined on the basis of the L50 criterion (King, 1995). The reproductive time was determined according to the monthly analysis of the gonadosomatic index (GSI), hepatosomatic index (HI) and condition factor K. The reproductive period was established on the basis of the months when these indices reach maximum values within an annual cycle.

Results

A total of 334 specimens of *P. medius* were analyzed. Average sizes of animals in the sample were 22 and 21.87 cm Tl, for males and females, respectively, and 21.91 cm for sexes combined. The sex ratio was 1.17M: 1F (χ^2=1.94 p >0.05), while the length-weight relationship showed a negative allometric growth for both males and females: W =0.0498Tl$^{2.58}$, R^2=0.84 (t=6.86 p <0.05) (Figure 1).

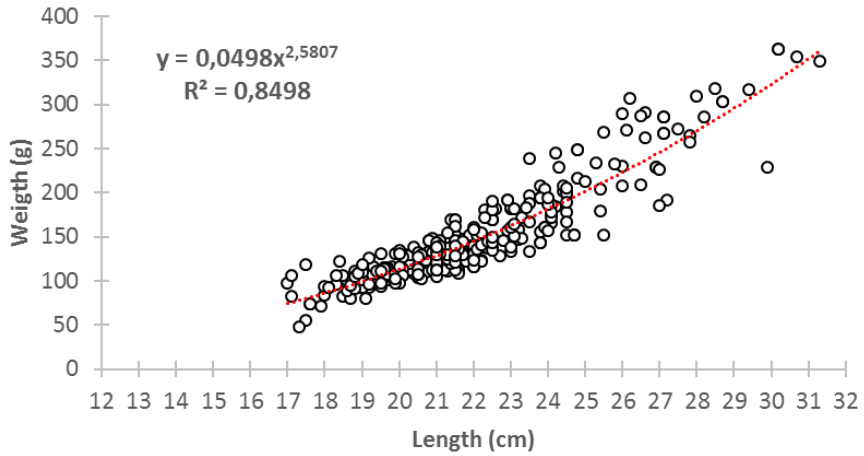

Figure 1: Length-weight relationship of combined sexes.

The size of sexual maturity L50 was estimated at 21.84 and 21.23 cm Tl, for males and females, respectively (Figure 2). These values were lower than the average sizes recorded in the capture in this study. The values of GSI, HI and Condition factor K, presented significant differences during the study (K-W p <0.05), with a marked seasonality in the reproductive period. The monthly values of the GSI showed an increase during the rainy season, having its highest values in January and February. A similar pattern was observed in the IH, while the condition factor K presented its lowest values in these months, coinciding with the highest value of the GSI (Figura 3) which could indicate energy costs to reproduce.

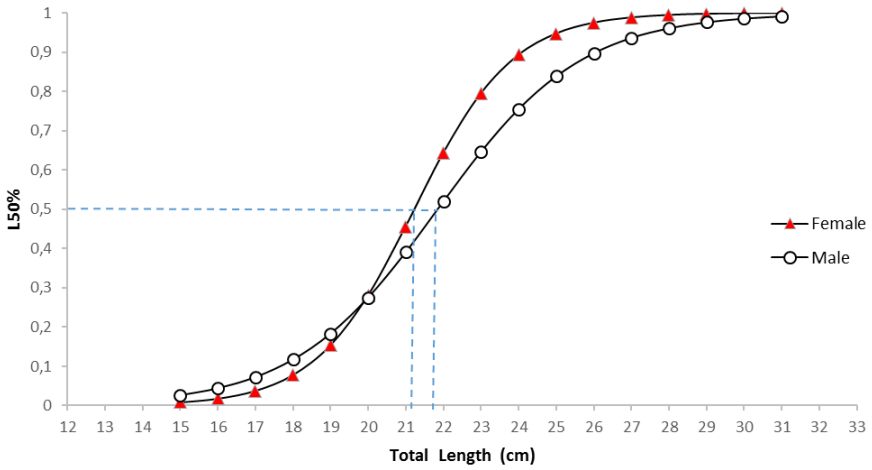

Figure 2: First size of sexual maturity for males and females.

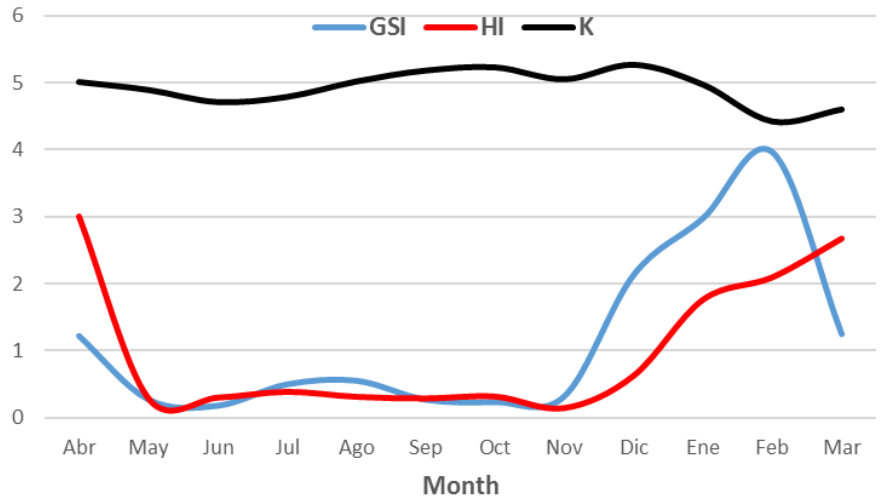

Figure 3: Monthly changes in the gonadosomatic index, hepatosomatic index and condition factor.

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Session 3

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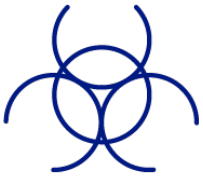

Plastic inputs into the ocean through the Guadalquivir river

Rocio Quintana Sepúlveda*[1]

*Corresponding author: roc.quintana@gmail.com
[1] University of Cadiz

Keywords: Plastic, River inputs, Guadalquivir, Marine pollution

Abstract

Densely populated developing economies, high rates of plastic waste generation combined with inadequate waste management infrastructures result in significant volumes of plastic waste leaking into the marine environment. Potentially, one of the main pathways of plastic inputs into the open ocean could be rivers, but there is no comprehensive information about the amount of litter transported. In this study, we are going to quantify the amount of plastic entering through the Guadalquivir river in the period of January 2th of 2014 to August 18th of 2015. The methodology used in this work is based on the imaging analysis using the ImageJ Fiji® system, with the final purpose of counting and measuring the entrance of microplastics (particles < 5 mm) and mesoplastic (particles between 5-25 mm).

Introduction

The presence of plastics in the marine environment has been identified as a major concern due of its persistent nature (Lebreton et al., 2017). Marine litter can be transported across long distances and remain in the marine environment for an undetermined length of time, where they can be found in the most remote areas of the Earth (Veiga et al., 2016). A substantial fraction of these marine plastic debris originates from land-based sources, where rivers potentially act as an important transport pathway for all sizes and types of plastics (Schmidt et al., 2017). Although it is estimated that the input of river litter is an important contributor to marine litter, there is still no complete information on the amount of litter transported through the rivers to open sea. In addition, there are no harmonized methodologies to provide quantitative data for comparable assessments of river litter (González et al., 2016). In order to establish future measures for the reduction of plastics and their possible impacts on the marine environment, it is necessary to quantify and identify the main sources, as well as their possible pathways to the marine environment, such as river inputs (Van der Wal et al., 2013). In this work, the main objective is the quantification of the entrance of plastics into the ocean through the Guadalquivir River, where we will focus of the entrance of microplastics (particles <5 mm) and mesoplastic (particles between 5-25 mm).

Methodology

Meso and micro litter on the Guadalquivir river were sampled from January 2th of 2014 to August 18^{th} of 2015, with 2 sampling points with the following coordinates: Sampling Point Number 3: Tarfia (36°57'30.4"N 6°'36.7"W); sampling Point Number 5: Bonanza (36°52'3.16"N 6°20'44.97"W). In this case, sampling was carried out for 24 continuous hours, with the aim of covering the entire tidal cycle, where tide number 1 and 3 correspond to the flow current, and

tide number 2 and 4 correspond to the ebb current. Samples were collected using 3 nets with 1 mm mesh size with a rectangular net opening of 3x3 meters. The net was towed at the top 20 cm of the river surface during 60-90 minutes, with an average filtered volume of 58 000 m^3. In the lab, samples were transfer into a 13 L jar, where only 1 L was analyzed for mesoplastic and 100 g was analyzed for microplastic. Floating plastic debris was carefully picked from the supernatant. Plastic items were weighted, counted and measured by imaging analysis using the ImageJ Fiji® system. In total, 3242 items of microplastic and 3194 items of mesoplastic have been examined in this work.

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Aerosol dry deposition events in the western Mediterranean

Helena Guerra Alcaide*[1] , Antonio Tovar Sánchez[2] , Marina Zamanillo Campos[3]

*Corresponding author: alcaidehelena@gmail.com
[1] Universidad de Cádiz
[2] Consejo Superior de Investigaciones Cientificas, CSIC
[3] Universitat de les Illes Balears

Keywords: Aerosol, Balearic Islands, Trace metals

Introduction

The aim of this study is to quantify and characterize chemically (primarily trace metals) the different events of dry deposition produced in the Balearic Islands (northwestern Mediterranean), and to examine their effect on marine phytoplankton. The study of aerosols is important because they have an impact on the climate system, human health and biochemical functioning of ecosystems. The importance of aerosols as a source of nutrients in the ecosystems depends on the large-scale variation in annual inputs, the magnitude of that input relative to other sources of nutrients and the short-term temporal and spatial variability in inputs (Donaghay et al., 1991). In the euphotic zone at the ocean surface, phytoplankton growth is controlled by the supply of nutrients. The primary nutrients that limit marine phytoplankton growth rates may include N, P, Fe and/or Si. Atmospheric transport and deposition provide a source for each of these nutrients to the oceans (Krishnamurthy et al., 2010). On the other hand, aerosol particles also can release to seawater elements that are highly toxic at relatively low concentrations, producing toxic effects on some taxa of phytoplankton, such as Cu (Moffett and Brand, 1996). A lot of research studies have been carried out to quantify atmospheric inputs of trace metals to the marine environment but none of them have focused on the Balearic Islands, and their effect on the phytoplankton.

Material and methods

Trace metals concentrations (Al, Cr, Cu, Fe, Mn, Ni, Pb and Zn) of atmospheric dry depositions samples were measured to characterize their sources and the spatial and temporal variability of these events. Weekly integrated atmospheric particulate samples were collected at Cabrera National Park (Cabrera), Es Amunts d'Eivissa (Ibiza) and S'Albufera des Grau Nature Park (Menorca) onto an acid-washed Teflon filter, from July 2012 to November 2013. The filter digestion was carried out at the microwave MARS Xpress (CEM Corp., Matthews, NC). After that, metal concentrations were determined by ICP-MS (i-CapQ model Thermo Fisher). A total of 69, 25 and 30 aerosol samples collected at Ibiza, Cabrera and Menorca, respectively (including blank filters), were digested and analyzed. Finally, to analyze the source of aerosols, the crustal enrichment factor (EF crustal) for the trace metals in the aerosols was calculated following Taylor (1964) and Ikegawa et al. (1990).

Figure 1: Location of atmospheric dry deposition sampled and surface seawater collection.

Results

The results indicated that, while Al, Cr, Fe and Mn have a natural origin (from African dust), Cu have a local anthropogenic origin. On the other hand, Zn and Ni present both a natural and local anthropogenic origin depending on the time. The response of coastal planktonic communities to the addition of natural and anthropogenic aerosols was analyzed through phytoplankton growth experiments conducted in May 2013 with surface nearshore water collected at Ses Salines Cape (South of Mallorca Island). The experiment demonstrates that Fe deposition events in the western Mediterranean Sea promote the growth of phytoplankton, whereas maximum and strong Cu deposition events seem to inhibit the phytoplankton growth.

Acknowledgments

This work has been developed as part of the Concorda project (Contribution of remote nutrients and pollutants from atmospheric and biotic deposition to the Cabrera Archipelago) (Ref: 384/2011), which aims to evaluate the effects of materials (nutrients, pollutants and biological materials) on marine and terrestrial ecosystems of the Cabrera National Park. Such knowledge is essential not only for understanding the general ecological functioning of Cabrera Archipelago, but also to design and implement management plans that would preserve and improve the health of various ecosystems of the National Park.

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Effects of micro- and nano- plastics in N_2 fixing bacteria associated with the Mediterranean seagrasses *Posidonia oceanica*

Xabier López Alforja*[1] , Victor Fernández Juárez[1] , Nona Sheila Agawin Romualdo[1]

*Corresponding author: xabier.lopez1@estudiant.uib.cat
[1] Área de Ecología, Departamento de Biología, Facultad de Ciencias, Universitat de les Illes Balears

Keywords: Nanoplastic, Microplastic, Diazotrophes, *Posidonia oceanica*, N_2-fixation

Introduction

The seagrass *Posidonia oceanica* is a key factor to the health and quality of coastal Mediterranean marine ecosystems, not only because the meadows host a diversity of macro and micro- organisms, but for their role in biogeochemical processes such as the nitrogen cycle in the Mediterranean (Agawin et al., 2014). It is widely accepted that *P. oceanica* meadows constitute one of the most productive ecosystems in the Mediterranean and hypothesised to be an important source of new atmospheric nitrogen (N_2) to the system. This may be due to the vast variety of N_2-fixing epiphytic microorganisms that could contribute up to 50% of the nitrogen requirements of the plant (Agawin et al., 2016). Also known as diazotrophs, these N_2-fixers can be highly influenced by environmental factors, either natural or anthropogenic, limiting their growth and their N_2 fixation rates. Marine litter has become a dominant threat to marine ecosystems, particularly in the Mediterranean Sea, recently considered to be one of the most contaminated seas with plastic concentrations of about 900-2,500 g·km^{-2} (Cózar et al., 2015). Moreover, the concern about smaller size plastics such as micro- and nano-plastics have risen over last decades due to their alarming abundance in marine systems (Mediterranean: 0.18 particles·m^{-2}) and their high bioavailability throughout the food-web (Alomar et al., 2016). Their physico-chemical characteristics (composition and surface area) allow them to act as transmission vectors for organic pollutants and toxic plasticizers (Cole et al., 2011). However, investigations on the effects of marine plastic contamination are relatively new and has not yet described in marine bacteria.

Materials and methods

The present work is part of a pioneering research on studying the repercussions of the most abundant microplastics and additives (i.e. Fluoranthrene) to N_2-fixing microorganisms. Recent molecular unpublished analysis of the *nifH* genes (which encode an operon subunit that codes for nitrogen reductase) in the microbial community in the phyllosphere of *P. oceanica* revealed several new *nifH* bacterial sequences. The aim of this study is to test the effect of nano/microplastics in two photoautotrophic strains that appeared to be associated with *P. oceanica* (filamentous *Fischerella muscicola* PCC 73103 and unicellular *Cyanothece* sp. PCC 7418). In order to assess the consequences of nano/microplastics, fluorescent polystyrene [PS] beads (nanoparticles) and polyethylene [PE], polypropylene [PP] and polyvinyl-chloride [PVC] were added to cultures of these N_2-fixers growing under two different inorganic phosphate and iron availabilities (Nutrient limiting treatment: 0.1 μM P_i +2 nM Fe; Non-limiting treatment: 0.045 mM P_i +0.0075 mM Fe) previously tested. The response variables measured were changes in cell abundances and growth, pigments, cell oxidative stress, metabolic activity, visual cell viability and N_2 fixation rates.

Results and conclusion

Preliminary results indicate that low levels of PS fluorescent nanobeads (4.55·105 particles·mL^{-1}) did not have a significant effect on N_2 fixation rates nor other biochemical response variables measured in the cultures of diazotrophs (filamentous *F. muscicola* PCC 73103 & unicellular *Cyanothece* sp. PCC 7418) tested. With regards to microplastic exposure, high concentrations (100 mg·L^{-1}) appeared to solicit differing responses varying with type of plastic, showing even beneficial effects in the growth of *Cyanothece* sp. PCC 7418. Both photoautotrophic bacterial culture responded significantly positive in terms of growth to PVC addition, but negatively to PP. This may be due to the use of PVC as carbon source. Moreover, PS fluorescent nanoplastic beads (1 μm) showed a similar positive slight effect on cell growth of *Cyanothece* sp. PCC 7418. In other parameters such as oxidative stress, the differences observed with the addition of nanoplastic beads may be due to the variation in nutrient availabilities with nutrient limitation treatments showing negative response to the unicellular cyanobacteria species tested. Further studies are needed to evaluate *in situ* the effects of plastic contamination in *P. oceanica* meadows and their associated microogranisms.

Acknowledgments

This work was supported by funding to NSRA trough the Agencia Estatal de Investigación and the European Regional Development Funds project (CTM2016-75457-P). We acknowledge the support and help of Scientific Technical Service (Dr. Guillem Ramis Munar and Maria Trinidad Garcia Barceló) of the University of the Balearic Islands for cytometry and gas-chromatography analyses, respectively. We also thank Pere Ferriol Buñola and Alba Coma Ninot for the help in the acquisition of the cultures.

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Microplastics present in the seawater surface of Los Gigantes in the southwest of Tenerife, Canary Island

Carmen Meléndez Díez*[1] , Alicia Andrea Herrera Ulibarri[2]

*Corresponding author: info@proyectofarfalle.com
[1] Proyecto FarFalle
[2] Universidad de Las Palmas de Gran Canaria (ULPGC)

Keywords: Microplastics, Synthetic fibers, Los Gigantes, Tenerife, Canary Islands

Abstract

Microplastics smaller than 5 millimeters have become an important ecological problem due to the large amount of plastic waste that ends up in the sea. The main impact is the ingestion of microplastics by marine species, the particles enter the tropic chain causing unpredictable effects on humans (Alicia Herrera, 2017).
This paper presents a method for automatic counting and classification of microstopic pasticles. Use of artificial vision techniques to analyze the acquired images of the samples.
They have been determined in the area of Los Gigantes, southwest of Tenerife, Canary Islands, in October 2018, MP / Km^2 in the seawater surface taking into account the position of four submarine emissares along the coast. The highest value obtained during the collection of samples with plankton net was 36429 MP / / Km^2, the remains found mainly were synthetic fibers (13) and fragments (9). However, in the other sampling points an average of 24646 MP / Km^2 was obtained being the most abundant fragments (average 13) followed by synthetic fibers (average 3).
The highest value obtained coincides with the situation of the main submarine emissary of the town of Puerto Santiago, Santa Cruz de Tenerife, Canary Islands.
The sampling, analysis and data provided are the result of the collaboration of Proyecto Microtrofic and Proyecto FarFalle.

Acknowledgments

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Effects of sewage discharge on macrofaunal communities nearby the underwater sewage duct of Trieste (northern Adriatic Sea) on spatial scale

Seyed Ehsan Vesal*[1] , Federica Nasi[2] , Rocco Auriemma[2] , Jessica Pazzaglia[1] , Paola Del Negro[2]

*Corresponding author: seyedehsan.vesal@phd.units.it
[1] Department of Life Science, University of Trieste,Trieste, Italy
[2] Oceanography section, National Institute of Oceanography and Applied Geophysics - OGS, Trieste, Italy

Keywords: Biodiversity, Eutrophication, Macrofauna, Sewage discharges

Introduction

The human exploitations of marine environments have led to rapid degradation, variation of food supplies and loss of habitats with a consequent reduction in species diversity (MEA, 2005). Excess organic matter enters the marine environment principally as sewage. It leads to the well-known symptoms termed eutrophication (Nasi et al., 2018). Due to their capacity to retain contaminants from different sources, the sediments act as a sink in the marine environment (Cardellicchio et al., 2007). Benthic communities in coastal environments are under increasing stress due to anthropogenically disturbances. Macroafuanal organisms due to high biomass and species diversity, could be considered as key biological components, which drive an important process such as nutrient cycling, oxygenation of deeper sediment layers and sediments reworking (Snelgrove, 1998). The main aim of this project is to investigate responses of coastal macrofaunal community influenced by wastewater discharge in the Gulf of Trieste.

Materials and methods

Sediments were sampled at 18 stations (in November 2017) along the sewage duct (at 5, 100 and 200 meters at increasing distance from the main outfall).

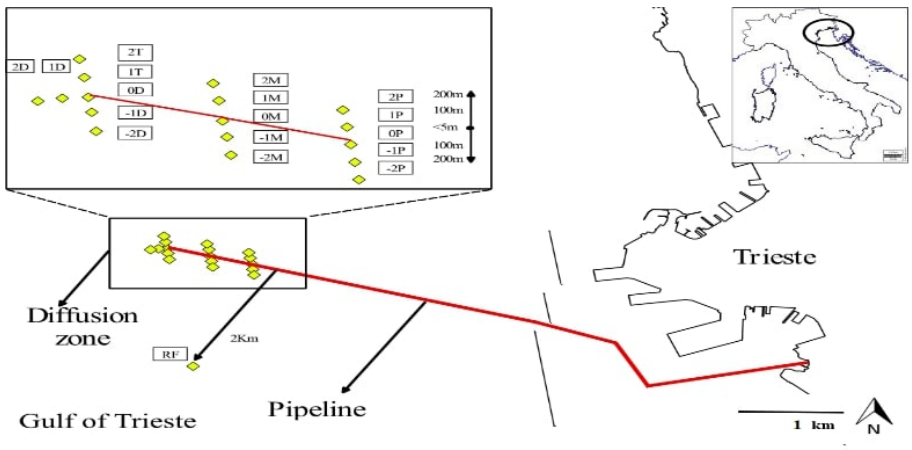

Figure 1: 3 transects along the pipeline (18 stations): Distal (2D, 1D, 2T, 1T, 0D, -1D, -2D), Medium (2M, 1M, 0M, -1M, -2M), Proximal (2P, 1P, 0P, -1P, -2P) and RF (Reference station)

Biogenic Remain (BR) for each station was collected and its volume was measured. The macrofaunal invertebrates were identified to the lowest possible taxonomical level. Biomass of each taxon was measured by wet weight (WW). Moreover, the whole sampling area was characterized by a fine grain size distribution (clay-silt sediments) and the redox potential (Eh) measured, showed low values (-255 mV) nearby the main sewage outfall, suggesting hypoxic condition (Figure 1).

Result and discussion

The present study did not show an evident pattern of organic matter variation, expressed as total organic carbon (TOC) and total nitrogen (TN), depending on the distance from the pipeline, or rather, a decreasing content of TOC toward the stations located at 200 meters from the duct were noticed. The macrofaunal community abundance was dominated by the taxa Polychaeta (≥80% in all the investigated stations) followed by Mollusca (7.3%), Crustacea (4.7%), Echinodermata (4.0%) and others (2.8%). Overall, greater macrofaunal numbers were detected at stations located at the medial transect (M stations) and at stations located at 200 meters, and, in opposite direction to the average sea bed current, a high biomass values were measured. The dominance of polychaetes (in particular *Capitella capitata*) was due to the high abundance of this taxon at St. 0D, whereas molluscs and crustaceans reached the highest percentage at St. 0P and St. 2P. Differences in biodiversity indices along a distance gradient from the pipeline was graphed using four box-plots. No evident patterns were displayed by the Box-Plot graphs, though the total number of species (S) and the Margalef index (d) seemed to show a tendency of a slightly decline toward the station far from the duct (Figure 2).

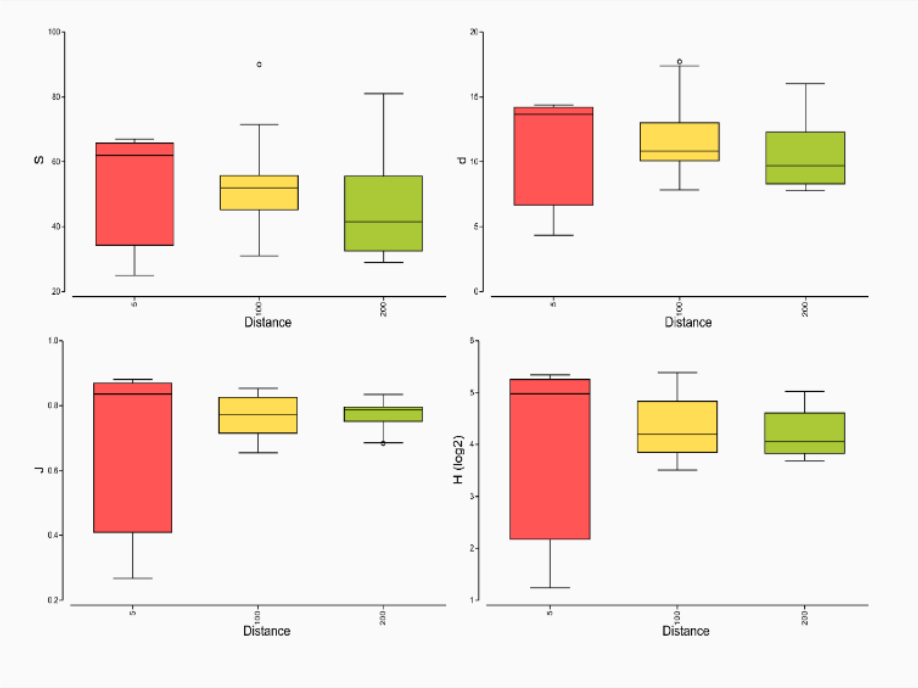

Figure 2: Box-plot of species number (S), Margalef' (d), Pielou' (J) and Shannon Wiener (H' log2) indices of the macrofaunal communities analysed. The stations are grouped by distance factor (≥5 m, 100 m and ≤200 m) from the duct.

This variability is principally due to the lowest evenness, diversity and richness values observed at St. 0D. The non-metrical multidimensional scaling ordination (nMDS) showed a gradient from

stations placed near the pipeline (5m) to ones located farther (200m). In this ordination plot, 0D was positioned at the right side of the plot at the maximum distance in opposition to -2P and RF. The nMDS output showed are the main environmental parameters (sand, silt, TN, Eh and BR) that contributed to macrofauna distribution, confirming DistLM analysis.The vector fitting nMDS analysis based on the main species selected by SIMPER analysis showed a high variation in taxa composition on an spatial scale along an increasing gradient from the pipeline (Figure 3). Specifically, a variation in taxa distribution was observed from the lower right corner of the graph (0D, 0M and 0P) to the left one (-1M, -2M and 1M). This strong changing was mainly due to the high abundance of *Capitella capitata* nearby the pipeline (particularly in the St. 0D) that was positioned at the right side of the plot.The environmental parameters overlaid on this ordination highlighted the same distribution pattern of the abiotic vector in the previous MDs plot (Figure 3).

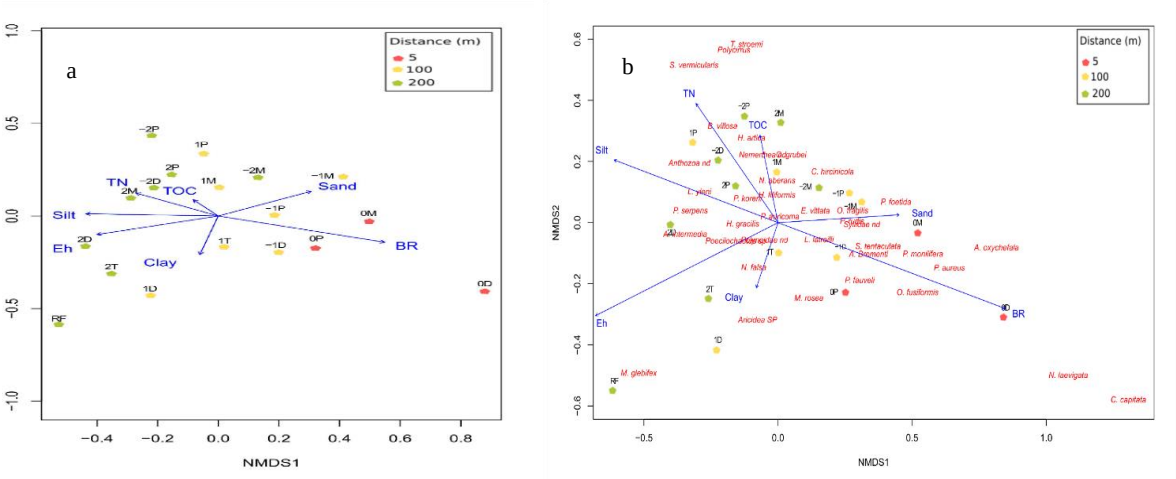

Figure 3: Non-metric multidimentional scaling (nMDS) ordination plot based on the whole macrofaunal community (a) and the taxa obtained by SIMPER analysis (cut-off: <35%) (b). Grain-size, chemical and biogenic remain (blue arrows) were plotted using vector fitting ordination (stress score:0.11 and 0.13, respectively). TOC = Total Organic Carbon; TN = Total Nitrogen; Eh= redox potential; BR=Biogenic remain. Station are indicated with different colours.

A variation in structural composition of macrofaunal community, in terms of biodiversity and species composition, was observed from stations placed nearby the pipeline to those located farther. Low values of diversity indices and high dominance of stress-tolerant species with low biomass (polychaete *Capitella capitata*) were observed at stations located nearby the main underwater outfall. This worm is the most common opportunistic species known as r-strategist (George, 1984). However, the whole species composition of the macrofaunal community and high diversity values observed at the stations located farther from the duct (100 and 200m) indicated a medium impact of the sewage discharge to macrofaunal community.

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Response to abiotic stress produced by heavy metals in the microalga *Chlorella sorokiniana*

Antonio León-Vaz*[1] , Inmaculada Giráldez[1] , Rosa León[1] , Javier Vigara[1]

*Corresponding author: antonioleonvaz@gmail.com
[1] Laboratory of Biochemistry, Faculty of Experimental Sciences, International Campus of Excellence in Marine Science (CEIMAR) and CIECAMBIO, University of Huelva, 21007, Huelva, Spain

Keywords: Microalgae, Biorremediation, Heavy metal, *Chlorella sorokiniana*, Abiotic stress

Introduction

The contamination of aquatic environments is a widespread concern nowadays. Heavy metals are one of the main contaminants because of their persistence and the troubles caused in the trophic chain and human health. This is more accurate in aquatic environments due to the contamination produced by industrial and mining activities. Moreover, the presence of these elements in the environment can modify the cellular metabolism, alter metabolic routes, produce oxidative stress or cause DNA damages (Ouyang et al., 2018). Nevertheless, recent studies have suggested the potential of microorganisms, such as bacteria and microalgae, as bioacumulators in order to avoid the contamination produced by heavy metals (Mantzorou et al., 2018). In this context, microalgae have been reported as excellent bioacumulator organisms compared to bacteria and fungi, because of their tolerance and accumulation capacity, being able to grow up with high concentrations of these elements (Debelius et al., 2009).

Objectives

In this study we tested the effect of different heavy metals and metalloids, such as Cu, Cd or As (III and V) on *Chlorella sorokiniana*, evaluating the growth rate of the microalga, the assimilation of nitrogen source (ammonium) and the intracellular accumulation of the indicated elements, under different metal concentrations. In addition, the effect of these elements on several metabolic pathways, such as nitrogen and sulphur metabolism or the antioxidant response has been evaluated by activity assay and RT-PCR.

Results

The results showed that *C. sorokiniana* has a high tolerance to these heavy metals, being able to grow with concentrations over 500 μM of Cu, 200 μM of Cd, 500 μM of As (III) and 5 mM of As (V), without significant differences in the final biomass. Additionally, the differences in nitrogen consumption are less accurate in all the metals except As (V), where significant differences appeared in the culture with a concentration of 5 mM of the metalloid. Moreover, we have demonstrated that *C. sorokiniana* can accumulate high concentrations of Cu and Cd, whereas it did not appear intracellular As. Finally, the RT-PCR and the activity assays showed different alterations in the metabolic pathways studied, which conclude that, although the microalga can grow, their cellular pathways are affected by these heavy metals.

Acknowledgments

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Water quality monitoring of coastal water in La Herradura Bay (Granada, South-Western Mediterranean)

Miguel María Granados Fernández*[1] , Jorge García Márquez[2] , Salvador Arijo[2] , Andreas Reul[1] , Begoña Bautista[1] , María Muñoz Muñoz[3]

*Corresponding author: granadosm@uma.es

[1] Departamento de Ecología y Geología, Universidad de Málaga, Campus de Teatinos s/n, 29071 Málaga, Spain.

[2] Departamento de Microbiología, Universidad de Málaga, Campus de Teatinos s/n, 29071 Málaga, Spain.

[3] Instituto Español de Oceanografía, C.O. Málaga., Puerto Pesquero s/n, Fuengirola, 29640 Málaga, Spain.

Keywords: Monitoring, Water quality, Management, Coastal water

Introduction and objectives

The evaluation of the quality level of 45 beaches along the Andalusian coast by Williams et al. (2012) considers 8 human variables and 18 physical variables, showing that 11 beaches are classified as the worst beaches and 7 are classified as the best beaches on a scale from 1 to 5 (where 1 is the best category and 5 the worst category). Furthermore, wastewater treatment is deficient along the Andalusian coast, particularly in small villages (Del Moral et al., 2015). This is especially important in summer, when most of the coastal villages along the Andalusian coast increase its population due to tourism, and wastewater treatment plants can be overloaded. The resulting coastal eutrophication can enhance the appearance of red tide blooms, affecting the composition of phytoplankton. Moreover, if red tides are dominated by harmful algae, local fishery can also be affected negatively.

La Herradura (Costa de Granada, southern Spain) is a small town located between the Site of Community Importance (SCI) Maro-Cerro Gordo and the Special Area of Conservation (SAC) Punta de la Mona. The possible contamination of this bay by the anthropogenic pressure can affect those Marine Protected Areas.

The objective of this study is to determine the spatial and temporal fluctuations in the phytoplankton community and the water quality in La Herradura Bay during high and low seasons, with the aim of detecting possible algal blooms, eutrophication processes and contamination by wastewater effluents. All of it by applying a new simple sampling method.

Materials and methods

The study was performed in La Herradura Bay during 7 months from winter to summer in 2019. Two types of sampling were carried out, one fixed stations in coastline and offshore, and other with zigzag surveys through a kayak.

In order to detect algal blooms, a biweekly sampling at three coastal stations (Stn. 1, Stn. 2, Stn. 3, Figure 1) was carried out along the beach of La Herradura from winter to summer. At each sampling station, water temperature was measured, and phytoplankton composition was analysed by flourospectrometry and FlowCAM. In addition, a zigzag survey with Kayak

and fluorospectrometer was carried out (Figure 2) for evaluating the patchy distribution of phytoplankton in the Bay.

To detect the potential contamination of coastal water by urban wastewater, seawater samples were collected at the four coastal stations (St1, St2, St3 and Stn4, Figure 1) and filtered for faecal bacteria enumeration on Chromocult agar (count of *Escherichia coli*) and on Slanetz-Bartley agar (count of fecal enterococcus). The determination of faecal contamination was sampled twice at low and high seasons. In addition, other stations were also sampled in the bay during the kayak surveys in order to cover representatively the whole Bay. For it between the beach and the submarine wastewater outfall two stations were sampled (Stn. 5 and Stn. 6, Figure 1) and other two offshore the wastewater outfall (Stn. X1 and Stn. X2, Figure 1).

Figure 1: Sampling stations in La Herradura Bay (see explanation in text).

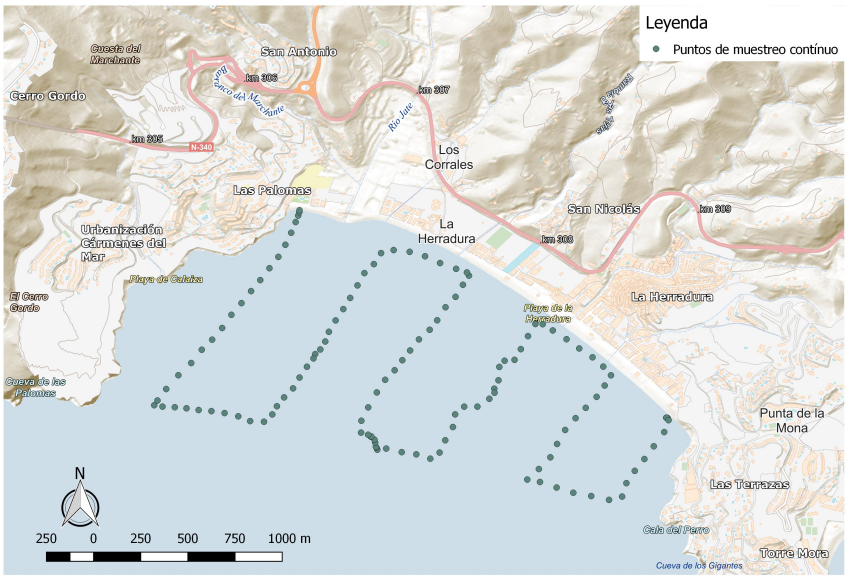

Figure 2: Zigzag survey with Kayak in La Herradura Bay (see explanation in text).

Results and discussion

The 10 biweekly sampling of total chlorophyll and phytoplankton composition with the fluorospectrometer, revealed significant differences among stations and throughout the time. The differences among stations could be due to patchy distribution of phytoplankton in the Bay, as detected during the Kayak survey. The data also suggested a direct relationship between changes in the spatial distribution of chlorophyll *a* concentration and the predominant wind regime.

Concerning the water quality, most of the stations were free of faecal contamination, except for station four (Stn. 4, Figure 1) which was next to a river runoff, where elevated concentrations of *E. coli* and faecal Enterococcus were detected. Faecal bacteria concentration increased during high season and overcame the legal concentration allowed for bathing waters (BOE, 2007).
These results suggest that a viable standard monitoring protocol for Andalusian coastal waters is needed, especially for the detection of faecal contamination of small coastal villages, as this diffuse contamination along the Andalusian coast can be harmful for bathers. Moreover, the anthropogenic nutrient supply to coastal stratified waters, can enhance red tides in summer, just during high season.
Although the submarine wastewater effluents of small village are small, most of them are close to the coast or not deep enough to avoid the contamination of swimming areas and coastal ecosystems. The Andalusian economy is based on tourism and it can not tolerate diffuse faecal pollution sources that could turn coastal waters unsuitable for bathers. Hence, detailed studies on diffuse contamination by small villages wastewater and possible harmful algal blooms should be carried out routinely.
In conclusion, this work shows preliminary results on water quality and phytoplankton composition in coastal areas by applying a simple, economic and contamination free sampling method; which is especially suitable for studies in marine protected areas.

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Pump-underway ship intake: an opportunity for Marine Strategy Framework Directive (MSFD) monitoring needs. First observations of microplastics on oceanic and coastal waters off the Canary Islands (Subtropical NE Atlantic)

Tania Montoto-Martinez*[1] , Jose Joaquin Hernandez-Brito[2] , Maria Dolores Gelado-Caballero[1]

*Corresponding author: tania.montoto@ulpgc.es
[1] Research group on Environmental Technologies, Management and Biogeochemistry. University of Las Palmas de Gran Canaria (ULPGC)
[2] Oceanic Platform of the Canary Islands (PLOCAN)

Keywords: Microplastics, Surface waters, Monitoring, Pump-underway, MSFD

Abstract

Broad scale sampling methods for microplastic monitoring in open ocean waters is a current challenge in oceanography. Large amount of samples is required to understand distribution, abundance and fate of these particles in the environment. The underway water system of research vessels (RVs) has been approached for microplastic sampling in some studies up to date (Desforges et al., 2014; Enders et al., 2015; Kanhai et al., 2017; Lusher et al., 2014; Setala et al., 2016). In this study, we present a sampling methodology founded on the design of a microplastic sampling device connected to the pump-underway ship intake system as an opportunity for oceanic monitoring needs concerning microplastic abundance and distribution (Figure 1).

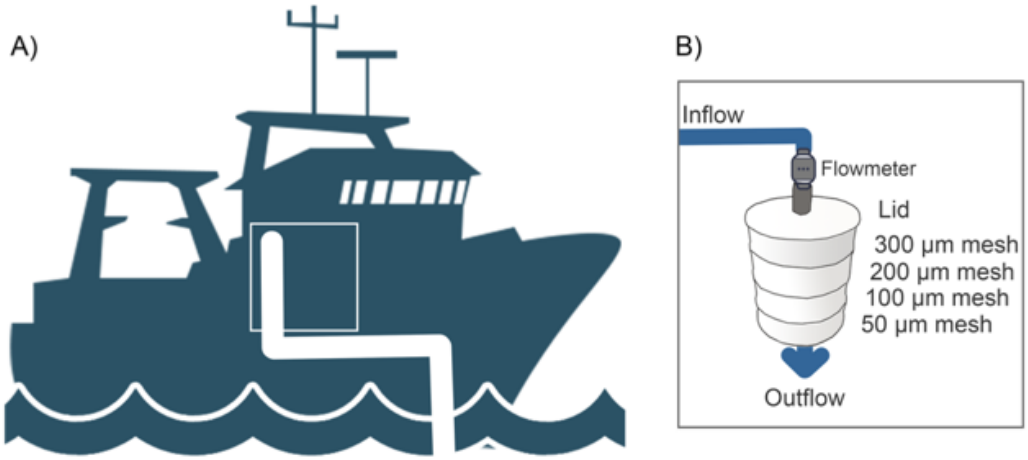

Figure 1: Sampling set up diagram, showing (a) the pump-underway system of the RV; and (b) the filtering device diagram.

This methodology provides four main advantages: (1) the sampling device is fully made with standard materials, cost-effective and affordable, and it can be self-mounted by the researchers without additional skilled personnel or equipment on-board; (2) it can be employed to report data

taking advantage of oceanographic campaigns without interfering their regular vessel activities; (3) it is highly versatile and reduces the time needed to recover each sample; (4) it is suitable to sample microplastic particles (both fibres and fragments) down to 50 μm. As preliminary field application results and first reported data from the Canary Islands oceanic and coastal waters, we investigated microplastics (0.05 – 1 mm) sampled from subsurface waters (- 4 m depth) using the pump-underway system of RV Ángeles Alvariño in the Subtropical NE Atlantic. Sampling was performed in three consecutive oceanographic campaigns over a year, repeating the same procedure, retrieving water while on navigation and while on coastal and oceanic stations. Microplastic particles (> 50 μm) were found in the total stations and transects sampled (Figure 2). Fibres (64.42%) were predominant over fragments (35.58%), being the concentration values over the data reported in other areas in the Atlantic.

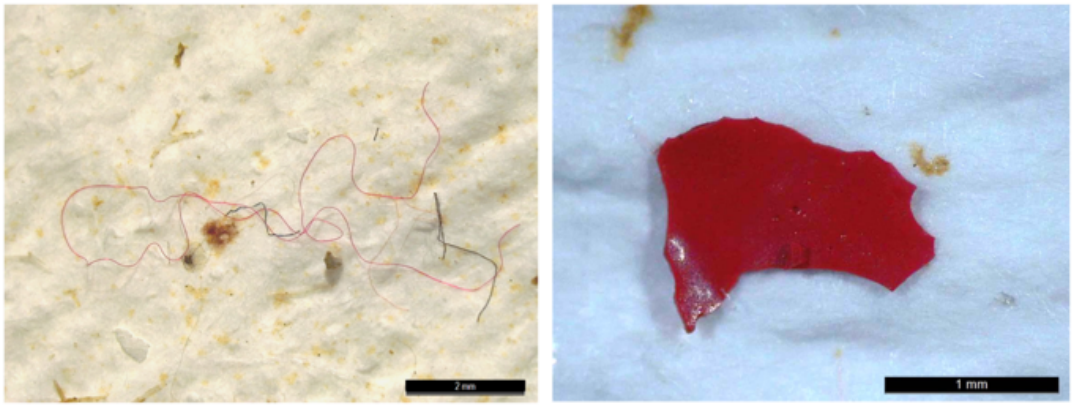

Figure 2: Pictures showing the typical microplastic particles found: (a) a specially long red fibre, and (b) a red microplastic fragment, showing characteristics for microplastic identification such as irregular edges.

The pump-underway system represents an opportunity as a sampling method to increase monitoring efforts and increase reported data for the implementation of the Marine Strategy Framework Directive (MSFD). More research is needed in order to assess the method efficiency and to develop proper strategies to address the threat that microplastics pose to the marine environment and ecosystems.

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Ballast water management: How bacteria from different sources respond to UVc radiation?

Javier Moreno-Andrés*[1] , Leonardo Romero-Martínez[1] , Asunción Acevedo[1] , Enrique Nebot[1]

*Corresponding author: javier.moreno@uca.es
[1] Department of Environmental Technologies. INMAR-Marine Research Institute. University of Cádiz.

Keywords: UV disinfection, Ballast water, Naturally occurring bacteria

Abstract

The shipping industry and the associated management of ships' ballast water is currently considered a global challenge to the health of the oceans due to the transport of invasive species and the significant impact that the discharge of ballast water can have on ecosystems and human activities. To avoid this problem, ballast water treatment might be implemented on ships, as required by the International Convention for the Control and Management of Ships' Ballast Water and Sediments of the International Maritime Organization (IMO, 2004). This convention establishes the maximum admissible concentrations of certain organisms that must be complied with the discharge of ballast water, in order to minimize the risk of the spread of invasive species. The bacteriological standards that appear in the convention focus mainly on typical microbial indicators affecting human health and not those that may affect ecosystems or associated maritime activities. Considering the great diversity and microbiological abundance in seawater, it is considered necessary to study the behavior of these microorganisms, since the capacity to cause great impacts in the receiving environments is high (Hess-Erga et al., 2019). Accordingly, the main objective of this work is to evaluate the response of different bacteria (from different sources: faecal and marine origin) to the UVc treatment of ballast waters. Special attention will be paid to the scenarios that take place after the discharge into the receiving water; studying the evolution of the organisms in a post-treatment scenario. The response of the different bacteria will depend directly on their sensitivity to UV radiation and this will be specific to each organism. Accordingly, different experiments have been carried out with a Collimated Beam Reactor and different type of bacteria, distinguishing between natural occurring bacteria (*Roseobacter sp., Pseudomonas litoralis, Kocuria rhizophila*) and typical indicators established in ballast water regulations (*Escherichia coli, Enterococcus faecalis*). According to the Dose-Response curves obtained (see Figure 1), the UV sensitivity of the bacteria can be defined as follows: *E. coli* > *Roseobacter sp.* > *E. faecalis* ≈ *P. litoralis* > *K. rhizophila*. The capacity of re-growth was also analyzed as a biological response, in order to evaluate the evolution of the bacteria once they have been treated. The results obtained show that there is a capacity of bacterial recolonization in the days after the treatment, which substantially differs from typical indicators (% regrowth ∼1-5%) in front of naturally occurring marine bacteria (% regrowth ∼20-40%).

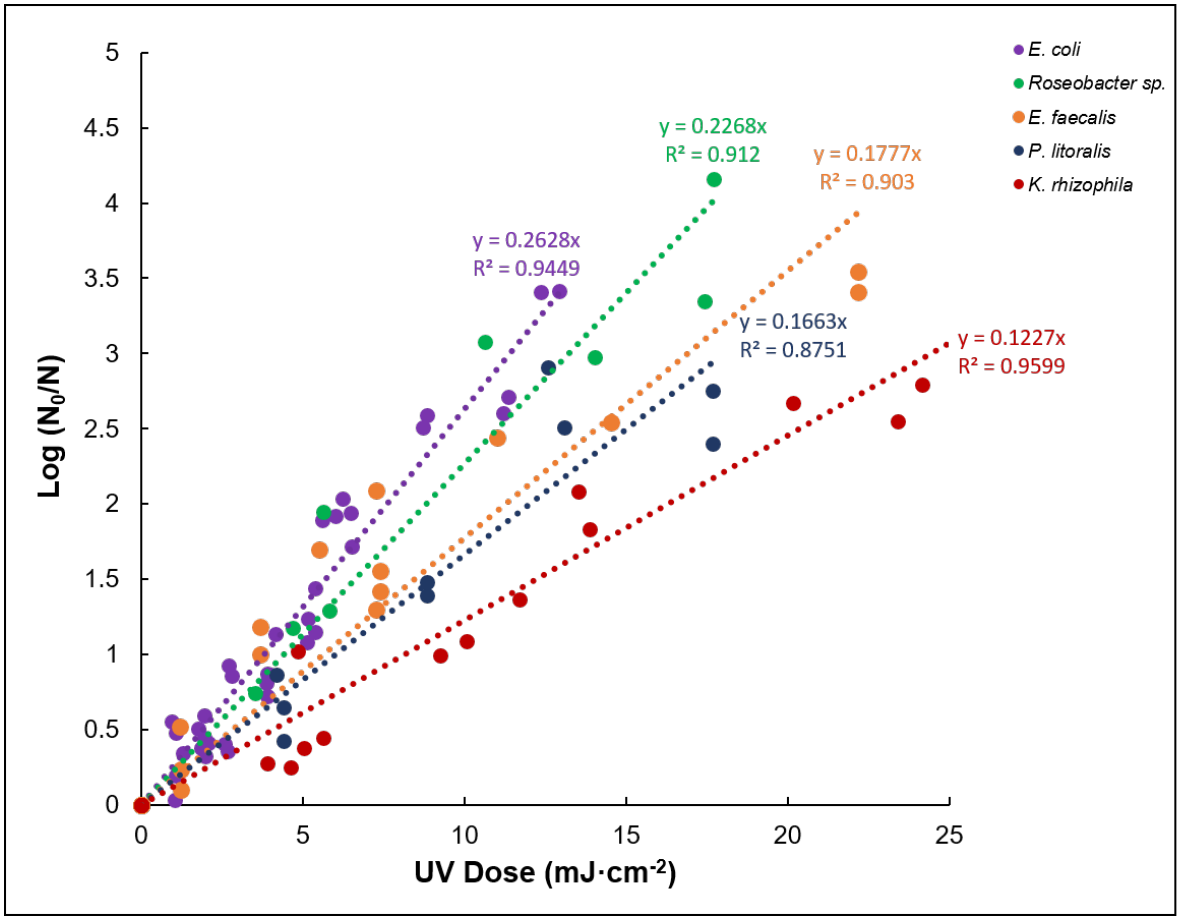

Figure 1: Dose-response curve obtained for the different microorganisms evaluated against UV treatment.

Based on the results obtained, it has been concluded that marine microorganisms have a high recovery capacity once they are treated and can achieve significant re-growth within 5 days. It might indicate that wild marine bacteria can develop important repair mechanisms that allow them to survive and evolve in a post-treatment scenario (Wennberg et al., 2013). In this scenario, important factors need to be taken into account, such as the characteristics of the receiving environment, or the different adaptation strategies in the face of stress phenomena. In this sense, future studies are necessary for this type of applications: i) studying the behavior of different specific microorganisms and ii) promoting treatments with greater oxidative power that are capable of inhibiting the phenomena of re-growth after treatment.

Acknowledgments

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Plastic inputs into the ocean through the Guadalquivir river

Rocio Quintana Sepúlveda*[1]

*Corresponding author: roc.quintana@gmail.com
[1] University of Cadiz

Keywords: Plastic, River inputs, Guadalquivir, Marine pollution

Abstract

Densely populated developing economies, high rates of plastic waste generation combined with inadequate waste management infrastructures result in significant volumes of plastic waste leaking into the marine environment. Potentially, one of the main pathways of plastic inputs into the open ocean could be rivers, but there is no comprehensive information about the amount of litter transported. In this study, we are going to quantify the amount of plastic entering through the Guadalquivir river in the period of January 2th of 2014 to August 18th of 2015. The methodology used in this work is based on the imaging analysis using the ImageJ Fiji® system, with the final purpose of counting and measuring the entrance of microplastics (particles < 5 mm) and mesoplastic (particles between 5-25 mm).

Introduction

The presence of plastics in the marine environment has been identified as a major concern due of its persistent nature (Lebreton et al., 2017). Marine litter can be transported across long distances and remain in the marine environment for an undetermined length of time, where they can be found in the most remote areas of the Earth (Veiga et al., 2016). A substantial fraction of these marine plastic debris originates from land-based sources, where rivers potentially act as an important transport pathway for all sizes and types of plastics (Schmidt et al., 2017). Although it is estimated that the input of river litter is an important contributor to marine litter, there is still no complete information on the amount of litter transported through the rivers to open sea. In addition, there are no harmonized methodologies to provide quantitative data for comparable assessments of river litter (González et al., 2016). In order to establish future measures for the reduction of plastics and their possible impacts on the marine environment, it is necessary to quantify and identify the main sources, as well as their possible pathways to the marine environment, such as river inputs (Van der Wal et al., 2013). In this work, the main objective is the quantification of the entrance of plastics into the ocean through the Guadalquivir River, where we will focus of the entrance of microplastics (particles <5 mm) and mesoplastic (particles between 5-25 mm).

Methodology

Meso and micro litter on the Guadalquivir river were sampled from January 2th of 2014 to August 18^{th} of 2015, with 2 sampling points with the following coordinates: Sampling Point Number 3: Tarfia (36°57'30.4"N 6°'36.7"W); sampling Point Number 5: Bonanza (36°52'3.16"N 6°20'44.97"W). In this case, sampling was carried out for 24 continuous hours, with the aim of covering the entire tidal cycle, where tide number 1 and 3 correspond to the flow current, and

tide number 2 and 4 correspond to the ebb current. Samples were collected using 3 nets with 1 mm mesh size with a rectangular net opening of 3x3 meters. The net was towed at the top 20 cm of the river surface during 60-90 minutes, with an average filtered volume of 58 000 m^3. In the lab, samples were transfer into a 13 L jar, where only 1 L was analyzed for mesoplastic and 100 g was analyzed for microplastic. Floating plastic debris was carefully picked from the supernatant. Plastic items were weighted, counted and measured by imaging analysis using the ImageJ Fiji® system. In total, 3242 items of microplastic and 3194 items of mesoplastic have been examined in this work.

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Session 4

The Sea Knowledge

Physical Oceanography and Marine Geology

Oceanographic interpretation of planktonic foraminifera assemblages sampled in a transect from Mediterranean Sea to Atlantic Ocean (MEDWAVES cruise, 2016)

Albert Fuster Prohens*[1] , Beatriz Alvira[1] , Anna Khokhlova[1] , Guillem Mateu Vicens[1]

*Corresponding author: albertfuster95@gmail.com
[1] Càtedra Guillem Colom. Departamento de Biología, Facultad de Ciencias, Universitat de les Illes Balears. Palma (Balearic Islands).

Keywords: Azores Current, Mediterranean outflow water (MOW), Planktonic foraminifera, Portugal Current

Introduction

The Foraminifera are an enormously successful phylum of amoeboid protists. Despite the predomination of benthic species, few of them are planktonic. There are only about 50 planktonic species, which have calcium carbonate test. Nevertheless, recent genetic and molecular analyses reveal the existence of some cryptospecies (de Vargas et al., 2001). Despite their low diversity, these organisms dwell practically in all oceans as a result of a set of adaptations. They were classified in three different groups related to their bathymetry. Shallow water species live between the surface and 50 meters deep. Intermediate water species inhabit in waters up to 100 meters deep. Finally, deep water species live below 100 meters deep. Planktonic foraminifera are also divided in five biogeographic provinces. The tropical province is ubicated in the equatorial zone and it is the region with the greater diversity. The subtropical province is found in higher latitudes and has slightly lower diversity. Between these two warm-waters provinces and cold-water regions there is the transitional zone, which is considered like an ecotone. The cold-water regions are constituted by subpolar and polar provinces where the diversity is low. Furthermore, currents play an important role in the transport of these organisms. In the Northeast quadrant of Atlantic Ocean, warm-water species are transported by different currents such as Azores Current. On the other hand, cold water species are displaced by North Atlantic and Portugal-Canarias Current (Be, 1977). When these organisms die their calcium carbonate test resist in the sediment during thousands of years. For this reason, the study of the thanatocoenosis of planktonic foraminifera has been widely used in the study of the long-term oceanographic conditions. In this context, the present study tries to reconstruct the oceanography in four seafloor edifices using planktonic foraminiferal shells of the sediment, focusing on Mediterranean outflow water (MOW).

Materials and methods

The collection of samples was done in four seafloor edifices, explored during the MEDWAVES cruise (2016). They are located along a distance gradient from the Mediterranean Sea (Seco de los Olivos in the Alboran Sea) towards the Atlantic Ocean (Gazul - Gulf of Cádiz, Ormonde and Formigas). Thirty-three sediment samples were collected using Van Veen grab and Box core. Then, samples were sieved and the grain size above 65 micrometers was used for foraminiferal assemblage analysis. Individuals were randomly collected from surficial sediment samples. Species richness was captured from 100 individuals per sample. Individuals were identified and grouped in their biogeographic

provinces. Moreover, specimens were classified as warm-water or cold-water species, where transitional species were included. The proportion of dextral and sinistral forms in *Globorotalia truncatulinoides* (d'Orbigny, 1839) populations were also determined. Then, abundance from each species and each association were calculated. Finally, non-metric multidimensional scaling (nMDS) was used to interrelate the similarity of the samples. Additionally, the proportion of warm and cold-water species and the proportions of sinistral and dextral forms of *Gr. truncatulinoides* were included in nMDS as a vector.

Results and discussion

Up to 4,210 foraminiferal shells, corresponding to 31 planktonic taxa, were randomly collected from superficial sediment samples, identified and counted. Clear differences are observed between planktonic community from Seco de los Olivos respect to the other Atlantic areas in the nMDS matrix. Gazul, situates in Gulf of Cadiz, is ubicated in an intermedium position between Seco de los Olivos and the other Atlantic areas, Ormonde and Formigas, which result similar (Figure 1). These patterns are due to the grater abundance of cold-water related species in both Seco de los Olivos (79 % of the total specimens) and Gazul (49 % of the total specimens). This observation reflects the influence of the Portugal Current, which transports cold-water taxa into the Gulf of Cadiz and the Alboran Sea (Be, 1977). Conversely, warm-water taxa dominate in Ormonde and Formigas seamounts, representing 63 % and 65 % of the planktonic individuals, respectively. In this case, the composition of this assemblage is derived from the transport by the Azores Current (Be, 1977). On the other hand, Shannon diversity value from Seco de los Olivos is low (H' = 1.6). It is a result of the great abundance of *Globoconella inflata* (d'Orbigny, 1839) and *Globigerina bulloides* (d'Orbigny, 1826) in Seco de los Olivos (58 % and 18 % of total specimens, respectively), both species are related to cold water and high food availability. Indeed, it is corresponded with the upwelling conditions, previously documented in Alboran Sea (Mallo et al., 2017). This oceanographic context enhances the dominance of few opportunistic species, resulting in lower relative abundance of all other species (Ottens and Nederbragt, 1992). It contrasts with the higher values obtained in Gazul (H' = 2.2), Ormonde (H' = 2.5) and Formigas (H' = 2.3). Convergence of warm-water and cold-water species result in a higher diversity in the Gulf of Cadiz (Ottens and Nederbragt, 1992). Additionally, this result could also reflect oligotrophic conditions in Ormonde and Formigas, which favors the coexistence of a greater number of specialist species. However, no evidence of MOW is observed in the foraminiferal community. This is a consequence of deep circulation of MOW which contrasts with the shallow distribution of the planktonic foraminifera. For this reason, the present study has focused in *Gr. truncatulinoides*, which is a deep-water planktonic species (Be, 1977). Despite being only considered as one morphospecies, genetic analysis revealed that it is a set of four cryptopsecies. *Globorotalia truncatulinoides* type II is exclusive of the Atlantic Ocean and the Mediterranean Sea. It is the only one which has dextral and sinistral forms (de Vargas et al., 2001). Northeast quadrant of the Atlantic Ocean is dominated by dextral forms (Ericson et al., 1955). It contrasts with the dominance of sinistral forms in Mediterranean Sea. As a result, sinistral morphotype can be exported from Mediterranean Sea to Atlantic by MOW. Hence, this study has proposed left-coiling *Gr. truncatulinoides* as an indicator to the Mediterranean-Atlantic connectivity. When the proportion of sinistral and dextral forms is analysed, the relative abundance of the sinistral form in *Gr. truncatulinoides* populations is maximum in the Mediterranean area (94 %). This proportion decreases progressively with the distance from the Mediterranean towards the Atlantic (Figure 1). Indeed, its minimum value has been obtained in Formigas (36 %), which is the most westward area. All in all, the present study concludes that the high abundance of cold-water species and also transitional species in Seco de los Olivos and Gazul reflects the influence of the Portugal Current. Furthermore, the abundance of warm-water species in Ormonde and Formigas is derived from the transport of these species by the Azores Current. Finally, sinistral

morphotype of *Gr. truncatulinoides* seems to be exported from the Mediterranean Sea to the Atlantic Ocean, where dextral forms dominate. Therefore, this species is an adequate indicator of the Mediterranean influence in the Atlantic.

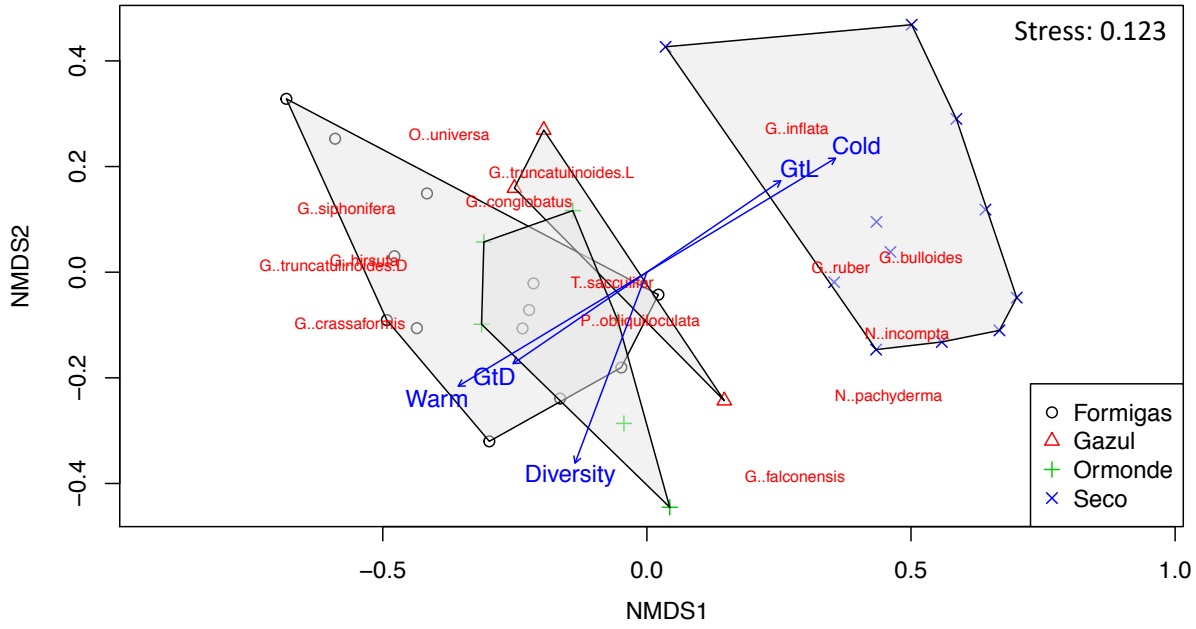

Figure 1: Non-metric multidimensional scaling (nMDS) matrix made from planktonic foraminifera species with abundances greater than 3 % in at least one sample. The vectors correspond to the Shannon index (Diversity), the proportion of cold and warm species and the proportion of sinistrals (GtL) and dextrals (GtD) forms in *Globorotalia truncatulinoides* populations.

Acknowledgments

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Circulation patterns on the continental shelf of the Gulf of Cadiz from measures of HF coastal radars

Sara Sirviente Alonso*[1] , Marina Bolado Penados[1] , Miguel Bruno Mejias[1]

*Corresponding author: sara.sirvientealonso@alum.uca.es
[1] Universidad de Cádiz

Keywords: HF Radar, Countercurrent, Gulf of Cadiz, ADCP

Introduction

The Gulf of Cadiz (GoC) is a sub-basin of the North Atlantic Ocean which connect with the Mediterranean Sea through the Strait of Gibraltar. It is a special complex system, where a two-layer exchange is allowed between the Atlantic Ocean and the Mediterranean Sea. Several studies have described and characterized a warm coastal countercurrent (CCC) flowing from east to west. The authors attribute this CCC to a different physical forcing mechanism such as the wind or the alongshore unbalance pressure gradient. To date, most of the studies have used remotely sensed Sea Surface Temperature, current time series and reanalysis to analyze the surface circulation pattern of GoC (Criado-Aldeanueva et al., 2009). Garel et al. (2016) were the first to describe the main characteristics of CCCs inside the platform of GoC from the observation and analysis of annual current time series from an ADCP. The aim of this work is to assess the surface circulation pattern of GoC based on velocity time series provided by HF coastal radar. The measurements supplied by the instrument will be validated with *in situ* velocity time series registered by an ADCP.

Materials and methods

The current speed data used in the present strudy have been collected from the system SeaSonde CODAR (Coastal Ocean Bynamics Applications Radar) HF radars belonging to the TRADE 2010-2013 (Trans-Regional RADars for Enviromental applications) project deployed in the Gulf of Cadiz. The system features 2 antennas, one located in the port of Mazagón (Huelva) and the other in Vila Real de San Antonio. Both stations have a transmission frequency of 13.5 MHz, a range of 75 km, a spatial resolution of 2 km and a time sampling interval (1h). The registration period used was from 15/10/2013 to 16/01/2014. Times series of surface current velocities acquired by the HF radars have been reconstructed by the application of Empirical Orthogonal Function (EOF). Furthermore, the signal has been preprocessed, by applying a low pass band FFT filter (cut-off period of 30 h) in order to separate the low frequency signal (subinertial signal) from the high frequency signal (the tidal signal). Real Empirical Orthogonal Function Decomposition (EOF) in which the components of the current velocity are included as scalar variables has then been applied to the subinertial signal. ADCP data period covers from 15/10/2013 to 16/01/2014. The ADCP was anchored in the point 37°6' 40" N and 7° 14' 19" O. It was anchored at a depth of approximately 20 m. The closed data to the bottom is recorded 2 m from it, the transducer is 2 m high, the blanking area or no register is a 0.4 m and there is 0.5 m to the first cell. The current profiler presents a sampling interval of 10 minutes. Noise and error data were discarded, being the cell at a depth of 7 m as the most superficial cell with reliable measurements. To assess whether the high pressure center located in the Ligurian region influences the pattern of GoC surface circulation, Mean Sea Level Pressure (MSLP) data from the ERA5 reanalysis

(Hersbach and Dee, 2016) were analyzed. In the same way, wind velocity reanalysis from MM5 where assessed as a possible physical forcing variable. Both atmospheric variables period cover from 15/10/2013 to 16/01/2014.

Results

In Figure 1 you can see qualitatively the comparison between the time series obtained by the radar system and in red that obtained by the current profiler. A high correlation can be seen between the two, thus reflecting that the measurements recorded by the radar have a high reliability and that is therefore a good instrument to study the surface circulation pattern. The differences in both series may be due to the limitations of the technique used in their treatment and the difference in depths to which both instruments record. The radar reaches a maximum spread of 1 m and the shallowest ADCP register at 7 m.

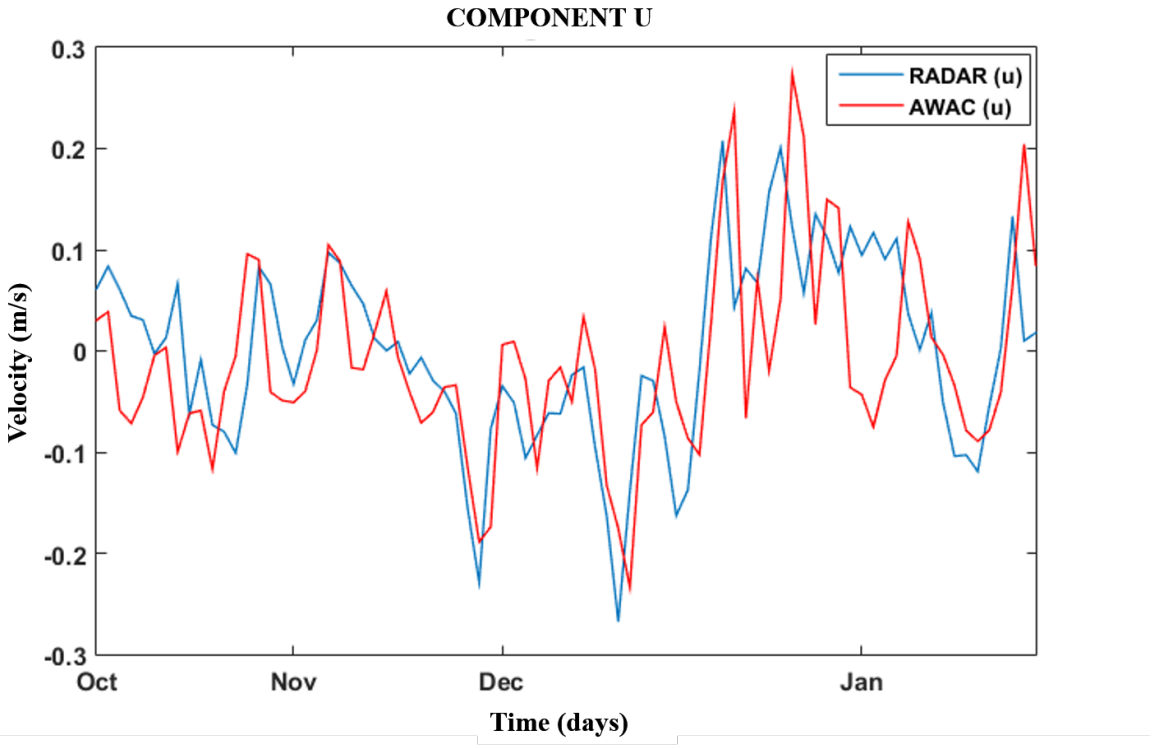

Figure 1: Zonal component of the velocity (m/s) calculated from the EOF analysis (blue) and the zonal component of the current (m/s) recorded by the ADCP (red) during period I. The black continuous line delimits the value 0 m/s of speed.

Mode 1 is the most energetic mode which explains more than 90% of the variance of the series. When spatial weights multiply by a positive time weight value, the current will preferably be directed westward. However, if it is multiplied by a negative time weight it will be directed east. Therefore, from the point of view of time weight (mode), a CCC event corresponds to a positive time weight. You can see how the mode captures CCC events on November 29 and December 12 well. The result show that mode 1 radar speed fields are very correlated with MSLP in Liguria (R = 0.5210) and zonal wind (R= -0.6402) while mode 2 is closely related to the southern wind component (R= 0.4340; Figure 2).

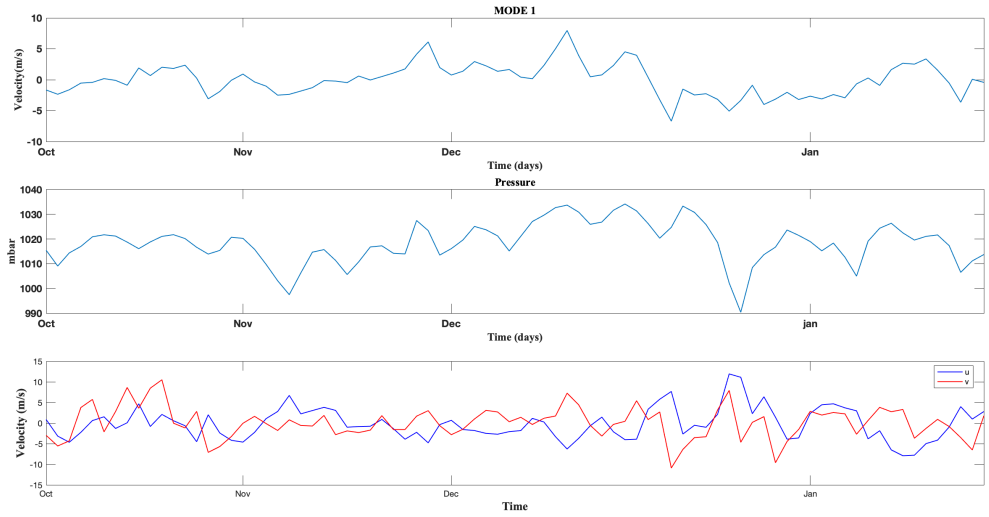

Figure 2: Results observed for study period I (15/10/2013-16/01/2014). a) Mode 1 series (HF radar). b) Zonal (red line, east-positive) and southern (blue line, north-positive) components of wind speed at 10 m (m/s) provided by MM5 model. Horizontal black line delimits the value of 0 m/s. c) Time series of atmospheric pressure (mbar) at sea level in the Liguria region provided by the ERA5 model.

In our case the mode 1 spatial weights for component or speed are negative. Therefore, a correlation coefficient between MSLP and positive mode 1 assumes when we consider the sign of spatial weight, a negative correlation with the component or current that is explained by mode 1. With this result, an increase in atmospheric pressure in Liguria would correspond to a decrease in the u component, i.e. an increase in the component or to the west. An uphill surge in Liguria causes the arrival of over lift waves to the Strait that reduce the incoming current towards the strait which could cause a pile of water in the western part of the strait (Candela et al., 1989). This stacking causes a longitudinal level gradient, which could feed the creation of a countercurrent. It can also be seen from the results of linear regressions that the variability of mode 1 can be explained by the zonal component of the wind. The MSLP and the wind zonal component are intimately related (R= -0.5465), making it impossible to sometimes separate them (Figure 2). Mode 2 is been related to the southern component of the wind. Soutwest winds generate a stack of masses of water on the coast, causing an imbalance in the balance of pressures and thus force a shift in the flow to the west (Figure 3).

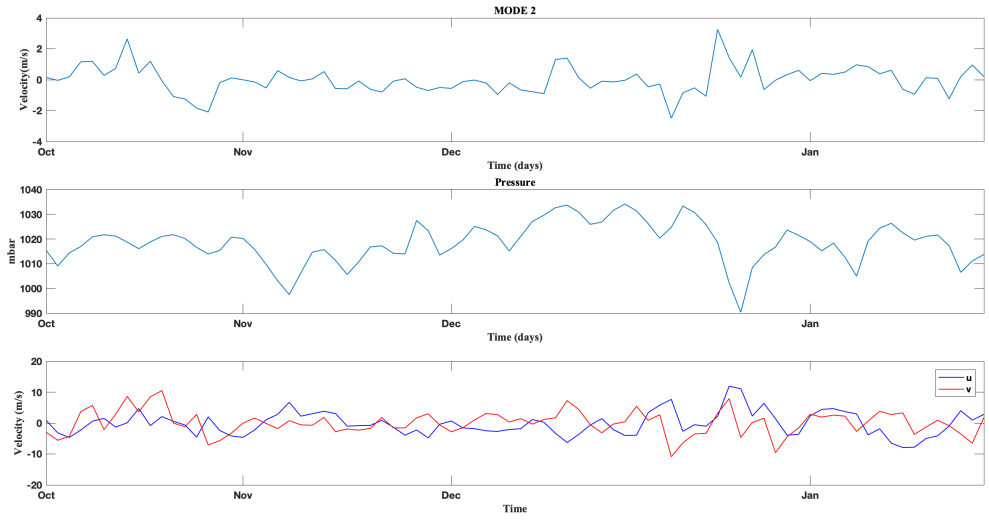

Figure 3: Results observed for study period I (15/10/2013-16/01/2014). a) Mode 2 series (HF radar). b) Zonal (red line, east-positive) and southern (blue line, north-positive) components of wind speed at 10 m (m/s) provided by MM5 model. Horizontal black line delimits the value of 0 m/s. c) Time series of atmospheric pressure (mbar) at sea level in the Liguria region provided by the ERA5 model

In conclusion, the measurements obtained with coastal HF radars provide reliable measures for the study and characterization of the GoC surface correlated pattern. Possible physical forcings that may explain the development of countercurrent events found are winds with a strong southern component and atmospheric pressure variations in the Ligurian area. The countercurrent has a high space-time variability, encompassing a greater extent in periods close to winter compared to the autumn period. Establishing counter-current events near winter introduces significant meso and submesoscale variability on the Gulf of Cadiz continental shelf.

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Spatial-temporal variability of the Almeria-Oran front using remote sensing

Sara Miquel*[1]

*Corresponding author: sa.miquelfer@alum.uca.es
[1] UCA

Keywords: Mesoscale, Oceanic fronts, Canny algorithm, Remote sensing, Alboran Sea

Abstract

In this report is presented the implementation of Canny algorithm for the detection of oceanic fronts using ocean colour images and SST in the Alboran Sea. The algorithm has been implemented using colour images from GlobColour in the period of 1998 to 2019. The analysis shows spatial and temporal variability of the Almeria-Oran front during the studied period, and a greater activity of the intensity of the front during winter. Although the front is intense there is a greater probability of fronts in the west Alboran gyre.

Introduction

Oceanic fronts are narrow zones in which the gradients of the physical, chemical and biological properties delineate borders between different types of water (Ullman and Cornillon, 2000). The fronts are considered dynamic structures susceptible to baroclinic instabilities that occur in a great variety of scales and are indicators of many oceanographic processes. During the last years, remote sensing has been used for the monitoring of oceanic fronts using satellite images. Alboran Sea is an ideal zone to study oceanic fronts, as it is an area in which there are plenty of this mesoscale structures due to the dynamics of the basin. Pelagic communities and the associated biogeochemical fluxes are often driven by the physical dynamics associated with mesoscale frontal structures. Hence, it is important to understand the long-term dynamics and the statistical properties of fronts and how these may be affected by the interannual and interdecadal climate dynamics. The Canny Algorithm Canny (1987) is based on the detection of horizontal gradients from convolution methods. It is used in the treatment for the detection of contours and the detection of oceanic fronts in images of low temporal and spatial resolution (Castelao et al., 2005) It can be useful on large-scale fronts as it is easy to implement. In the present work ocean colour oceanic data has been used, downloaded from the GlobColour Project (http://www.globcolour.info/). The study area for the detection of oceanic fronts in the Alboran Sea is limited between 38.5 °N and 35 °S, and 6 °W and 1 °E. The study period covers from August 1, 1997 to May 13, 2019. The spatial and temporal resolutions of these composite images are from 1 km to 8 days respectively.

Workflow

Canny Edge Detection (CED) algorithm is based on the detection of horizontal gradients and it has been applied in the field of oceanography for the detection of oceanic fronts. The version used in this study is the one proposed by Nieto et al. (2012) adapted from Oram et al. (2008) The algorithm implementation consists of four parts. Fist of all, the images of ocean colour

are smoothed with a Gaussian Filter in order to reduce the noise. Sigma parameter in Gauss Kernel is computed using Oram et al. (2008)equation. Then the gradient image is calculated using a Sobel edge enhancement kernel. The third step is based on the "non-maximal suppression" theorem, that it is based on the suppression of pixels that are not considered as edges, in order to avoid the detection of false edges. Lastly, a dual-threshold algorithm is adopted to detect and connect edges. These edges are determined by supressing all edges that are not connected to a very strong edge.

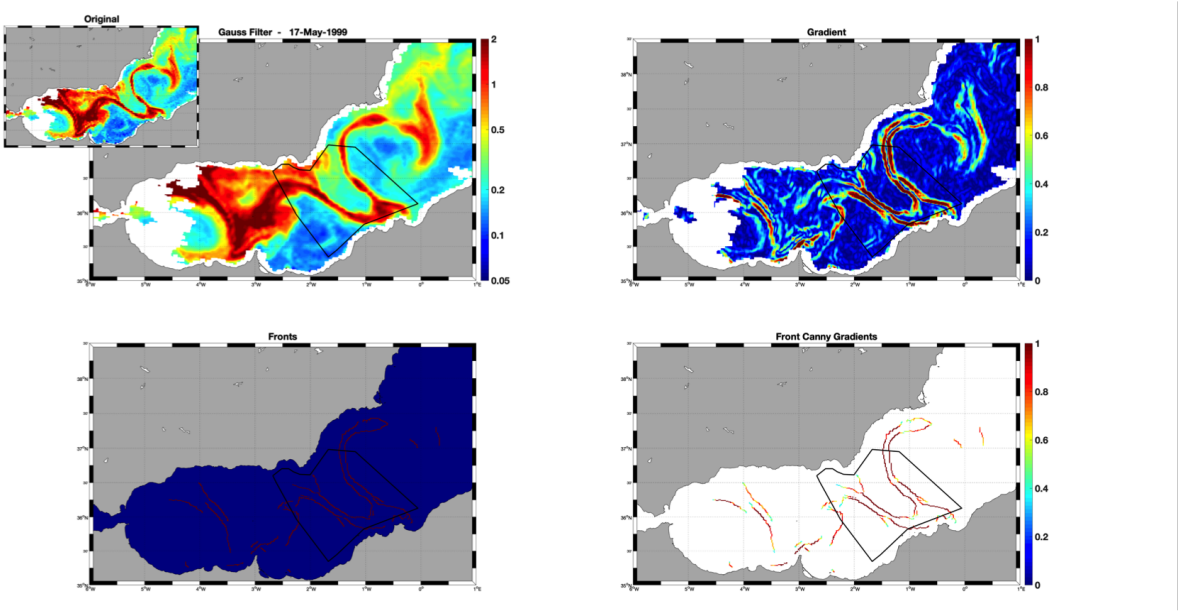

Figure 1: Daily example (17, May 1999). Ocean Colour image with 5 x 5 kernel and a sigma value (σ) of 3.

Following the proposed workflow, it has been possible to adapt the algorithm to the study zone and make preliminary assumptions about variability of the Almeria-Oran front.

Acknowledgments

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Study of Submesoscale processes in Cape Trafalgar

Marina Bolado-Penagos[1,2] , Iria Sala[2,3] , Águeda Vázquez[2,4] , Miguel Bruno[1,2]

*Corresponding author: marina.bolado@uca.es
[1] Departamento de Física Aplicada, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Puerto Real, 11510 Cádiz, Spain
[2] Instituto Universitario de Investigaciones Marinas (INMAR), Campus de Excelencia Internacional del Mar (CEI-MAR), Universidad de Cádiz, Puerto Real, 11510 Cádiz, Spain
[3] Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Puerto Real, 11510 Cádiz, Spain
[4] Departamento de Física Aplicada, Escuela Superior de Ingeniería, Universidad de Cádiz, Puerto Real, 11510 Cádiz, Spain

Keywords: Cape Trafalgar, Upwelling, Interface, Submesoscale, Winds

Abstract

Attending to satellite imagery, Cape Trafalgar can be distinguished as an area of both high chlorophyll-a concentration and cold sea surface temperature. The presence of a quasi-permanent upwelling off Cape Trafalgar has been previously characterized by the presence of both high nutrient and high chlorophyll concentration (e.g. Rubín et al. (1997); García et al. (2002)). Vargas-Yáñez et al. (2002) described from a bi-dimensional model that the tide-topography interaction is the main reason for the presence of cold upwelled water in the area. Recently, Sala et al. (2018) based their study on chlorophyll-a time series of satellite imagery. They correlated the existence of the upwelling with the spring-neap tides cycle. In the present work the dynamic of the aforementioned upwelling is assessed, as well as the importance and the role of the submesoscale processes in the area. For this purpose, in-situ data and drifter trajectories have been analyzed. Results from current meters moored on the coastal Trafalgar margin and in the centre of the channel (A1 and A2 respectively; Figure 1) along with a thermistors string (TH; Figure 1), show that the interface between Surface Atlantic Water and Eastern North Atlantic Central Water (SAW-ENACW; 17.5°C) is displaced to shallower depths at both phases of the tidal currents (Figure 2).

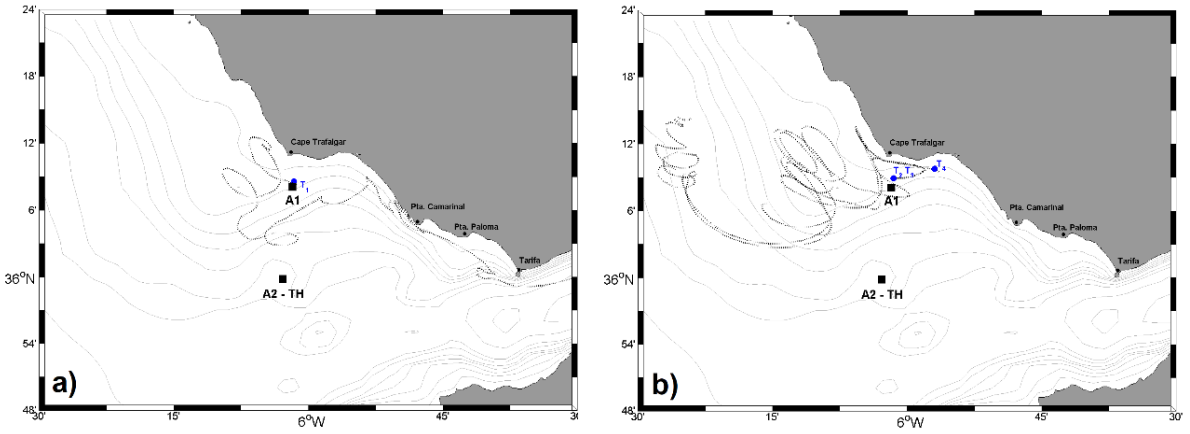

Figure 1: Map showing the location of the study area along with the trajectories (dotted black lines) of the drifters. Black squares show the position of the moored instruments: A1 and A2 current meters and TH thermistors string. Blue dots are the locations where the Lagrangian drifters were released. (a) Trajectory depicted by the drifter T_1 that was released under westerlies over the area, and (b) trajectories of the drifters T_2-T_4. By the time that these drifters were launched, easterly winds blew over the area. Grey contour lines represent the bathymetry of the Strait (20, 40, 80, 100, 150, 200, 400, 600 and 800 m).

Although oscillations are observed throughout the temperature register with a semidiurnal pattern, the highest are depicted during spring tides (i.e. full moon on 28th September 2015). Regarding the tidal phase, the highest oscillations of the interface take place when the flow is displaced eastwards (directed to the Strait of Gibraltar). When the flow is directed westwards (directed to the Gulf of Cadiz) the upraise is less intense than the former. Four Lagrangian drifters were released in the study area (T_{1-4}; Figure 1). The drifters' trajectories indicate two different surface circulation patterns. Only one drifter, which was deployed under westerly winds (T_1; Figure 1a) and during neap tides, was able to be displaced towards the Alboran Sea after being retained in the area for three days. The drifters deployed under easterlies (T_{2-4}; Figure 1b) remained within the Trafalgar area for the entire period. The presence of the quasi-permanent high productivity in this area may be explained from the high residence times depicted from the drifters' trajectories and the regularity of the upwelling showed from the temperature record.

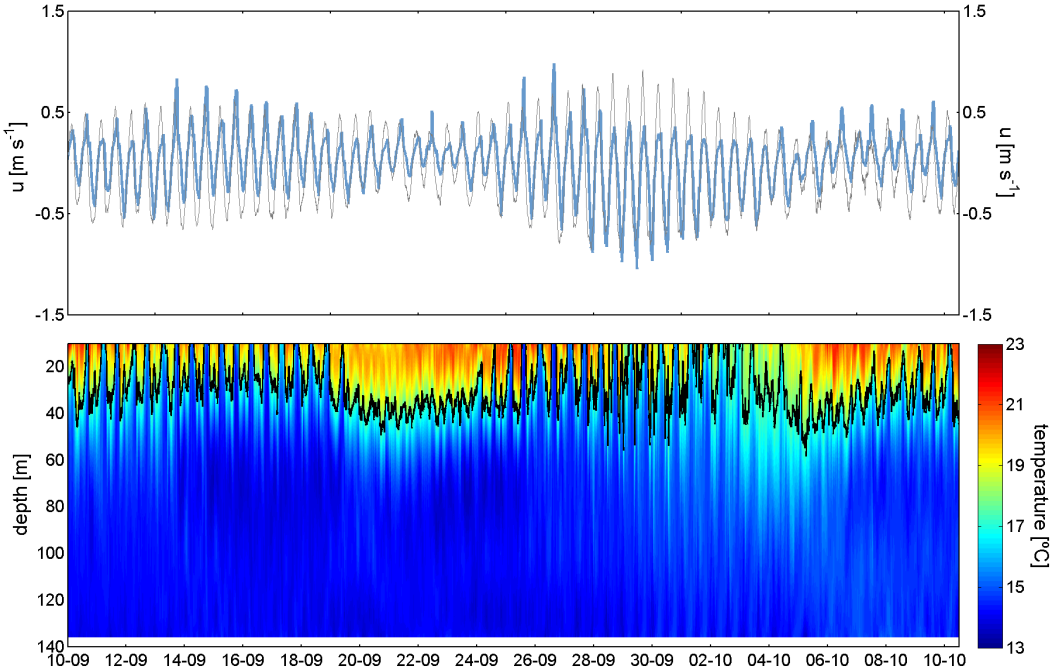

Figure 2: Current and temperature evolution in the Trafalgar area. (a) Barotropic current at A1 (light grey line) and A2 (light blue line). (b) Thermistor string records in the centre channel (TH). The black thick line corresponds with the position of the isotherm 17.5 °C.

Acknowledgments

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Dependence of wind-driven northwest African upwelling on large-scale features in CMIP5 models

Antonio Castaño Tierno*[1] , Elsa Mohino Harris[1] , Belén Rodríguez-Fonseca[1] , Teresa Losada Doval[1]

*Corresponding author: antcasta@ucm.es
[1] Department of Physics of the Earth and Astrophysics, Universidad Complutense de Madrid, 28040, Madrid, Spain. Institute of Geosciences (IGEO), 28040, Madrid, Spain.

Keywords: Coupled models, Upwelling, Biases, Ocean-atmosphere, Tropical Atlantic

Abstract

The Northwest African (NWA) upwelling system is located between 13° North and 35° North, in front of the North African coast (Wang et al., 2015). It is part of the larger Canary Eastern Boundary Upwelling System (sometimes referred to as the North Atlantic Eastern Upwelling System), which extends from Cape Blanche to the northern tip of the Iberian Peninsula. This is a region of great interest for the oceanographic community due to the abundance of fish and marine life derived from the cool and nutrient-rich upwelled water. The exact zone of study stretches from 10° N to 25° N and from 20° W to 15° W. In this work we assess coupled model behaviour in the simulation of NWA upwelling, as well as studying how large-scale biases affect that representation. The present work uses 24 state-of-the-art general circulation coupled models from the Coupled Model Intercomparison Project Phase 5 (CMIP5) to study which are the most salient features of the annual cycle of wind-driven upwelling in the model ensemble, and try to assess how intermodel differences in the representation of large-scale phenomena, such as subtropical anticyclones or Southern Ocean surface radiation budget, affect NWA upwelling simulation. The results point to intermodel variability being dominated by two modes (Figure 1), one centered around 23° North and other located south of 15° North. Intermodel variations of sea level pressure land-sea gradient in Northern Africa are the direct driver of enhanced or diminished upwelling. However, the first mode on intermodel variability seems to be ultimately conditioned by the radiation budget over Northern Africa (Figure 2), while the second mode depends on the position of the Intertropical Convergence Zone (ITCZ), and thus on the interhemispherical energy budget (Mechoso et al., 2016).

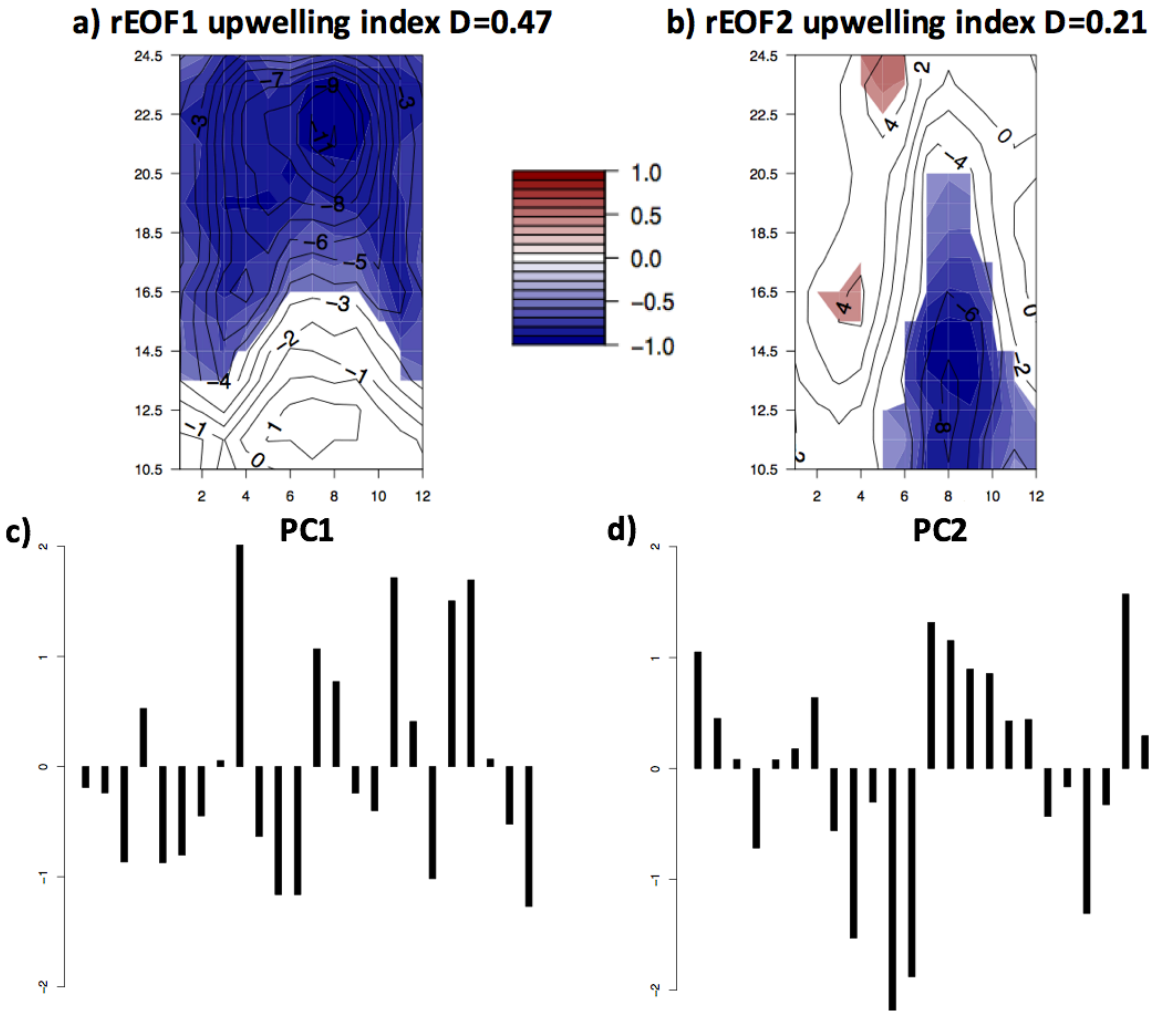

Figure 1: a) EOF1 and b) EOF2 of the intermodel variability of the month-latitude Hovmöllers of the upwelling index. The EOFs are represented as the regression of the standardized PC onto the upwelling index. The correlation between the PC and the intermodel variability in each point of the Hovmöller is calculated and shading areas represent the correlation in those regions and months in which they are significant at the 90% level. The fraction of variance explained by each mode is indicated at the top of each panel (D). c) PC1 and d) PC2 standardized scores for the upwelling index.

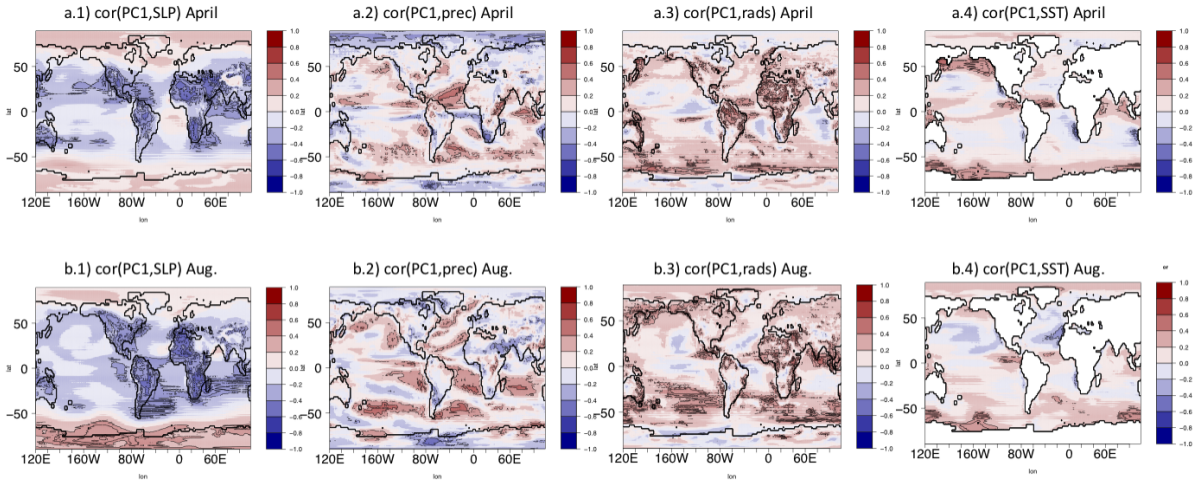

Figure 2: Top row: intermodel correlation between upwelling index (PC1 and climatological mean for April of SLP (a.1), precipitation (a.2), net radiation at the surface (a.3) and SST (a.4). Bottom row: intermodel correlation between upwelling index PC1 and climatological mean for August of SLP (b.1), precipitation (b.2), net radiation at the surface (b.3) and SST (a.4). Correlations significant at the 90% level (Student t-test) are marked in contours.

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Life and death on Tagoro submarine volcano (Canary Islands, Spain): eight years monitoring the nutrient release, oxygen levels, and fluorescence anomalies

Alba González-Vega*[1] , Eugenio Fraile-Nuez[1] , J. Magdalena Santana-Casiano[2] , Jesús M. Arrieta[1]

*Corresponding author: alba.gonzalez119@alu.ulpgc.es
[1] Instituto Español de Oceanografía, Centro Oceanográfico de Canarias, Santa Cruz de Tenerife, Spain
[2] Instituto de Oceanografía y Cambio Global, Las Palmas de Gran Canaria, Spain

Keywords: Dissolved inorganic nutrients, Dissolved oxygen, Hydrothermal vents, Natural fertilization, Submarine volcano

Abstract

The submarine volcano Tagoro erupted south of El Hierro island in October 2011 and has been intensely monitored during the last 8 years. The initial eruptive stage (first six months) produced abrupt physical-chemical perturbations in a wide area south and north of the island, including a marked acidification and severe deoxygenation, even reaching anoxic levels, which resulted in high mortality among the local biota. After March 2012, the eruptive process evolved into a low-temperature hydrothermal system with diffusive emission of heat, gases, metals, bioavailable iron, and inorganic nutrients. These emissions, which exhibit a stationary cyclic behavior, still produce important physical-chemical anomalies in the water column over the main and secondary craters. Here, we present a detailed study of the emissions of inorganic macronutrients (nitrate+nitrite, phosphate, and silicate) comprising a dataset of over 3300 discrete water samples collected through three different methodologies, as well as oxygen and fluorescence data from over 600 vertical profiles and 130 tow-yo transects. Our results show a significant nutrient enrichment throughout the whole studied period (Figure 1), up to 781% (nitrate), 296% (phosphate) and 1530% (silicate) in the water column, and larger enrichments of phosphate (946%) and silicate (32440%) in the samples collected directly from the vents. Additionally, positive oxygen and fluorescence anomalies were observed in the waters surrounding the main craters, suggesting a possible effect of these nutrient-rich emissions on the local marine biota.

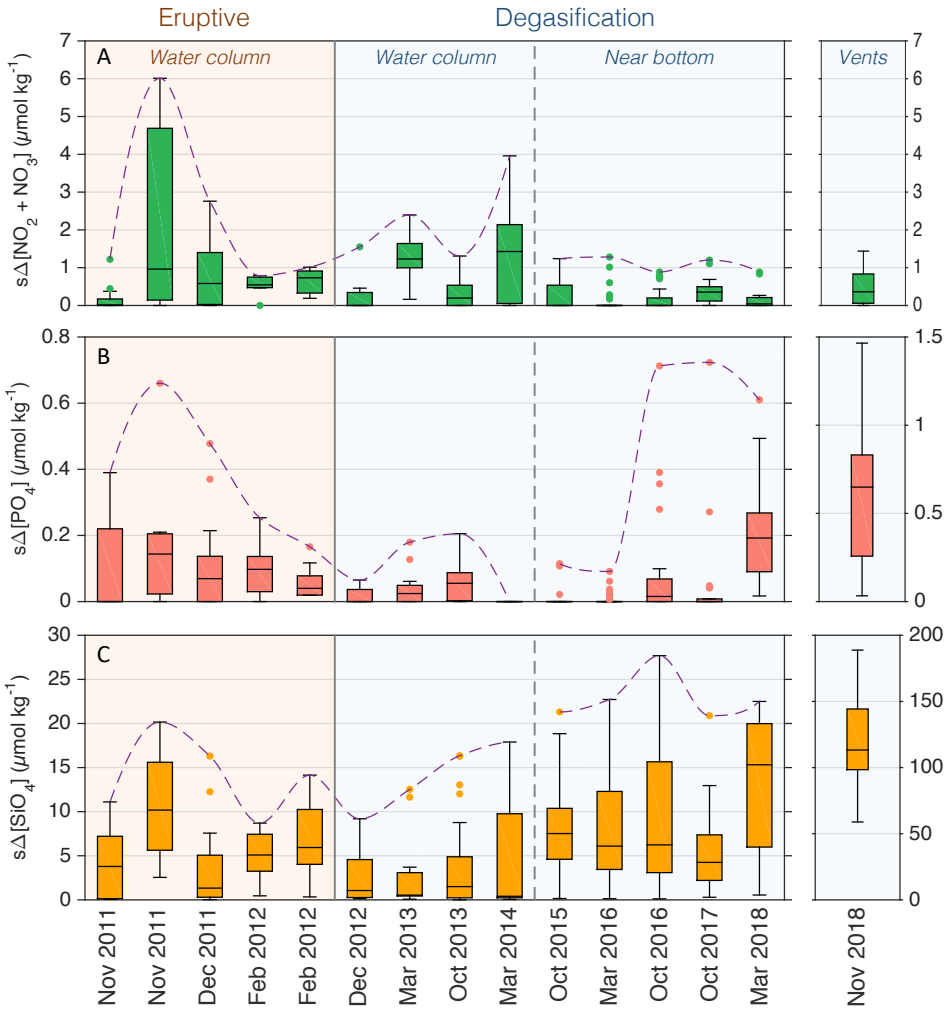

Figure 1: Significant anomalies of a) nitrate, b) phosphate, and c) silicate for each cruise. Boxplots show the median, quartiles, and outliers of the datasets. The dashed line shows the maxima. Volcanic stage (eruptive / degasification) and sample type according to the sampling methodology (water column / near bottom / vents) are specified.

Postglacial transgressive deposit associated to the retreating Guadiana river mouth under rapid sea-level changes

Álvaro Carrion-Torrente*[1] , Francisco J. Lobo[1] , Ángel Puga-Bernabeu[2] , Isabel Mendes[3] , María Isabel Reguera[4]

*Corresponding author: alvct@iact.ugr-csic.es

[1] Instituto Andaluz de Ciencias de la Tierra, CSIC-Universidad de Granada, Avenida de las Palmeras 4, 18100 Armilla, Granada, Spain

[2] Departamento de Estratigrafía y Paleontología, Universidad de Granada, 18002 Granada, Spain.

[3] CIMA-Centro de Investigaçao Marinha e Ambiental, Universidade do Algarve

[4] Instituto Geológico y Minero de España, C/ Ríos Rosas, 23, 28003 Madrid

Keywords: Sea-level change, Gulf of Cadiz, Transgressive deposits, Transgression, Global change

Introduction

The sedimentary record of transgressive shorelines exhibits a high variability due to the interplay of different controlling factors, such as the amount and type of sediment supply, the intensity of the hydrodynamic regime, varying sea-level rise rates and the influence of previous topography. Two types of transgressions can be defined according to the balance between sediment supply conditions and the intensity of ravinement in the shallow-water environment: erosional and depositional (Swift, 1968). According to the gradients of preexisting surfaces and the pattern of sea-level rise, transgressions may operate continuously due to the retreat of the shoreface, or instead be punctuated by periods of sediment accretion alternating with the drowning of coastal deposits (Cattaneo and Steel, 2003). As a consequence, a large number of transgressive architectures may be generated; the most complete case includes lower coastal and fluvial deposits and upper marine deposits separated by the ravinement (Saito, 1994). The postglacial sea-level rise provided ideal conditions to study the sedimentary response to flooded shelves, due to the alternation of periods with different rates of sea-level rise. In particular, two major periods of enhanced sea-level rise were associated to meltwater pulses due to exchange of water between ice sheets and ocean, and were punctuated by periods of slower sea-level rise such as the Younger Dryas interval (Stanford et al., 2011; Lambeck et al., 2014). Over most continental shelves, retreating shorelines have been dominated by erosional processes with very effective ravinement that generated well-marked erosional surfaces (Nordfjord et al., 2009) and/or coarse-grained lags (Lantzsch et al., 2010). In such settings, few transgressive prograding deposits have been observed, such as in wide portions of the California shelf (Grossman et al., 2006), the New Jersey shelf (Nordfjord et al., 2009) or the Galizia shelf (Lantzsch et al., 2010). Only in settings with continuous sediment supply the postglacial transgressive sedimentary record preserves an expanded record of backstepping deposits (Cattaneo and Steel, 2003). These conditions have been documented in several Mediterranean settings, such as the Gulf of Lions (Gensous and Tesson, 2003) or the Adriatic Sea (Maselli et al., 2011), where transgressive deposits comprise up to four parasequences. The development of such depositional units is thought to be driven by periods of decelerated sea-level rise in which enhanced sediment fluxes occurred (Gensous and Tesson, 2003; Maselli et al., 2011). The Gulf of Cadiz shelf contains a widespread record of the postglacial transgression. In particular, off the Guadiana River a limited grid of high-resolution seismic

profiles enabled the distinction of a set of backstepping deposits overlying the widespread erosional surface that formed during the Last Glacial Maximum (Lobo et al., 2001). In this contribution, we combine a substantial amount of additional data in order to define the stratigraphic architecture of postglacial deposits and to place them in the context of postglacial high-frequency climatic and glacio-eustatic fluctuations. The characterization of this deposit will contribute to understand the response of coastal systems to the increase rates of sea-level rise (Figure 1).

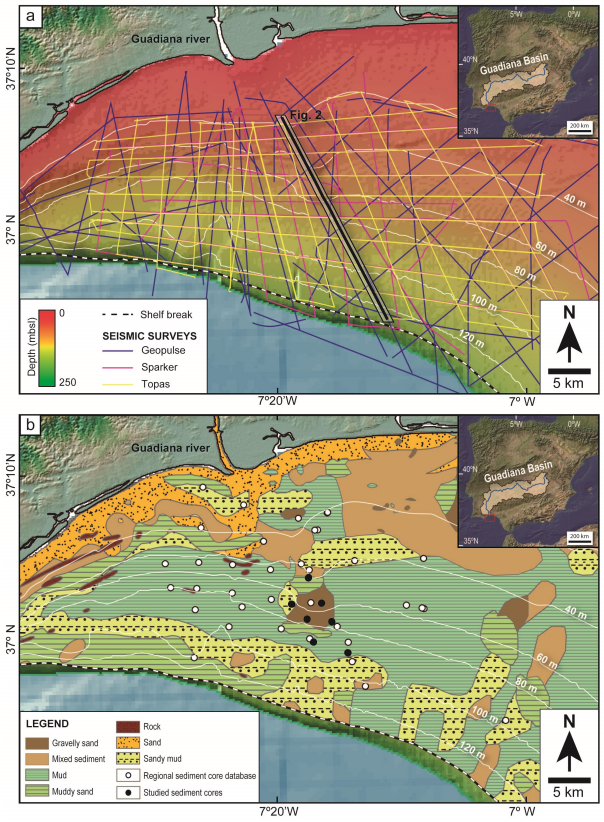

Figure 1: Seismic surveys database and bathimetry of the study area. b: Geological setting of the Guadiana river mouth and sedimentological database

Objectives and methods

The main objectives of this work are:

1. to characterize the distribution, morphology and composition of transgressive wedges
2. to integrate the formation of these deposits in the corresponding chronostratigraphic framework
3. to establish the relative importance of these units and their implications in relation to global change

To reach the proposed goals, two different databases will be used:

1. Seismic database, composed of a high-density grid of seismic profiles with diverse resolutions, collected in several oceanographic surveys.
2. Sedimentological database, composed of sediment cores collected in 45 different sites in the shelf off and around the Guadiana River.

Seismic profiles were interpreted following seismic stratigraphy concepts. Macroscopic properties of sediment cores were described, including sedimentary facies and contacts; in addition, several 14C datings provided the chronostratigraphic constrains for placing transgressive deposits in an evolutionary context.

Results, discussion and conclusions

The studied deposits comprise four main units (U2, U3, U4, U5), based on the seismic facies and the occurrence of seismic discontinuities. These units show wedge shape geometry, with about 10-15 m of maximum thickness and are arranged in a well-marked retrograding stacking pattern. Each unit can be also divided in two or more subunits according to their internal seismic facies. The lower part is composed of very gently clinoforms dipping southwestward (<0.5°). The upper boundaries of these clinoforms are highly eroded and covered by sheet-like subunits that show transparent to chaotic seismic facies with some weak internal reflections (Figure 2).
The sedimentary facies in each sub-unit exhibits a consistent pattern. The lower sub-units are dominated by fine-grained sands with intercalated silty layers. The upper sub-units are characterized by reworked facies composed of very fragmented granule- to pebble-sized bioclasts within a silty sand matrix (Figure 2a-c).

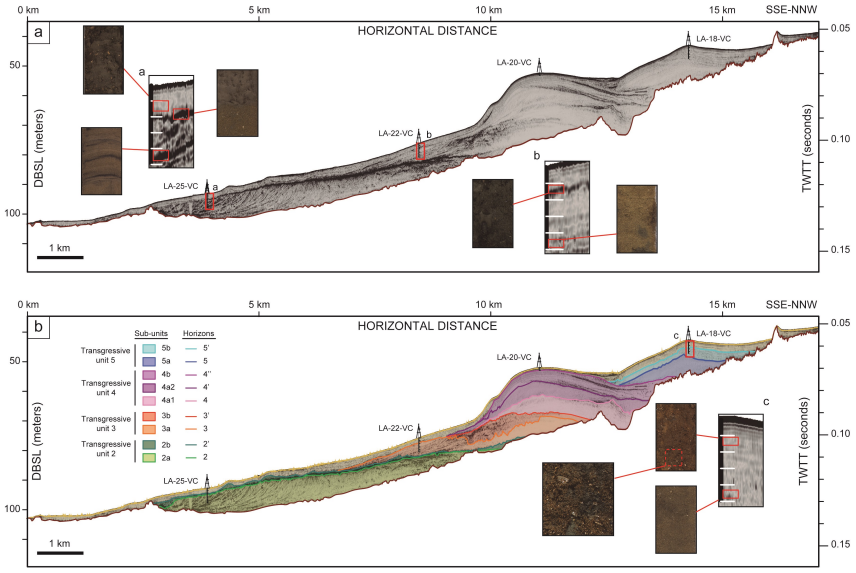

Figure 2: Seismic line of the trangressive unit (up) and its interpretation (down). a-c: detail of the sediment cores for each unit.

The internal structure of each wedge-shaped deposit reveals two major phases of development. The first phase was associated to the development of shallow-water, coarse-grained prodeltas. The occurrence of a net erosional boundary and the seismic and sedimentological characteristics indicate that the upper sub-units resulted from the reworking of the original clinoforms. Therefore, each deposit would be composed of both depositional and erosional elements. Under this interpretation, the boundaries between the lower clinoforms and the upper chaotic facies would be interpreted as ravinement surfaces. The preservation of both elements is relatively rare, as in other areas with similar development of transgressive parasequences, the occurrence of reworking facies is poorly documented (Gensous and Tesson, 2003; Maselli et al., 2011). The sedimentary architecture of the deposits resulted from the combination of relatively significant sediment fluxes and an energetic oceanographic regime, in contrast to most Mediterranean settings where hydrodynamic conditions are of lower energy. Age datings indicate a postglacial development driven by the

overall sea-level rise. Preliminary correlation with a suite of sea-level curves indicates that the formation of transgressive parasequence is framed by the 14-8 ka interval, when most of the units seem to be related to phases of enhanced sea-level rise mostly driven by meltwater pulses rather than by periods of reduced sea-level rise such as the Younger Dryas event. These correlations suggest that pulses of enhanced sediment fluxes are not necessarily coupled with phases of slow sea-level rise (Figure 3).

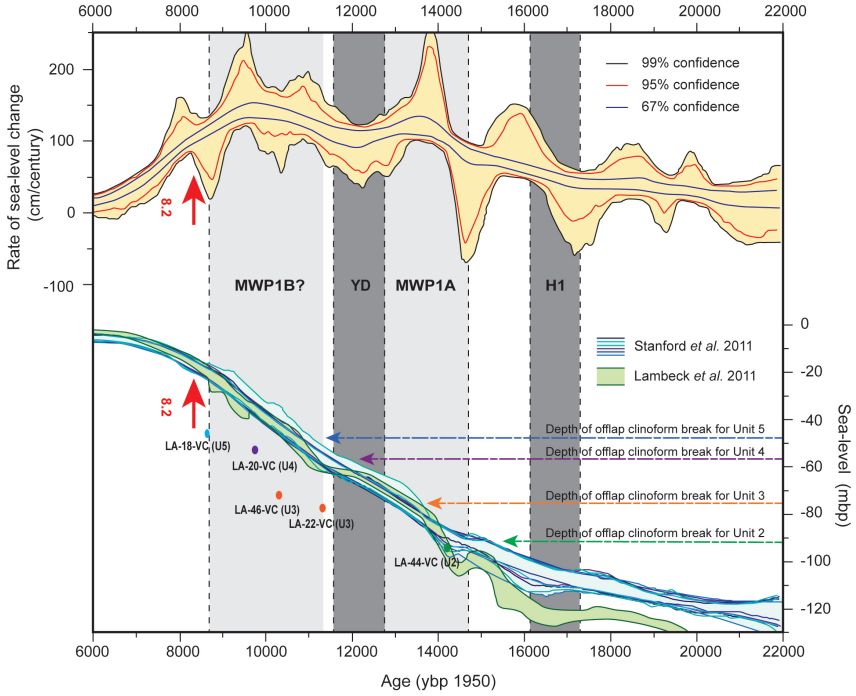

Figure 3: Datations of transgressive units related to the sea-level curve from the last 22 ky to 6 ky and the rate of sea-level change for this time.

Acknowledgments

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Geometry, geomorphology and stratigraphic architecture of subaqueous storm fans in the Galician margin

Natalia Martínez-Carrreño*[1] , Soledad García-Gil[2] , Víctor Cartelle[3]

*Corresponding author: natalia.martinez@ieo.es
[1] Instituto Español de Oceanografía, Centro Oceanográfico de Málaga, Spain
[2] Marine Geosciences Department, University of Vigo, Spain
[3] School of Earth and Environment, University of Leeds, UK

Keywords: Holocene, Sea-level change, Storm events, Subaqueous clinoforms

Introduction

The term clinoform is used to describe dipping, chronostratigraphic strata surfaces, observed from coastal to deeper areas at different scales – from tens to hundreds of meters (e.g., Patruno et al. (2015)). Clinoforms can have basinward-accreting (e.g. Patruno et al. (2015)) or landward-accreting slopes; being the last associated to infilling of lagoons from the back-barrier side (e.g. May et al. (2017)) or by advancing flood-tidal deltas (e.g. Allen and Posamentier (1993)). While landward-accreting clinoforms are built in shallow coastal or lagoonal zones, basinward-dipping ones are ubiquitous and appear from shallow, basin-margin areas, to deeper water areas. The Galician coast (NW Iberia) is characterised by the presence of several prolonged inlets along the shore, called rias. The Rías Baixas, located on the southwest margin of Galicia, have a characteristic funnel shape, with central axes depths ranging from ca. 40-70 m in the outer zone to 5-10 m in the innermost areas. The bathymetric map reveals a seafloor relief characterised by the presence of several rocky outcrops and islands. The presence of these islands has been an important factor of control in the genesis and preservation of some landward prograding sedimentary bodies whose singularities made them different from the landward-accreting clinoforms described until now. In this study, we combine geophysical data and vibrocores to map and study the origin, depositional conditions and evolution of three subaqueous prograding sedimentary bodies located in the Galician margin.

Materials and Methods

The present study is a compilation of geophysical data (multibeam and high-resolution seismic profiles) from different oceanographic surveys carried out in the northwestern margin of the Iberian Peninsula from the 1991 to 2015. Seismic data were acquired using a 'modified Boomer', which consists of a single Boomer source (AAE CSP 300) and two receivers (a ORE 3.5 kHz sub-bottom profiler and a AAE 8 single-channel streamer). Multibeam data from the Ría de Vigo were acquired with a multibeam echosounder (Reson 8125); while multibeam data from the rias of Potevedra and Arousa were provided by the General Secretary of Fisheries (Spanish Ministry of Agriculture, Fisheries and Food). In all surveys, a differential global positioning system (dGPS) was used for navigation and positioning, recording geographical information with HighPack® navigation software. In those cases, were high-resolution seismic records were available in a digital format, the data were integrated into IHS Kingdom Suite® (IHS Markit Limited) for visualization and interpretation. Four vibrocores were retrieved from the sedimentary bodies

located at the mouth of the rias of Vigo and Pontevedra. The vibrocorer consisted of a 4-m-long, 9-cm-diameter steel core barrel. All cores were subsequently analysed in the laboratory.

Results and Discussion

Three subaqueous prograding sedimentary bodies have been identified in the western Galician margin (Rías Baixas). These three sedimentary features appear associated to the presence of ridges or basement outcrops, which gave place to sheltered areas suitable for the genesis and preservation of these sedimentary bodies. They have total areas higher than 1 km^2 and are placed between the isobaths of 6 and 49 m. Their morphology in plan view is that of a symmetric fan. The axial lengths vary between 1269 and 2391 m (Table 1), while their maximum thickness are slightly higher than 20 m. Other relevant parameters to characterize these clinoforms are included in Table 1.

Table 1: Parameters used to characterize the clinoforms

Feature			Vigo	Pontevedra	Arousa
Total relief (H)	Height (m)	Hh	33.40	48.80	40.00
	Down-dipextent (m)	Hd	1952.00	2391.00	1269.50
Inflection zone slope gradient (º)		Is	22.00	1.50	13.08
Inflection point elevation (over toe point)		Ih	6.48	12.80	10.75
Foreset	Height (m)	Fh	18.00	24.11	19.00
	Down-dip extent (m)	Fd	190.00	1089.00	127.00
	Slope gradient (º)	Fs	16.00	1.21	6.07
Inner bottomset	Height (m)	Bh	2.01	0.94	1.78
	Down-dip extent (m)	Bd	126.00	200.40	136.00
	Slope gradient (º)	Bs	1.04	0.34	0.81
Outer topset	Height (m)	Th	2.38	6.41	4.32
	Down-dip extent (m)	Td	608.46	584.50	477.72
	Slope gradient (º)	Ts	0.44	0.63	0.64
Shape ratio (h/H; non-dim.)		h/H	0.19	0.26	0.27
Fan area (km^2)		A	1.79	5.01	1.08
Maximum thickness (m)		Mt	23.20	21.10	22.40
Net-to-gross (%sand + pebble)		N-g	61-97	74-91	71-94
Age (Kyr)		Age	∼8.2	∼8.2	∼8.2
Clinoform progradation rate (km/kyr)		P	0.093	0.108	
Clinoform trajectory (º)		CT	+0.4	+0.54	+0.31
Water depth of rollover point (m)		Wd	13.50	24.18	19.20

The position of the prograding clinoforms at the mouth of each of the Rías Baixas, and the progradation direction (i.e., toward the rias instead seaward) suggest that the sediment comes from the shelf, and is transported into the ria by wave action during storm events (Martínez-Carreño et al., 2017) The three subaqueous prograding sedimentary bodies are deposited directly over the basement (granitic or metamorphic) in its proximal zone and overlying older sedimentary units (of late Pleistocene age) in its distal part. The seismic analysis allowed to identify five clinothems of variable thickness and geometry conforming the internal architecture of those prograding sedimentary bodies. These clinothems, characterized by the presence of internal reflectors dipping towards the ria, are deposited over an older (∼9.4-8.2 kyr BP) and more extensive unit, interpreted as a shoreface deposit. The development of the five clinothems described in these study was associated with the sea level rise occurred during the Holocene. In general, basinward-accreting clinoforms result from the interaction of sediment supply, accommodation space and sediment dispersion by the action of waves and currents (e.g. Anell and Midtkandal (2017)), and therefore are sensible to eustatic sea-level changes. The analysis of the internal configuration of the sedimentary bodies at the rias mouths shows differences in the clinothem

geometry (sigmoidal *vs* oblique) and the clinoform trajectory, which may be related to differences in the accommodation space and sediment influx in each ria during the sea level rise. The comparison of these landward prograding sedimentary bodies with other landward-accreting features, such as washover fans, reveals great differences relative to their area, thickness and genesis, indicating that they correspond to different sedimentary processes. In fact, parameters such as time scale, sedimentation rates, foreset down-dip extent and clinoform trajectories, obtained from these clinoforms are similar to those measured by Patruno et al. (2015) to characterize subaqueous delta clinoforms ($\leq$ 20 kyr). However, critical differences in the forcing mechanisms and the direction of progradation (i.e. landward instead seaward) lead us to consider that the sedimentary bodies described in the present study represent a new type of subaqueous clinoform; therefore, we propose the term subaqueous storm fans.

Acknowledgments

The authors wish to thank the Spanish General Secretary of Fisheries for allowing us to to use the multibeam data. Thanks are also due to IHS for providing the Kingdom Software Suite® free of charge. This work was funded by the Spanish Ministry of Economy and Competitiveness and the European Regional Development Fund (project ECOMER, ref. CGL2012-33584) and by the Xunta de Galicia (project GRC2015/020).

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Magnetic signals at Easter Island during the 2010 and 2015 Chilean tsunamis compared with numerical models

Carlos Torres Echeverría*[1] , Ignacia Calisto Burgos[1] , Dante Figueroa Martínez[1]

*Corresponding author: catorrese@udec.cl
[1] Universidad de Concepción, Departamento de Geofísica, Avda. Esteban Iturra s/n - Barrio Universitario. Concepción, Chile

Keywords: COMCOT, Easter Island, INTERMAGNET, Magnetic field, Tsunamis

Introduction

As the electrically-conductive seawater flows through the Earth's main magnetic field, the interaction of the moving salt ions with the geomagnetic field induces electric currents within the ocean, which in turn set up small, but detectable, secondary magnetic fields in the range of a few nanotesla (nT). These ocean-generated perturbations are observable at land-based geomagnetic stations, on the seafloor, and even at satellite altitudes.
During the 2010 and 2015 Chilean tsunamis, periodic signals of about 1 nT in the vertical component of the geomagnetic field were captured by the Easter Island Observatory (code IPM, according to the geomagnetic observatory network INTERMAGNET), located in Easter Island, Chile, about 3500 km west of Chilean coast. Both tsunamis were produced after the earthquakes in Chile on February 27, 2010, at 06:34 UTC, and September 16, 2015, at 22:55 UTC, respectively. In both cases, the magnetic perturbations were recorded when the tsunami waves passed around Easter Island.
Tsunami-generated magnetic (TGM) signals recorded at a land-based observatory can be produced by electric currents not only within the ocean but also in the ionosphere, the latter generated via the Tsunami-Atmosphere-Ionosphere (TAI) coupling mechanism. Differences between existing studies regarding the origin of the recorded signals at IPM motivated us to compare magnetic records with computed TGM signals for the two events. Our main objective was to confirm that tsunami-forced electric currents within the sea caused the magnetic field perturbations at Easter Island during the 2010 and 2015 tsunamis. Recognizing the origin of a TGM signal would represent an advance in incorporating this physical mechanism into a warning system.

Methodology

Our methodology involved four main steps:
1. Obtaining the seawater velocities by using the software COrnell Multi-grid COupled Tsunami model (COMCOT), version 1.7. 2. Based on the Ohm's law for a moving conductor, and using the seawater velocities, computation of time series of tsunami-generated electric currents within a rectangular area around the Easter Island. 3. Computation of the TGM fields at IPM by using the Biot-Savart law, which allows to calculate an induced magnetic field at an observation point when the electric current distribution is a known function of position. 4. Comparison between the computed time series and magnetic records observed at IPM. The computed TGM and magnetometer signals were high-pass filtered with a period of 25 min, in order to focus the analysis only on the periods of the tsunami waves instead of those related to after-tsunami

processes, such as resonance phenomena and edge waves along coastlines. The choice of the cutoff period was based on the period content of the sea-level anomaly from a virtual mareograph located 2.5 km from IPM.

Results and Discussion

The computed TGM and recorded signals at IPM for the 2010 and 2015 events are shown in Figures 1 and 2, respectively:

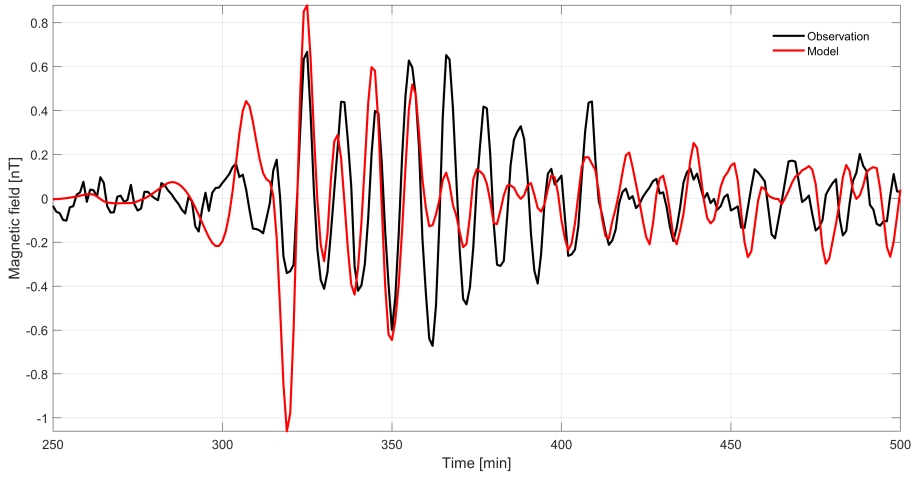

Figure 1: Comparison between the high-pass filtered magnetic field measurement (black line) and simulated TGM field (red line) at IPM for the 2010 Chilean tsunami. Time is measured relative to the earthquake origin time. From Torres et al. (2018).

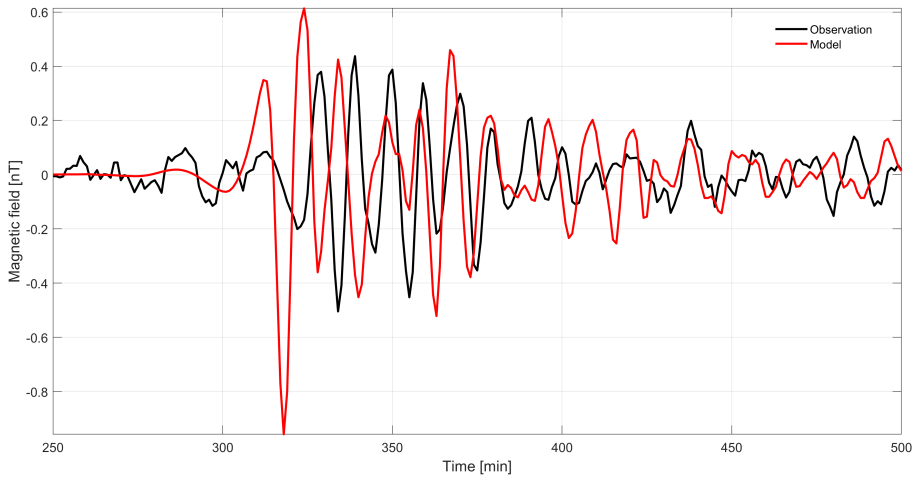

Figure 2: Comparison between the high-pass filtered magnetic field measurement (black line) and simulated TGM field (red line) at IPM for the 2015 Chilean tsunami. Time is measured relative to the earthquake origin time. From Torres et al. (2018).

Our magnetic model can reproduce the main features, but not all, of the observed magnetic fields. We hypothesize that the latter might be caused, in both events, by a combination of oceanic and ionospheric electric currents. The contribution of both effects could even validate the first oscillations of the calculated signals (where the differences are more noticeable), since magnetic disturbances generated via TAI coupling can appear both after and before a tsunami

arrival. These co- and ahead-of- tsunami traveling ionospheric disturbances were proposed by Klausner et al. (2016) using a wavelet analysis, as an explanation for the disturbances in data from ground-based magnetometer observatories -IPM, among them- during the 2015 event. Our results suggest that even a relatively small tsunami (for example, compared with 2010 Mw8.8 Chile and 2011 Mw9.0 Japan tsunamis), such as the one produced by the 2015 Mw8.2 earthquake, induces an observable magnetic perturbation due to the water movement. In the case of the 2010 event, we consider that our results, based on a numerical model, represent an improvement to the work of Klausner et al. (2014), who proposed TAI coupling as responsible for the measurements based on a wavelet transform data-processing technique.
The numerical model used in this work can predict a good part of the trailing waves, at least for the 2010 tsunami. For purposes of warning, not only the tsunami wave front but also the trailing waves are important. When a train of waves approaches a coastal region, the varying topography refracts and traps part of the tsunami energy, triggering unexpected progressive and standing waves on continental shelves and coastal areas even hours after an earthquake, leading to episodes of confusion among the population. For example, the constructive interference of these kind of waves produced devastating effects three hours after the onset of the 2010 earthquake at Talcahuano, Chile, which was located 100 km south of the epicenter. Moreover, tsunami-island interaction causes that wavefronts travel around an island, extending the tsunami menace even to the coastline segments potentially protected from the main tsunami attack. Tsunami-triggered waves could also produce ionospheric disturbances and detectable perturbations in the geomagnetic field.
Especially important for our research group (Chilean Integrated Ocean Observing System, CHIOOS) is monitoring the activity in the complex coastal zone off Concepción, Chile. Since ocean-generated magnetic signals are sensitive to the depth-integrated and conductivity-weighted horizontal velocities, magnetometer data are likely to contain information about temporal variable distributions of salinity and temperature (Petereit et al., 2018). Encouraged by the results obtained in this work and in previous studies, a land-based magnetometer was installed in Bahía de San Vicente, close to Concepción, and its produced data will be presented in future papers.

Acknowledgments

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Photogrammetric techniques to obtain fine scale 3D models of the bedforms from submarine video imagery

Raúl Jiménez Romero*[1] , Desirée Palomino[2] , Luis Miguel Fernández Salas[3] , Yolanda Vila[3]

*Corresponding author: raul.jimenezromero@alum.uca.es
[1] Universidad de Cádiz, Facultad de Ciencias del Mar y Ambientales, Dpto. Ciencias de la Tierra
[2] Instituto Español de Oceanografía, Centro Oceanográfico de Málaga
[3] Instituto Español de Oceanografía, Centro Oceanográfico de Cádiz

Keywords: Bedforms, Fine scale, Photogrammetry, 3D Models, Gulf of Cádiz

Introduction

On deep marine environments, bottom currents generate different fine-scale (cm) sedimentary bedforms generally associated to large scale (km) morphologies (Friedman et al., 2013). The fine-scale bedforms have been poorly studied due to the great difficulty of obtaining accurate information that allows for quantitative measurements at great depth. Increasingly the use of OTVs (Observation Towed Vehicles) or ROVs (Remote Operate Vehicles) to obtain photographs and videos of the marine seabottom is frequent. Since the beginning of this century, the use of photogrammetric techniques in a variety of marine scientific disciplines has been enhanced (Gerdes et al., 2019; Lyons et al., 2002). These techniques not only allow the accurate analysis of the seabed on a fine-scale, they also allow the analysis of vertical and flat surfaces in a single model and they can be also applied to the study of marine habitats (Stow et al., 2009). Our goal in this work is to design a workflow that allows to create high resolution 3D models from submarine video images, using photogrammetric techniques. The models must be sufficiently precise to perform quantitative measurements on centimetric scale bedforms.

Workflow

The video images used in this work have been obtained during the surveys ISUNEPCA0615, ISUNEPCA0616 and ISUNEPCA0517, where video transects of the seabed were made between 200 and 700 m depth, using the THT-HORUS photogrammetric sled. This sled is an OTV with two high-definition video recording cameras (1080x1920), two linear laser projectors with a separation of 75 cm and two laser pointers with a separation of 7.5 cm. The acquisition angle of the video images is 45° with the respect to the sea floor. For the construction of the 3D models, frames corresponding to 3 s of video were taken, representative of the complete transect, using the software Blender 2.79b. These frames were exported to Agisoft PhotoScan 1.4.5, which uses the "Structure from Motion" (SfM) technique to perform a photogrammetric reconstruction of the filmed surface. The software allows to generate a dense point cloud in .las format and the corresponding orthophoto in .tif format (Figure 1). With the ArcGIS 10.6.1 software, the .las files of the different photogrammetric reconstructions were processed to create digital terrain models (DTMs). For this goal, we used the interpolation method by the inverse of the weighted distance (IDW) with different cell size, depending on the characteristics of the point cloud (Figure 1). The MDTs generated were scaled using sled lasers and used to perform quantitative surface measurements and generate 3D models.

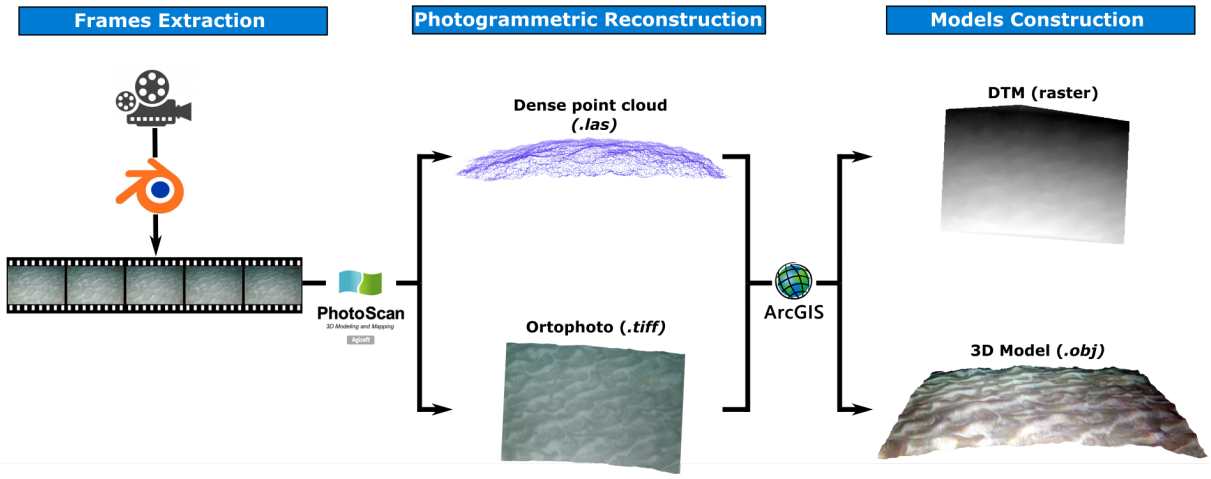

Figure 1: Workflow followed for 3D reconstruction of video imagery.

Quantitative measures

Following the proposed workflow, it has been possible to create DTMs with a resolution of 5 x 5 mm, as well as 3D models of some sections of the video transects (Figure 2). From the DTMs it is possible to obtain derived information through, for example, the slope, the aspect, the topographic roughness index, the curvature, the topographic profiles, etc. All this information allows very detailed geomorphological and morphometric studies of the seabed at a fine-scale.

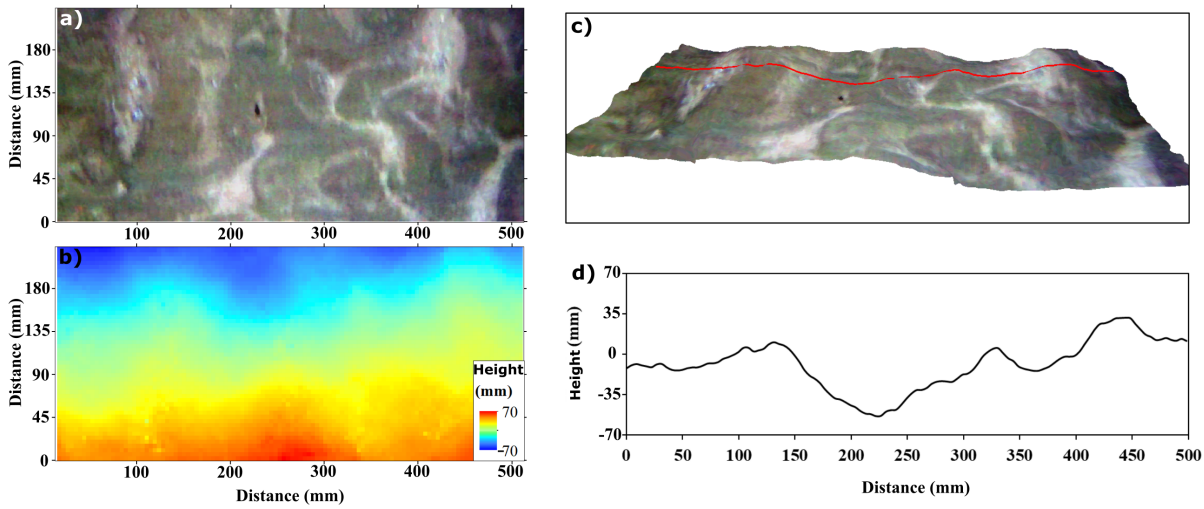

Figure 2: a) Video image of the sandy sea floor where ripples can be observed; b) Digital Terrain Model (DTM) of the video image; c) 3D reconstruction of the video image. The red line indicates the location of the topographic profile shown in (d).

With this methodology it is possible to obtain quantitative information of the video images that otherwise was not possible. They can also benefit from this methodology in the studies of habitats and underwater archaeology, as well as for monitoring the seabed. It is important to take into account that technical improvements can be made in the workflow to obtain more precise models.

Acknowledgments

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Session 5

The Sea Knowledge

Marine Wildlife

Usefulness of semi-automatic image analysis for the assessment of zooplankton community structure in a highly dynamic area of the Alboran Sea (SW Mediterranean).

Nerea Valcárcel-Pérez*[1] , Lidia Yebra[1] , Inma Herrera[1,2] , Jesús M. Mercado [1] , Francisco Gómez-Jakobsen[1] , Soluna Salles[1]

*Corresponding author: nerea.valcarcel@ieo.es
[1] Instituto Español de Oceanografía, C. O. Málaga
[2] Present address: IU - ECOAQUA, Universidad de las Palmas de Gran Canaria

Keywords: Alboran Sea, Biodiversity, Ecotaxa, Image analysis, Zooplankton

Introduction

The Alboran Sea is the westernmost basin in the Mediterranean Sea, which is connected to the Atlantic Ocean by the Strait of Gibraltar. The entrance of the Atlantic Jet through the Strait results in a system of two quasi-permanent anticyclonic gyres dominating the entire basin. This circulation pattern coupled with westerly winds also produces an intermittent upwelling of nutrient-rich Deep Mediterranean Water in the northern coast (Figure 1). Previous studies have shown the strong influence of the hydrodynamics on the planktonic communities, dividing the Mediterranean basin in areas with marked different productivities. In the coastal area, the enrichment in inorganic nutrients produced by the upwelling makes the Alboran Sea one of the most productive areas of the Mediterranean Sea (Mercado et al., 2007; Yebra et al., 2017) whereas the western central basin is dominated by an oligotrophic anticyclonic gyre (WAG). Besides, the frontal area between the Atlantic Jet and the edge of the WAG enhances plankton productivity in open waters (Yebra et al., 2018). In this work we tested the usefulness of semiautomatic image analysis to assess the variability in taxonomic composition and normalized biovolume size spectra (NBSS) of the zooplankton communities associated to those different mesoscale hydrological structures.

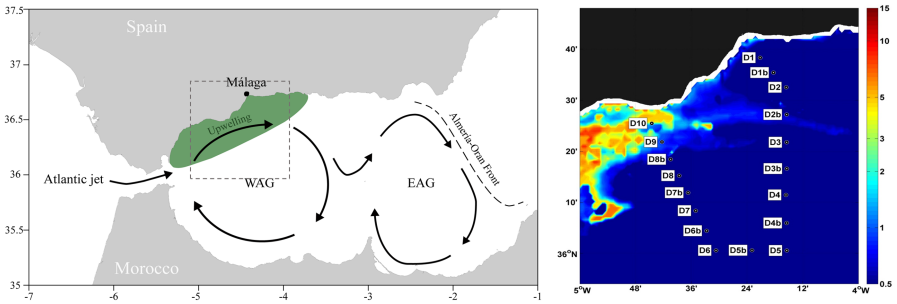

Figure 1: Study area (dashed box), Left: Alboran Sea surface circulation, WAG: western anticyclonic gyre, EAG: eastern anticyclonic gyre, green area: upwelling influence zone; Right: stations location (b stations were only sampled with CTD) and Chl a composite 9–19 July 2014 (mg Chl $\cdot$ m^{-3}).

Methods

Ten stations were sampled in summer 2014 by day and night, on board R/V García del Cid, following a transect perpendicular from the Spanish coast towards the WAG (Figure 1). Zooplankton samples were obtained with double WP2 net (200 μm mesh size) in vertical tows (maximum depth 100m). The samples were fixed with buffered formalin (f.c. 4%) and scanned in aliquots with an Epson Perfection v850 scanner, in glass trays at 2400dpi. The images were processed with Zooprocess software developed for Zooscan (https://sites.google.com/view/piqv). Resulting vignettes and associated data were imported to Ecotaxa (Picheral et al., 2017). We used an initial selection of 4,069 vignettes to create our initial training set for the identification of images in the study area. We classified all the vignettes resulting from the image processing, including zooplankton and some large phytoplankton taxa, detritus and artifacts (scratches, shadows and borders of the scanning tray). Several cycles of prediction and validation over the whole set of samples were carried out until achieving an adequate precision in relation to the abundance of each group.

Group	Precision	Recall
Bacillariophyceae	72.3 (*)	81.5
Dinoflagellata	-	-
Ceratium	85.4	100
Noctilucales	99.4	92.1
Pyrocystales	96.6	65.1
Appendicularia	66	82.4
Copepoda	-	-
Calanoida	73.9	78.8
Oncaeidae	62.6	64.7
Oithonidae	27.4 (*)	65.5
Harpacticoida	100 (*)	2.7
Corycaeidae	0 (*)	0
Poecilostomatoida	0 (*)	0
Copepoda (undet.)	0 (*)	0
Copepoda nauplii	100	2.8
Cladocera	-	-
Evadne	61.4	90.2
Penilia	86.1	76.7
Podon	1.9 (*)	3.2
Cladocera (undet.)	7.1 (*)	3.2
Doliolida	90.6	91.7
Chaetognatha	30.5	81.2
Cirripedia	0 (*)	0
Cnidaria	18.1	28.3
Decapoda	0 (*)	0
Echinodermata	0 (*)	0
Egg (others)	16.7	2.4
Euphausiacea	3.3 (*)	17.4
Fish	-	-
Fish larvae	18.5 (*)	21.7
Fish egg	46.4 (*)	46.4
Foraminifera	30.9	79.2
Mollusca	-	-
Bivalvia	16.8 (*)	24.1
Limacinidae	63	11.9
Ostracoda	100	5.3
Polychaeta	0 (*)	0
Bryozoa	0 (*)	0
Radiolaria	16.5 (*)	47.1
Siphonophorae	2.8 (*)	2.1
Tintinnida	0 (*)	0
Others (undet.)	0 (*)	0
Detritus	96.7	68.3
Artefact	99.2	86.5
Multiple	77.8	93.3

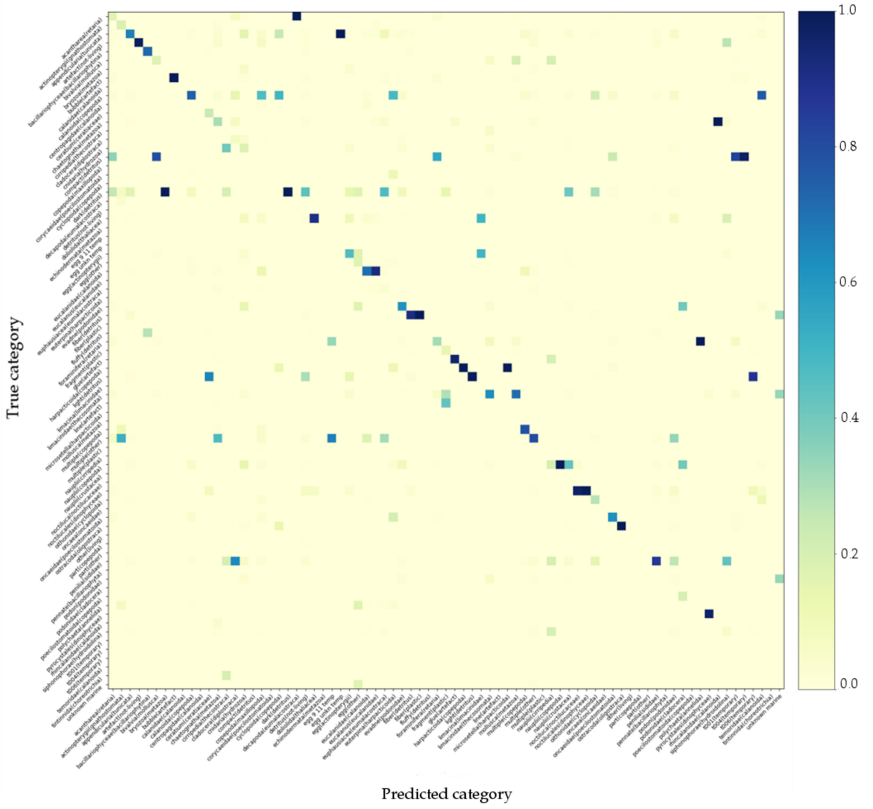

Figure 2: Accuracy parameters of the Alboran Sea training set. Left: Percentages of precision and recall for each group. Groups marked with (*) had all their vignettes validated. Right: Confusion matrix, the diagonal contains the precision rate. Blank rows represent groups from previous versions of the training set, final groups in the set are listed in the table.

Elliptical biovolume (EBv, mm^3), was calculated from major and minor axis computed by Zooprocess as follows:

$$EBv = \frac{4}{3} \cdot \pi \cdot \left(\frac{Major}{2}\right) \cdot \left(\frac{Minor}{2}\right)^2 \quad (1)$$

To calculate the NBSS, particle abundances were arranged on the x-axis in $\log_2$ size classes (mm^3). The y-values were the sum of the individual EBv for each interval normalized to the length of the size class ($mm^3 \cdot m^{-3} \cdot mm^{-3}$). Finally, the NBSS was calculated from the linear regression between the biomass of each size and the size classes expressed on a logarithmic scale (Blanco et al., 1994).

Results and Discussion

Due to the high productivity of the Alboran Sea, all our samples presented high percentages of detritus (40 to 60% of vignettes in the WAG and coast, respectively), making difficult to achieve high precision in the prediction of some groups (Figure 2). In addition, some shallow samples (D1 and N1) could not be processed, because the amount of detritus and mucus prevented a correct subsampling. Nevertheless, the identification accuracy of the main taxonomic groups ranged from 66.0% (*Appendicularia*) to 90.6% (*Doliolida*). Regarding the total abundance, we identified 95% of the total vignettes and 90% of the zooplankton images with a mean precision of 81.7% ± 15.1 SD (Figure 2). The application of the semi-automated image analysis allowed us to detect differences in the taxonomic composition (data not shown) and variations in the slope of the NBSS among the coast, the gyre and the Atlantic Jet. We observed steeper slopes in the stations belonging to areas of high productivity compared to the WAG (Figure 3). This is in agreement with the differences expected between eutrophic and oligotrophic areas.

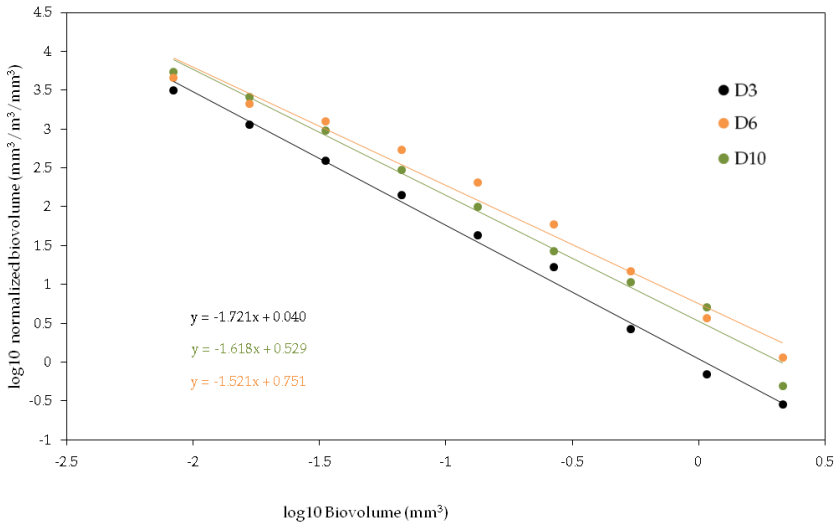

Figure 3: Slopes of the normalized biovolume size spectra at three stations from different productivity areas: coast (D10), WAG (D6) and Atlantic Jet (D3).

Our analyses demonstrate that semi-automated image analysis of plankton is an especially interesting tool for research studies with large spatial and temporal scales, because it allows to obtain results faster than the traditional methods. Despite presenting lower taxonomic resolution compared to microscopy, it is a useful tool to be implemented in ongoing long-term monitoring programs in our study area, which are necessary for the implementation of international initiatives such as MEDPOL and the EU Marine Strategy Framework Directive.

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Associated epifauna to syngnathids populations in the Cíes Islands Archipelago (National Atlantic Islands Park)

Sara Campos Rosende*[1] , Arturo Jiménez Solís[1] , Cristina Piñeiro Corbeira[2] , Miquel Planas Oliver[1]

*Corresponding author: sara94cr@gmail.com
[1] Instituto de Investigaciones Marinas (CSIC), 36208 Vigo, Spain
[2] BIOCOST, Facultade de Ciencias, Universidade da Coruña, A Coruña, Spain.

Keywords: Cíes Islands, Epifauna, Season and site variation, Stable isotopes, Trophic level

Introduction, Material and Methods

Epifauna associated with algae cover plays an important role in marine ecosystems, since it provides food for species that are higher up in the food chain. To characterize the potential prey of the syngnathids and their possible spatio-temporal variations, a study was conducted to characterize the epifauna associated with the main habitat-forming algae species in the Cies Islands. In addition, stable isotope analyses (SIA) were carried out to know the trophic structure of the community. Samples of *Asparagopsis armata*, *Codium* sp. and *Cystoseira baccata* were collected in three different zones (A, B, C) and subzones of the archipelago in spring, summer and autumn for two years (54 samples in total). The individuals collected were quantified and separated by the lowest possible level of identification. PERMANOVA analyses were made using the factors year (fixed), algae species (fixed), season (random, nested in year), zone (random, nested in algae species) and subzone (radom, nested in zone). From each of the groups that were formed, the most representative species were chosen for SIA.

Results

The results obtained showed that the most abundant groups were copepods, gammarids and gastropods. They also showed that the abundances of each group vary depending on the area and season of the year sampled. For example, in the three seasons, the dominant group was copepods, while in spring and autumn, the gastropods dominated on the gammarids, contrary to what was observed in summer. The abundance of the epifauna varies depending on the season and the sampling area, with a decrease in the abundance of species in autumn. On the other hand, the statistical analyses showed no significant differences according to the type of algae collected. Percentages of total macroalgae cover of the sampled transects ranged from 50 and 90%, with a tendency to increase from spring to summer (Ochoa, 2019).

Table 1: Selected OTUS for analysis of stable isotopes and weighted mean values ± SD for ^{15}N, ^{13}C and C/N ratio. The trophic level (TL) calculated from Post's equation (Post, 2002) is indicated.

	TAXA	OTUS	$\delta^{15}N$	$\delta^{13}C$	NT	C/N
FILTER	Bivalvia	*Musculus costulatus*	5,13 ± 0,50	-17,66 ± 1,32	2,00	2,93 ± 0,11
	Bivalvia	*Irus irus*	6,03 ± 0,48	-17,08 ± 1,21	2,26	3,05 ± 0,14
	Bivalvia	Outros Mitilidos	5,98 ± 0,45	-17,25 ± 0,86	2,25	2,97 ± 0,09
GRAZERS	Isopoda	*Cymodoce truncata*	7,03 ± 0,31	-19,28 ± 1,27	2,56	3,84 ± 0,50
	Isopoda	*Dynamene bidentata*	7,01 ± 0,86	-17,09 ± 1,91	2,55	3,56 ± 0,34
	Gastropoda	*Elysia spp*	7,38 ± 0,90	-18,41 ± 3,21	2,66	3,29 ± 0,12
	Gastropoda	*Peringia ulvae*	6,75 ± 0,44	-15,11 ± 1,21	2,48	3,35 ± 0,21
DETRITIVORES	Gastropoda	*Tricolia pullus*	7,36 ± 0,71	-15,27 ± 1,22	2,65	3,00 ± 0,14
	Amphipoda	*Caprella linearis*	6,49 ± 0,38	-17,30 ± 0,54	2,40	2,42 ± 1,61
	Decapoda	*Porcellana platycheles*	7,17 ± 0,32	-17,35 ± 0,88	2,60	2,69 ± 0,27
	Amphipoda	*Amphilochus manudens*	6,44 ± 0,84	-16,14 ± 0,52	2,38	3,73 ± 0,16
	Amphipoda	*Apherusa spp*	6,61 ± 1,00	-15,91 ± 1,08	2,44	4,13 ± 0,28
	Amphipoda	Outros Gammáridos	6,45 ± 0,43	-16,50 ± 1,53	2,39	4,15 ± 0,13
SUSPENSIVOROUS	Decapoda	*Pisidia longicornis*	7,10 ± 0,41	-17,05 ± 0,54	2,58	2,74 ± 0,12
	Copepoda	O. Harpacticoida	6,58 ± 0,50	-19,60 ± 1,48	2,43	3,36 ± 0,05
	Amphipoda	*Corophium spp*	6,32 ± 0,37	-16,40 ± 0,80	2,35	3,89 ± 0,23
	Holothuroidea	*Aslia lefevrei*	7,49 ± 0,55	-11,50 ± 1,20	2,69	4,91 ± 0,50
	Ophiuroidea	*Amphipholis squamata*	7,56 ± 0,67	-19,93 ± 1,06	2,71	2,50 ± 0,12
	Ophiuroidea	*Ophiotrix fragilis*	7,02 ± 0,56	-23,91 ± 3,09	2,55	1,98 ± 0,25
OMNIVOROUS	Gastropoda	*Bittium reticulatum*	7,08 ± 0,57	-16,76 ± 0,65	2,57	3,12 ± 0,16
	Decapoda	*Hippolyte varians*	9,05 ± 0,64	-16,25 ± 0,60	3,15	3,51 ± 0,55
	Amphipoda	*Caprella acanthifera*	6,49 ± 0,27	-16,17 ± 1,14	2,40	4,17 ± 0,32
	Polychaeta	F. Nereididae	7,67 ± 1,27	-21,41 ± 2,96	2,74	3,31 ± 0,19
	Gastropoda	*Calliostoma zizyphinum*	7,87 ± 0,96	-16,22 ± 1,35	2,80	3,01 ± 0,13
PREDATORS	Polychaeta	F. Polynoidae	8,92 ± 0,87	-16, 92 ± 0,82	3,11	3,20 ± 0,29
	Pycnogonida	*Endeis spp*	9,17 ± 0,77	-17,54 ± 0,98	3,19	2,26 ± 0,10
	Platyhelminthes	*Stylochoplana maculata*	7,18 ± 0,59	-16,59 ± 1,22	2,60	3,23 ± 0,11
	Gastropoda	*Dotofragilis*	9,11 ± 1,09	-14,80 ± 0,45	3,17	3,47 ± 0,14
	Mysidacea	*Siriella armata*	10,04 ± 0,41	-16,78 ± 0,63	3,44	3,39 ± 0,07

Taxonomic analysis of the epifauna and the bibliographic information consulted allowed us to establish a trophic structure formed by six groups: filter feeders, grazers, detritivores, suspensivores, omnivores and predators. The values of ^{15}N allowed us to calculate, using Post's equation (Post, 2002) the trophic level of each OTUS (operative taxonomic units) (Table 1). In addition, the isotopic characteristics were used to create a carbon-nitrogen graph (Figure 1), where filter feeders (lower levels of ^{15}N) are found in the lower part of the graph and predators in the upper part (higher levels of ^{15}N). The ANOVA performed for trophic group, season and sampling area for variables ^{15}N and ^{13}C showed that there are only significant differences in the trophic group and sampling season for nitrogen ($p<0.001$). Between sampling zones, no significant differences were found for any of the stable isotopes.

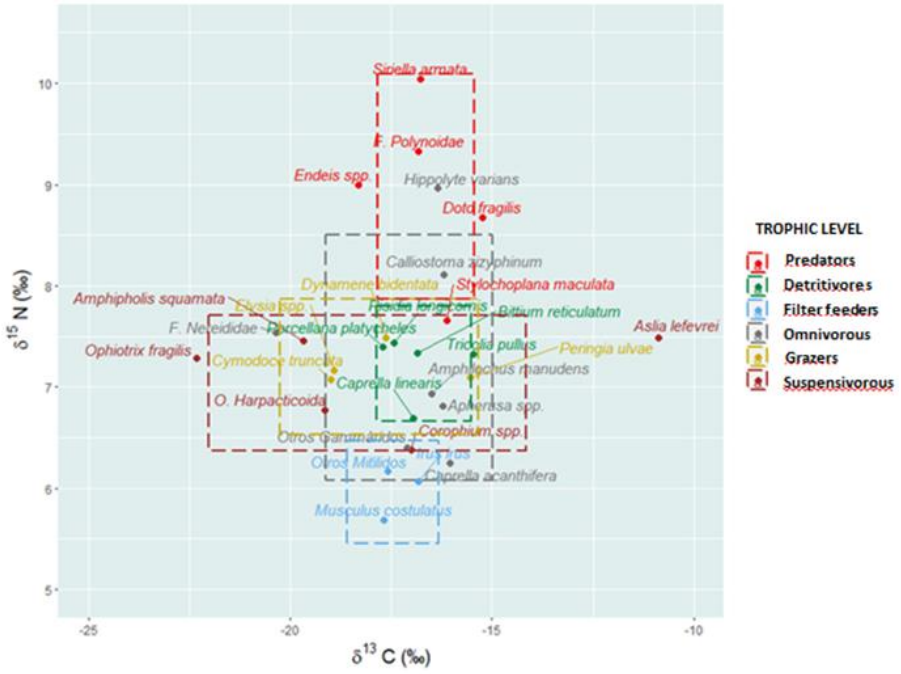

Figure 1: Mean values of ^{15}N and ^{13}C of the OTUS studied. *Musculus costulatus* was used as a baseline. The squares represent the mean and standard deviation of each trophic level.

Discussion

The most important differences were found in autumn, compared to spring-summer. These differences are due to an increase in copepods and a decrease in amphipods in autumn because some copepods species may have the reproduction period on this season (Asencio et al., 1993). On the other hand, the abundance decrease could be due to the algae showing limited growth and tissue loss at this season due to low light intensity, temperature, nutrient availability and adverse environmental conditions. This translates into a lower availability of habitat and food for the associated epifauna (Winkler et al., 2017). On the contrary, summer has the greatest abundance, and may be related to the periods of recruitment and/or reproduction that some species present during this season. Another reason that would explain these results is the upwelling phenomenon that occurs in spring-summer, which brings nutrients that allow the proliferation of planktonic organisms (Alvarez-Salgado et al., 2002). The results of stable isotopes of ^{15}N and ^{13}C allowed to establish the trophic structure of the epifauna and syngnathid community as well as to help characterize its diet. In addition, they also showed variability between species and seasons in the case of ^{15}N. These differences could be due to the trophic position of each taxa, that is, the type of diet of each one. Filter feeders showed the lowest level of ^{15}N since it is the first trophic chain step (used as a baseline in this study), while the predator group constituted the highest level. The rest of the groups formed the intermediate trophic levels, with different types of overlap, indicating the great food diversification of the primary consumers. On the other hand, seasonal differences could be related to the fluctuation that species may suffer over time; species of different trophic levels may appear or disappear. After these aspects studied, the ecological interest of the Cíes Islands is evident due to the great biodiversity that lives in these areas and that is constituted by a great variety of taxonomic groups.

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Differential space distribution of the *Balaenoptera* genus at the east tropical Atlantic Ocean

Maria del Camino García Ramos*[1] , José Carlos Báez Barrionuevo[2]

*Corresponding author: camino.garcia.ramos@gmail.com
[1] Universidade da Coruña
[2] IEO Málaga

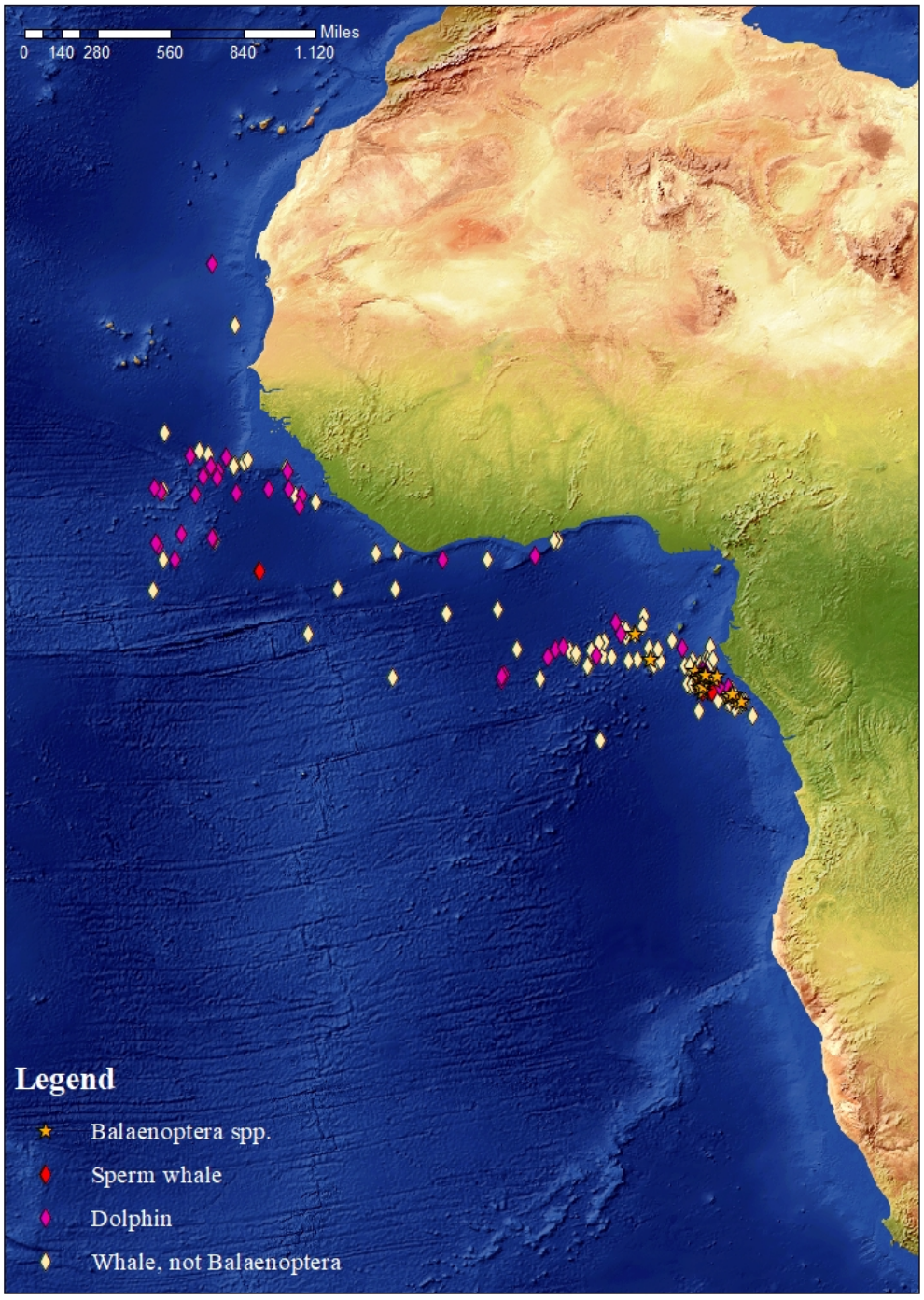
Keywords: Balaenoptera spp. habitat, Rorquals distribution, Balaenoptera-Atlantic Ocean, Whales- Area 34, SST and V-component

Introduction

Cetaceans are the group that has obtained more modifications, and over the years they have been persecuted by humans because of the great economic importance they possess (Fernández-Casado, 2000) in particular the biggest genres among which is *Balaenoptera* Lacépède, 1804. At present, *Balaenoptera* populations are protected by the International Whaling Commission (IWC), but in many cases their location and status is unknown. In previous works with data from opportunistic sightings (Fernández et al., 2018) the differential presence of a species has been used in comparison with the rest of the sightings to determine “preferential" habitats.this reason, the objective of this project is to improve knowledge of the spatial distribution of *Balaenoptera* spp. using low-cost techniques, such as the opportunistic sighting from purse seiners in tropical Atlantic Ocean areas.

Material and methods

The Spanish Institute of Oceanography (IEO) observers program on board commercial purse seine vessels follows a scientific programme, under the EU Data Collection Framework (Figure 1). The main aim of observer program is obtaining direct information on bycatch species, as well as on discards of target species (e.g. catch and bycatch species identification, number of individuals, size, and other biological data). In the present study, we used data recorded by IEO from 2003 to 2016 from the above-mentioned programme. These data included 189 cetaceans sightings observed during the fishing operation: 19 *Balaenoptera* spp., 39 dolphins and Ziphiidae, and 131 other cetaceans.



Avistamientos oportunistas área 34

Figure 1: Opportunistic sighting map.

Table 1: Number of opportunistic sightings, for each group and year.

Año	B	WNB	D	SW
2003	0	9	4	0
2004	0	5	0	0
2005	0	1	1	0
2006	2	14	1	0
2007	0	5	0	0
2008	6	18	5	0
2009	1	18	3	0
2010	0	22	9	0
2011	0	10	7	1
2012	0	2	4	0
2013	0	2	1	0
2014	9	8	3	3
2015	0	10	0	0
2016	1	3	1	0
N° de AO	19	127	39	4
Varianza	7,478	45,302	7,7198	0,681
Media	1,357	9,071	2,7857143	0,2857
CD	5,5101	4,994	2,771	2,3846

As explicative environmental variables we used: Sea Surface Temperature, Sea Surface Height, Meridional surface velocity, zonal surface velocity, Mixing layer depth, Dissolved oxygen, and Sea surface salinity. Moreover, we included the spatial variables: longitude and latitude. In our project it is very important to define Hutchinsonian fundamental niche (1957), which is defined mathematically as the set of different environmental variables (both biotic and abiotic factors) and limit values within which a specific species can survive. Each of the variables is independent of the others and their limit values define Ndimensional space, where each dimension corresponds to a possible state of the environment in which the species can exist, at a certain moment (T) (Vázquez, 2005). For all of this, the Generalized Linear Models (GLM) is one of the most used methods for the adjustment of statistical niche ecological models (Báez et al., 2014).

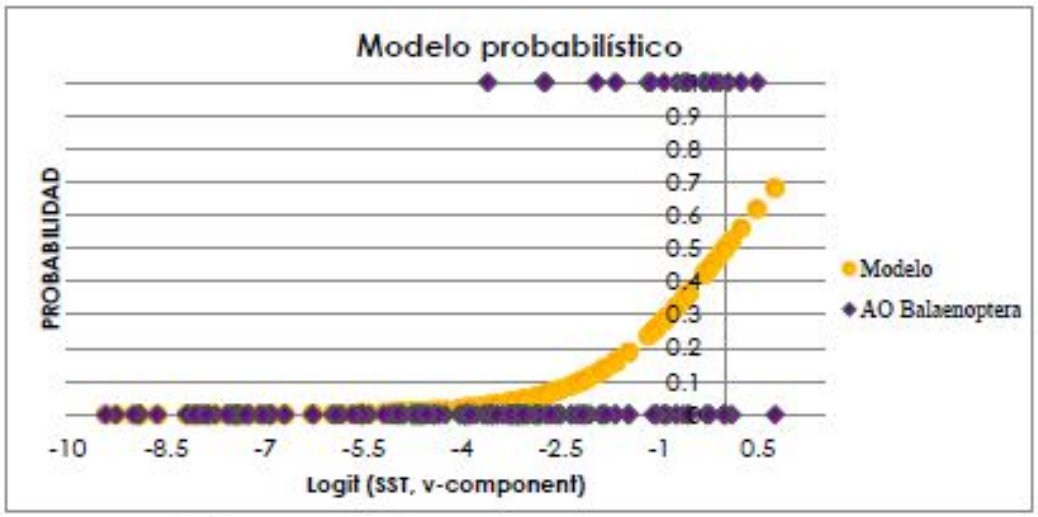
Results

Model coefficients were assessed by means of an omnibus test and the goodness-of-fit between expected and observed proportions of by-catch events along ten classes of probability values and evaluated using the Hosmer and Lemeshow test (which also follows a Chi-square distribution; low p-0.05 would indicate lack of fit of the model). On the one hand, the Omnibus test examines whether there are significant differences between the -2LL (less than twice the natural logarithm of the likelihood) of the initial step, and the -2LL of the model, using a Chi-squared test with one degree of freedom. On the other hand, the Hosmer and Lemeshow test compares the observed and expected frequencies of each value of the binomial variable according to their probability. In this case we expected that there are no significant differences for a good model fit. In addition, the discrimination capacity of the model (trade-off between sensitivity and specificity) was evaluated with the receiving operating characteristic (ROC) curve. Furthermore, the area under the ROC curve (AUC) provides a scalar value representing the expected discrimination capacity of the model. The relative importance of each variable within the model was assessed using the Wald test. The most parsimonious model obtained presented the Sea Surface Temperature (SST), and Meridional surface velocity (V-component, Table 2) as independent and explanatory variables. According to the Omnibus test, the adjusted model is significant (X^2 = 39.678, 2 degrees of freedom, P <0.001). In addition, no significant differences were observed between the observed values of presences and absences, and those expected by the model, by means of the Hosmer & Lemeshow test (X^2 = 2,802, 8 degrees of freedom P = 0,946). The likelihood (-2LL) was 83.642

and R2 of Nagelkerke = 0.395. The logit connection function is expressed as:

$$Y = 25,189 + [(-1,147) * SST] + [(-9,440) * Vcomponent] \quad (1)$$

Table 2: Model results.



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Effects of non-native species over the settlement process of the European spiny lobster (*Palinurus elephas*)

Anabel Muñoz*[1] , Maria Elena Cefalì[2] , Juancho Movilla[2] , Raquel Goñi[1] , David Díaz[1]

*Corresponding author: anabel.mcaballero@ieo.es
[1] Instituto Español de Oceanografía, Centro Oceanográfico de Baleares, Muelle de Poniente s/n, Palma, Spain
[2] Instituto Español de Oceanografía, Centro Oceanográfico de Baleares, Estación de Investigación Jaume Ferrer, Mahón, Spain

Keywords: Non-native species, Habitat preference, *Palinurus elephas*, *Lophocladia lallemandii*

Introduction

In the Mediterranean, the non-native red macroalga *Lophocladia lallemandii* was introduced from the Red Sea via the Suez Canal (Boudouresque and Verlaque, 2002) and is nowadays widespread. This filamentous macroalgae can colonize most kinds of hard substrata and has been considered an important threat to autochthonous benthic assemblages (Kersting et al., 2014). In 2006 a small spot of *L. lallemandii* was detected for the first time in the MPA (Marine Protected Area) of Columbretes Islands (Western Mediterranean), an area mostly dominated in the infralittoral communities by the brown alga *Dictyopteris membranacea* (photophilic facies) and the green alga *Halimenda tuna* (sciaphilic facies). Fortunately, since 2002 a long-term monitoring of spiny lobster (*Palinurus elephas*) settlement has been carried out around all the islands in the MPA. Until that moment, the ratio between the settlement index of spiny lobster in the main islands (Illa Grossa) was in average 3 times higher in comparison to other surrounding smaller islands. In 2008, a massive bloom of *L. lallemandii* was detected in the Illa Grossa bay, and the ratio declined to 1.1 in comparison to close areas, all of them without the presence of *L. lallemandii*. The aim of this study is to investigate how an invasive species as *L. lallemandii* could modify the settlement strength of *P. elephas*.

Material and Methods

In order to test our objective, we implemented experimental Y-maze tanks that allowed the specimens to choose between two shelter options. A total of 99 early benthic juveniles (EBJs) of *P. elephas* of sizes between 13 and 18 mm (Carapace Length - CL) were used to test their preference for the invasive alga versus an autochthonous one (*H. tuna*) also the physical, chemical, physicochemical effects of the two algae in the shelter selection. To cover all the interactions, a total of 10 treatments and one control with 9 replicates each one, were performed. In the 99 trials carried out with a duration of each one of 30 minutes, the spiny lobster could move freely around the Y-maze while being videotaped for the posterior analysis. In each trial we registered three results, 1) the first shelter option selected by the individual, 2) the shelter option where the spiny lobster was found at the end of the trial, and 3) time spent by the spiny lobster in each shelter. Chi-square test was used to analyse whether the first shelter selected by the lobsters differed from their final choice. Binomial test was used to check if the final choice of the lobster was randomly selected. We tested whether the difference of time spent in one or the other shelter

was significantly different from zero using a Wilcoxon signed-rank test (two-tailed). Differences between treatments regarding the time spent in the shelter with presence of each alga were tested using ANOVA tests.

Results and Conclusions

As results, fidelity to the first shelter selected was observed in 76 out of the 94 lobsters that made any choice (a total of 6 trials were removed from the analysis because the individuals did not move from the starting point of the Y-maze) (Chi-squared test $p < 0.001$). Only 33.3% of the lobsters chose *L. lallemandii* as option against *H. tuna*, but this result does not offer a significant differences between algae (Binomial test, $p > 0.05$), probably due to the low number of replicates. There were no significant differences between treatments in the time spent in the shelters with each of the two algae (*H. tuna*: $F_2 = 1.32$, $p > 0.05$ and *L. lallemandii*: $F_2 = 0.42$, $p > 0.05$) so no chemical, physical or physicochemical effect could be detected. In conclusion, a clear reduction of the settlement was observed when the invasive macroalga was present in the natural environment, but our experimental design does not offer clear results to determine that the causality of the decline of the relative abundance of EBJs of *P. elephas* is *L. lallemandii*. Probably many other causes could lead to modify the ratio between areas with a presence or absence of invasive species. Further studies are required to find out more factors affecting the settlement of *P. elephas* in a global change marine environment.

Acknowledgments

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Malacofauna associated with the sedimentary bottom in the Cerro-Gordo submarine cave (Granada)

Lidia Pino de la Torre*[1] , Serge Gofas[1] , Carlos Navarro Barranco[2]

*Corresponding author: lidia_pino@outlook.es
[1] Animal Biology Department, University of Malaga
[2] Marine Biology Laboratory, University of Seville

Keywords: Underwater cave, Molluscs, Sediment, Spatial gradient, Taxonomy

Introduction

Underwater caves are unique and vulnerable habitats, listed in Annex I of Directive 92/43/EEC of the European Community, and in the Barcelona Convention. Caves provide the opportunity to study in situ species generally living in deeper environments thanks to scuba diving (Vacelet, 1999). Although underwater caves are abundant in Mediterranean coastal habitats (Giakoumi et al., 2013), studies have focused mainly on specific taxonomic groups (Gerovasileiou and Voultsiadou, 2015b). Caves in the Alboran Sea are among the least studied in the Mediterranean, with the fewest species described (Gerovasileiou and Voultsiadou, 2015a) In this study, we focus on Cerro-Gordo karstic cave, located on the Paraje Natural of Maro Cerro-Gordo, in Granada. This is one of the largest caves and with the greatest biological interest in this area (Navarro Barranco, 2015). The main objective of this work is the study of the malacofauna associated with the sediment of Cerro-Gordo cave. As a secondary objective, we will try to identify the origin of the skeletal remains deposited inside the cave.

Materials and methods

Samples were collected by scuba diving in three different zones of Cerro-Gordo cave (36° 43.78'N, 3° 45.97'W, depth 15 m). Sediment samples were taken with a depth of 3 cm. In addition, another 4 samples were collected by scraping algae from the walls next to the cave entrance, in order to identify the possible origin of the skeletal remains found inside the cave. Four replicates from each station were taken (Figure 1).

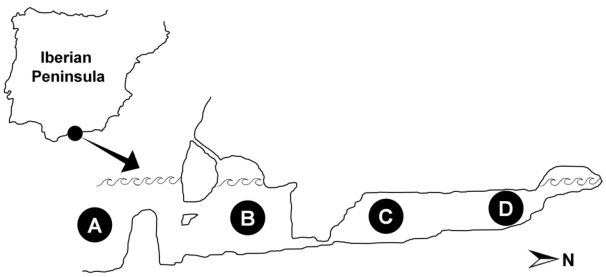

Figure 1: Location and longitudinal section of Cerro-Gordo cave, showing the sampling stations: Station 1 and 2 (dark areas), Station 3 (semidark area) and the Station 4 (outside the cave). Image modified from (Navarro Barranco, 2015).

In the laboratory, all the samples were washed through a 0.5 mm mesh sieve and fixed with 70% ethanol. The fauna was examined under the stereomicroscope and molluscs were identified.

For the samples collected outside the entrance, the volume of algae was estimated from water displaced in a graduated cylinder, and dry biomass was determined.

Results and discussion

In the semidark area of the cave, there was a heterogeneous substrate with coarse and fine particle size, however, this granulometry becomes finer in the dark areas because of low energy water movement. This sediment contains very few living molluscs and low species richness, especially in the innermost muddy areas. Indeed, Navarro Barranco (2015) confirmed granulometric differences inside the cave, and also that living organisms in the innermost areas are very scarce, because sediment is anoxic just a few centimeters below the surface. *Emarginula tenera* (Figure 2) and *Diodora* spp. were the most abundant skeletal remains of molluscs inside the cave, but *Barbatia barbata* was also abundant in the semidark area. *Nucula perminima* and *Oudardia compressa* were the most abundant living molluscs (Tables 1 and 2); both are detritivores rather than filter-feeders, which could explain why they are dominant in this low energy and oligotrophic environment.

Figure 2: *Emarginula tenera* collected in the dark area, station 1.

Table 1: List of the representative skeletal remains of mollusc species recorded inside Cerro-Gordo cave: Station 1 and 2 (dark areas), and Station 3 (semidark area).

SPECIES OF DEAD MOLLUSCS		
STATION 1	STATION 2	STATION 3
Emarginula tenera Locard, 1891	*Emarginula tenera* Locard, 1891	*Emarginula tenera* Locard, 1891
Diodora spp.	*Diodora* spp.	*Diodora* spp.
Crenella arenaria Monterosato, 1875	*Gregariella semigranata* (Reeve, 1858)	*Gregariella semigranata* (Reeve, 1858)
Gregariella semigranata (Reeve, 1858)	*Barbatia barbata* (Linnaeus, 1758)	*Barbatia barbata* (Linnaeus, 1758)
Barbatia barbata (Linnaeus, 1758)	*Hiatella arctica* (Linnaeus, 1767)	*Nucula nitidosa* Winckworth, 1930
Nucula nitidosa Winckworth, 1930	*Serratina serrata* (Brocchi, 1814)	*Serratina serrata* (Brocchi, 1814)
Nucula perminima Monterosato, 1875	*Oudardia compressa* (Brocchi, 1814)	
Hiatella arctica (Linnaeus, 1767)	*Gouldia minima* (Montagu, 1803)	

Table 2: List of the representative living mollusc species recorded inside Cerro-Gordo cave: Station 1 and 2 (dark areas) and Station 3 (semidark area).

SPECIES OF LIVING MOLLUSCS		
STATION 1	STATION 2	STATION 3
Nucula perminima Monterosato, 1875	*Oudardia compressa* (Brocchi, 1814)	*Nucula nitidosa* Winckworth, 1930
Diodora gibberula (Lamarck, 1822)	*Nucula nitidosa* Winckworth, 1930	*Gouldia minima* (Montagu, 1803)
Odostomia sp.		*Lucinella divaricata* (Linnaeus, 1758)

Regarding skeletal remains from other groups, brachiopods such as *Novocrania anomala* and *Megathiris detruncata* were more abundant in the dark stations than semidark one, and several sipunculids and polychaetes worms were recovered too. Corals such as *Thalamophyllia gasti* or *Caryophyllia inornata*, and large bryozoans were more abundant in the semidark area. Bryozoans were also found in great quantity on the walls near the entrance and covering algae such as *Halopteris scoparia*, the most commonly found. This study also revealed that some shells recovered from the sediment could have been brought into the cave from outside. Representative species as *Gregariella semigranata* or *Hiatella arctica* were found alive close to the entrance, but also as skeletal remains inside the cave. However, most skeletal remains collected from the innermost two samples, including *Emarginula tenera*, *Crenella arenaria* or *Barbatia barbata*, may proceed from the cave overhangs, because they were not found alive at the entrance.

Acknowledgments

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Syngnathids in the Cíes Islands (National Park of the Atlantic Islands): Distribution and trophic structure of the habitat

Arturo Jiménez Solís*[1] , Sara Campos Rosende[2] , Miquel Planas Oliver[3]

*Corresponding author: jimenezarturo1994@gmail.com
[1] Universidad de Vigo
[2] Instituto de Investigaciones Marinas (IIM-CSIC)

Keywords: Syngnathidae, Stable isotopes, Epifauna, Trophic chain, Cíes islands

Introduction

Syngnathids are a family of marine teleost fish that includes seahorses, pipe horses, pipefish and sea dragons. These are vulnerable species, with different levels of protection, which mostly live in association with algae communities (IUCN, 2019).
Epifauna associated with algae communities is very important since they are the food pantry for many species, including syngnathids. The knowledge of the trophic organization of a community is fundamental for the study of trophic connectivity and the determination of the trophic position of the species. In the case of syngnathids, there are not many studies carried out, with relatively different results depending on the species, the geographical area and the type of habitat, although certain groups of crustaceans seem to be an important part of the diet.
Stable isotopes are used as a non-invasive methodology (avoiding animal sacrifice) that, taking into account the assumption "you are what you eat", tries to determine the trophic relationships and to reconstruct animal diets (Sokołowski et al., 2014). In the case of syngnathids, isotopic techniques were used for the first time by Valladares et al. (2017) to infer the components of the seahorse diet.

Material and methods

The fieldwork consisted of day dives in three zones (A, B and C, Figure 1) in the National Park of the Atlantic Islands of Galicia (NO Spain) in spring, summer and autumn of 2018. The following tasks were carried out:

- Sighting and sampling of syngnathid fish (weight, size, dorsal fin for isotopic analysis).
- Abundance and diversity of macroalgae in algae communities.
- Obtaining epifauna samples associated with macroalgae.
- Sampling of the sediment.

After the analysis of the epifauna associated with macroalgae, There was a selection of Operational Taxonomic Units (OTUS), which were analyzed to determine their stable isotope profile of $\delta^{15}N$ and δ^{13} C. In molluscs with shell, this was extracted previously to the isotope analysis (SIA), which was carried out in Research Support Services of the University of A Coruña using continuous flow mass spectrometry (IR - MS). Since the samples were not subjected to lipid extraction, their own correction factors were also applied, depending on the type of organism. The samples of

syngnathid fin were processed for SIA as indicated by Valladares and Planas (2012). The trophic level of the different OTUS and syngnatids was obtained according to the equation developed by Post (2002).

$$NT = \lambda + ((\delta^{15}Nsecundaryconsumer - \delta^{15}Nbase) \div \Delta n) \quad (1)$$

λ is the trophic position of the organism used as δ^{15}N base and Δ_n which is the enrichment of δ^{15}N by trophic level. A value of 3.4 $^{o}/_{oo}$ was considered for Δ_n(Post, 2002). It is a generic value that should be calculated for each species, but specific values for species of Syngnathids are unknown. Both for the calculation of the δ^{15}N secondary consumer and for the calculation of δ^{15}N base the average values of the stations and the zones were used in order to obtain the isotopic signature of the individual integrating the spatial and temporal scale.

Results and discussion

Two syngnathid species were identified: *Syngnathus acus* (Linnaeus, 1758) and *Entelurus aequoreus* (Linnaeus, 1758). The first was the most abundant and most widely distributed, with large specimens, while the second showed low abundances and a more selective distribution, limited to zone C (Borrón). This area is the most abundant in syngnathids, whose presence was considerably reduced in autumn (Figure 1).

Figure 1: Catches made in the samplings of spring, summer and autumn in 2018 in zones A, B and C. Each circle represents a specimen of *S. acus* (green) and *E. aequoreous* (blue).

The population of *S. acus* was constituted fundamentally by sexually mature specimens (60% in females and 89% in males). The breeding season was extended to spring and summer, while in autumn none of the specimens was sexually mature (Figure 2).

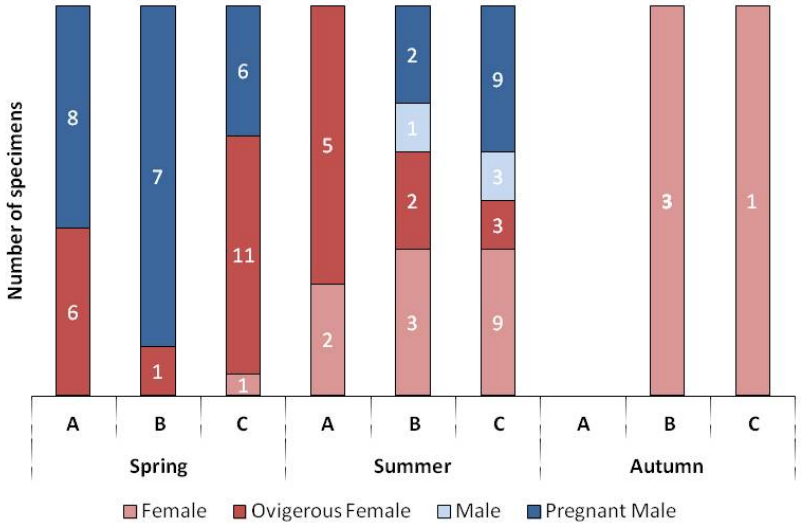

Figure 2: Catches of *S. acus* distributed by their reproductive status, in the zones and stations sampled during the year 2018. The relative proportion of each reproductive state is represented in the y axis.

The analysis of the epifauna allowed to establish a trophic structure formed by six groups: filter feeders, grazers, detritivores, suspensivores, omnivores and predators (including syngnathids), with syngnathids occupying the top trophic position (Figure 3).

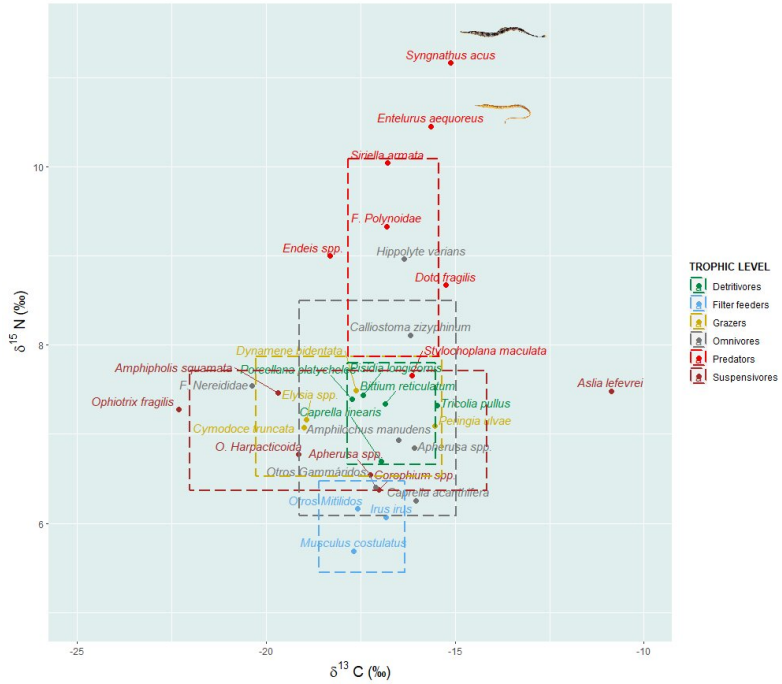

Figure 3: Mean values of δ^{15}N and δ^{13}C of epifaunal OTUS and the two species of syngnathids studied. The rectangles represent the standard deviations of each trophic group. The bivalve *M. costulatus* was used as a baseline.

The results confirmed that the two species of syngnathids are primary carnivores with a similar diet and trophic position (NT = 3.61 in *E. aequoreus* and 3.76 in *S. acus*) in the three

zones studied, although with slight seasonal variations. The estimation of the most important components of the diet is currently in the analysis phase. Zone C (Borrón) is especially important because of the lower exposure to ocean currents, the characteristics of its algae community that makes up a food pantry (epifauna) and the abundance and diversity of syngnathids. For these reasons, this area is considered of interest as a future special integral protection zone.

Acknowledgments

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Morphological changes in the gills of mussels *Mytilus galloprovincialis* caused by the pea crab *Afropinnotheres monodi*

Marta Perez-Miguel*[1] , Erica Sparaventi[1] , Javier Sepúlveda[1] , Marta Sendra[1]

*Corresponding author: msendra@iim.csic.es
[1] Instituto de Investigacións Mariñas (ICMAN-CSIC)

Keywords: Mussel, Pinnotherid, Infestation, Feeding, Parasitism

Introduction

The mussel *Mytilus galloprovincialis* is the most important bivalve for the aquaculture in Spain. This country is the second global producer of mussels after China. Therefore, the study of their health status requires special attention. Pinnotherid live as symbionts in other invertebrates, very often in the paleal cavity of bivalve mollusks as mussels. It is not frequent that have high levels of pea crab populations in bivalve's beds. Nevertheless, recently a large number of pinnotherid, until 45.5% of infestation, have been detected in the intertidal zone of the Gulf of Cádiz (Perez-Miguel et al., 2018). The species corresponds to *Afropinnotheres monodi* Manning 1993, that is originally from Morocco and Mauritania coast. Besides, it has been shown that *A. monodi* is a parasite causing around 15% of condition index reduction. Due to the fact that this parasite constitutes a potential risk for the mussels causing economic losses in the culture of mussels, it is important to understand the impact of this pea crab in the mussels feeding. The aim of this study is to clarify if there is a relation between the infestation and the filtration rates and also to study if morphological changes in the gills of mussels are triggered by *A. monodi* infestation.

Material and methods

The wild mussels used in this study were collected within the Bay of Cadiz (southwestern Spain) by hand (scuba diving) from the submerged chains of five of the buoys used as lateral marks of the navigable channel of the Bay of Cadiz. The collected mussels were quickly taken to the laboratory where they were acclimated for 24 hours in a filtrated seawater tank at 17 ° C under 12L/12D light conditions. The experience of filtration was carried in 200 mussels with shell length between 80 and 30 cm. Individual mussels were placed in 1L beakers filled with 0.5 μm filtered seawater and continue aeration. The animals were fed one time with the flagellate algae *Isochrysis galbana* Parke 1949, and the cell density was about 104 cells·ml^{-1}. Sampling times of microalgae cell density were: after 30 minutes (T1), 1 hour (T2), 2 hours (T3), 3 hours (T4), 5 hours (T5) and 20 hours (T6). At each time, the cell density was measured by flow cytometry. After finished the feeding experience, the shell length, width and deep of each mussel was measured to the nearest 0.1 mm with a dial calliper (Tesa Cal IP65). The two valves of the shell were then open with a scalpel and the flesh was carefully inspected and, if present, pea crabs were removed and recorded. The pea crab cephalothorax width was with the same dial calliper and its sex established on the basis of the presence (male) or absence (female) of gonopods. Photos of the mussels' gills were taken with a Canon D1100 reflex camera to know its surface with the software Image-J. Finally, the bivalve flesh was removed from the shell and its wet weight estimated in the digital balance (precision 0.1 mg).

Results and conclusions

The infestation rate was 52.57%. We are currently working on the data obtained in this experiment. However, the gills of the infested mussels have a surface area apparently smaller than the gillls of the mussels not infested with *A. monodi*. Nerveless, there are no apparent differences between the filtration rate of the infested and non-infested mussels. This may indicate that the infested mussels spend more energy on the filtration than non-infested mussels, which would explain the decrease in their fitness.

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Stranding records of the loggerhead turtle *Caretta caretta* (Linnaeus, 1758) at the Balearic Islands in the last 25 years

Maria Febrer-Serra*[1] , Emanuela Renga[2] , Gloria Fernández[2] , Antoni Sureda[3] , Guillem Mateu-Vicens[4] , Samuel Pinya[4]

*Corresponding author: maria.febrer@uib.es

[1] Interdisciplinary Ecology Group. University of the Balearic Islands. Palma (Balearic Islands, Spain)

[2] Fundación Palma Aquarium. Marine Fauna Recovery Centre. Balearic Islands, Spain.

[3] Interdisciplinary Ecology Group. University of the Balearic Islands. Palma (Balearic Islands, Spain) / Research Group on Community Nutrition and Oxidative Stress (NUCOX) and CIBEROBN. University of the Balearic Islands. Palma (Balearic Islands, Spain)

[4] Interdisciplinary Ecology Group. University of the Balearic Islands. Palma (Balearic Islands, Spain) / Museu Balear de Ciències Naturals. Balearic Islands, Spain

Keywords: *Caretta caretta*, Entanglement, Longline, Recovery, Strandings

Introduction

Sea turtles (Reptilia, Testudines) are distributed in tropical and subtropical oceans worldwide. Juveniles are mainly found in oceanic waters, while subadults and adults spend most of the time in neritic areas. *Caretta caretta* (Linnaeus, 1758) is listed as Vulnerable in the IUCN Red List, while the Mediterranean subpopulation is classified as Least Concern. Several human factors, such as bycatch and marine pollution have been described as threats to *C. caretta* (Tomás et al., 2008). *Caretta caretta* is the most abundant sea turtle species in the Mediterranean Sea. The western Mediterranean is used as a foraging ground by immature loggerhead turtles from the Northwest Atlantic and Eastern Mediterranean rookeries (Carreras et al., 2006). Despite the wide variety of monitoring methods available, there is still a lack of information concerning the biology and ecology of sea turtles. This fact emphasises the importance of the long-term stranding records (Bellido López et al., 2018). In these studies, the collaboration with Wildlife Recovery Centres is essential, not only for the recovery of the injured turtles but also for the extensive collection of information about the animals admitted at the centre. In this context, the aim of the present work was to analyse the demographic parameters and the main threats of the population of *C. caretta* in the Balearic Islands. For this purpose, the stranding records collected by the Marine Fauna Recovery Centre of Palma Aquarium Foundation between 1993 and 2017 (25 years) were used.

Materials and methods

The staff from the Recovery Centre registered the information concerning the location and the date in each case of stranding during the period 1993-2017. Biometric parameters such as straight carapace length (SCL), weight and sex (when possible) were also taken in the individuals captured. In addition, the initial state (alive or corpse), stranding cause (see Figure 1), time of stay at the Recovery Centre and the final state of the individuals after the recovery period (released, dead or other cases) were registered. Specimens were classified in three age classes determined by their SCL, known as pelagic juveniles (SCL < 42 cm), subadults (42 cm $\leq$ SCL < 70 cm) and adults (SCL ≥ 70 cm) according to Orós et al. (2016) and references therein.

Results and discussion

A total of 876 strandings were recorded. A greater number of juveniles (n = 200) and subadults (n = 500) in comparison with adults (n = 45) was obtained, as is also observed in Mediterranean Andalusian waters (Bellido López et al., 2018). It would indicate that the Western Mediterranean and, in particular, the Balearic Sea are foraging areas for immature individuals (Carreras et al., 2006; Báez et al., 2019). Mean and standard deviation (SD) (range) of SCL was 49.97 ± 13.28 cm (13.09 – 92.00 cm). The most frequent causes of stranding were unknown (40.18 %), ingestion of hooks (31.51 %), entanglement (13.70 %) and traumatism (5.48 %). The causes of stranding were similar to those observed in other studies (Tomás et al., 2008; Orós et al., 2016; Bellido López et al., 2018) and reflect the high prevalence of anthropogenic threat factors that affect sea turtles. Regarding the seasonality, up to 54.29 % of strandings occurred in summer as observed in Tomás et al. (2008) and Bellido López et al. (2018), followed by autumn (24.8 %), spring (14.17 %) and winter (6.74 %). In terms of interannual variation, there was an annual average and SD (range) of 35.04 ± 19.42 (4 - 73) turtle strandings. It is worth noting a considerable decrease in the cases of strandings caused by long-line hooks since 2009 (Figure 1). According to Báez et al. (2019), the decrease in the bycatch of *C. caretta* by the Spanish surface fleets is due to the replacement of the Spanish traditional surface longline by a semipelagic longline in 2007. Although these measures were only introduced for improving fishing strategies, they leave the longline inaccessible to turtles (Báez et al., 2019). In reference to the spatial distribution, the greatest number of strandings was observed in the UTM 5 x 5 km squares near the fishing harbours of the Balearic Islands (Figure 2). Finally, the cause entanglement in fishing gears and plastics obtained the highest release rate (87.62 %) in the Rescue Centre, while traumatism got the highest mortality rate (50.00 %). The total release rate (67.31 %) was considerably higher than the total death rate (27.88 %), as observed in Orós et al. (2016). This fact emphasises the relevance of the Recovery Centres in the management and conservation of sea turtles.

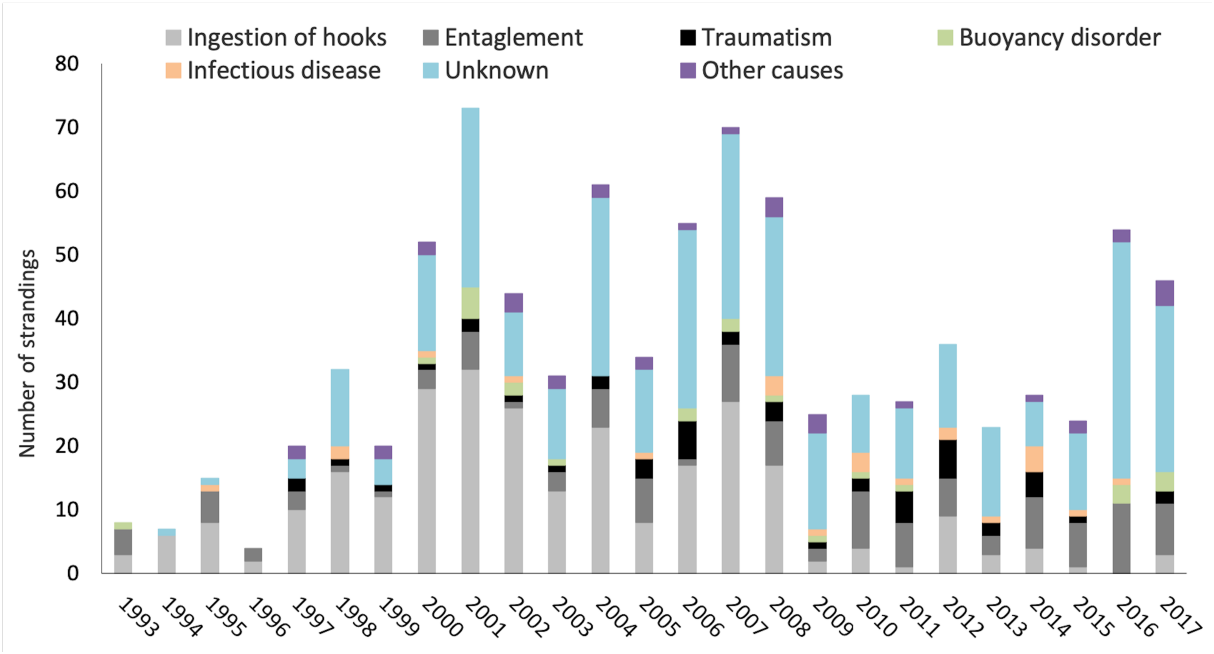

Figure 1: Inter-annual variability in causes of stranding among the period 1993 – 2017.

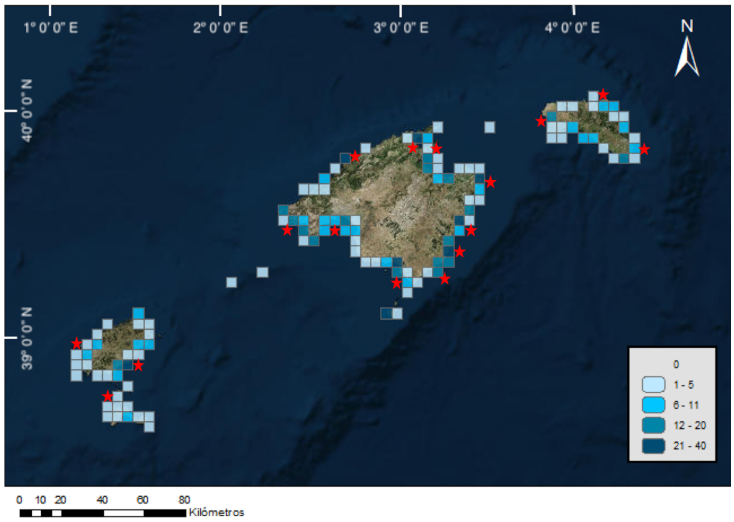

Figure 2: Spatial distribution of *Caretta caretta* strandings in UTM 5 x 5 km squares during the period 1993 – 2017 (ArcGis ©). The colour code indicates the number of strandings. Red marks show the main fishing harbours of the Balearic Islands.

Acknowledgments

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Pathogens and parasites in *Donax trunculus* L., 1758 from the littoral of Málaga

Noemí López-García*[1] , Juan Diego Castro-Claros[1] , Raquel Juanes-Segura[2] , Carmen Salas[1]

*Corresponding author: noemilg93@gmail.com
[1] Departamento de Biología Animal, Universidad de Málaga, Campus de Teatinos s/n, 29071-Málaga (Spain).
[2] Centro Superior de Formación Europa-Sur, S.A (CESUR), C/ Severo Ochoa 27-29, Parque Tecnológico, 29590-Málaga

Keywords: *Donax trunculus*, Mediterranean, Bivalves, Pathogens, Parasites

Introduction

The Mollusca are a major component of the marine benthos, with approximately one fourth of all species (Appeltans et al., 2012). Among its different classes, bivalves are those that tend to have higher abundances. Although many species of bivalves are epibenthic on hard substrates, most species live buried in soft bottoms, forming dense populations. Among the numerous pathogens and parasites that affect bivalves, some tend to lodge more frequently in particular organs, or directly only parasitize a specific organ. Some parasites may represent a risk to fisheries, causing serious economic losses (Villalba et al., 1993). The objectives of the present study are: A. to identify by histology the pathogens and parasites present in specimens of *Donax trunculus* (coquina) from the littoral of Málaga (southern Spain); B. to check which organs are affected and, C. to analyze the relationship between prevalence and sex or seasonality.

Material and methods

A total of 328 specimens of *D. trunculus*, collected in the coast of Málaga, have been analyzed; of these, 238 were collected monthly from February 1990 to January 1991 in the littoral between Benalmádena and Fuengirola, and 90 were collected monthly from February to April 2019 in the littoral of Velez-Málaga. For histological processing, specimens were anesthetized with $MgCl_2$, fixed in 10% of formaldehyde, embedded in paraffin, sectioned at 10 μm and stained with Haematoxylin of Carazzi- Eosin or Haematoxylin of Carazzi-VOF (Light green, orange G and acid Fuchsin).

Results and discussion

Two prokaryotes, *Rickettsia*-like and *Chlamydia*-like organisms have been identified (Figure.1A-B). *Rickettsia*-like mainly parasitize the gills, with a prevalence of 35.45% while *Chlamydia*-like appears in the digestive gland with a prevalence of 9.74%.

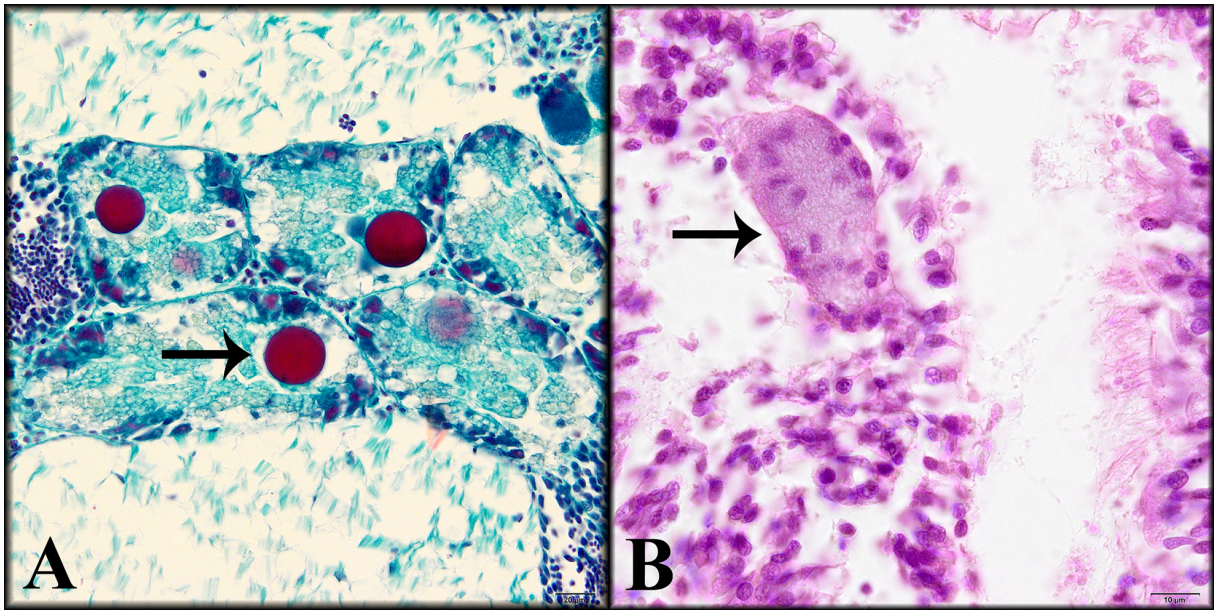

Figure 1: Pathogens Prokariotes of *Donax trunculus*. A. *Rickettsia*-like organisms. B. *Chlamydia*-like organisms.

Among the protists, we found *Marteilia* sp. that parasitizes basically the digestive gland with a prevalence of 100%, and *Nematopsis* sp. and *Porospora* sp. that parasitize the muscular tissues of the foot with a prevalence of 45.30% and 33%, respectively (Figure.2A-C). Finally, as metazoans we found larval stages of Digenean Trematodes in gonads (where they produce castration), palps, gills and foot, although with little presence (1.8% prevalence) (Figure.2D). Only *Nematopsis* sp. and *Porospora* sp. showed significant differences according to the sex of the hosts, with a higher prevalence in females than in males. Both species present seasonality since they are more frequent in spring and autumn. Regarding prokaryotes, *Rickettsia*-like present a peak of prevalence in the spring, followed by summer, whereas this presence is lower in autumn and winter. Conversely, we have not found the *Chlamydia*-like organism in spring, and its prevalence varies little in the other seasons (10-16%). Finally, the Trematodes present a small peak in spring (4.35% of prevalence).

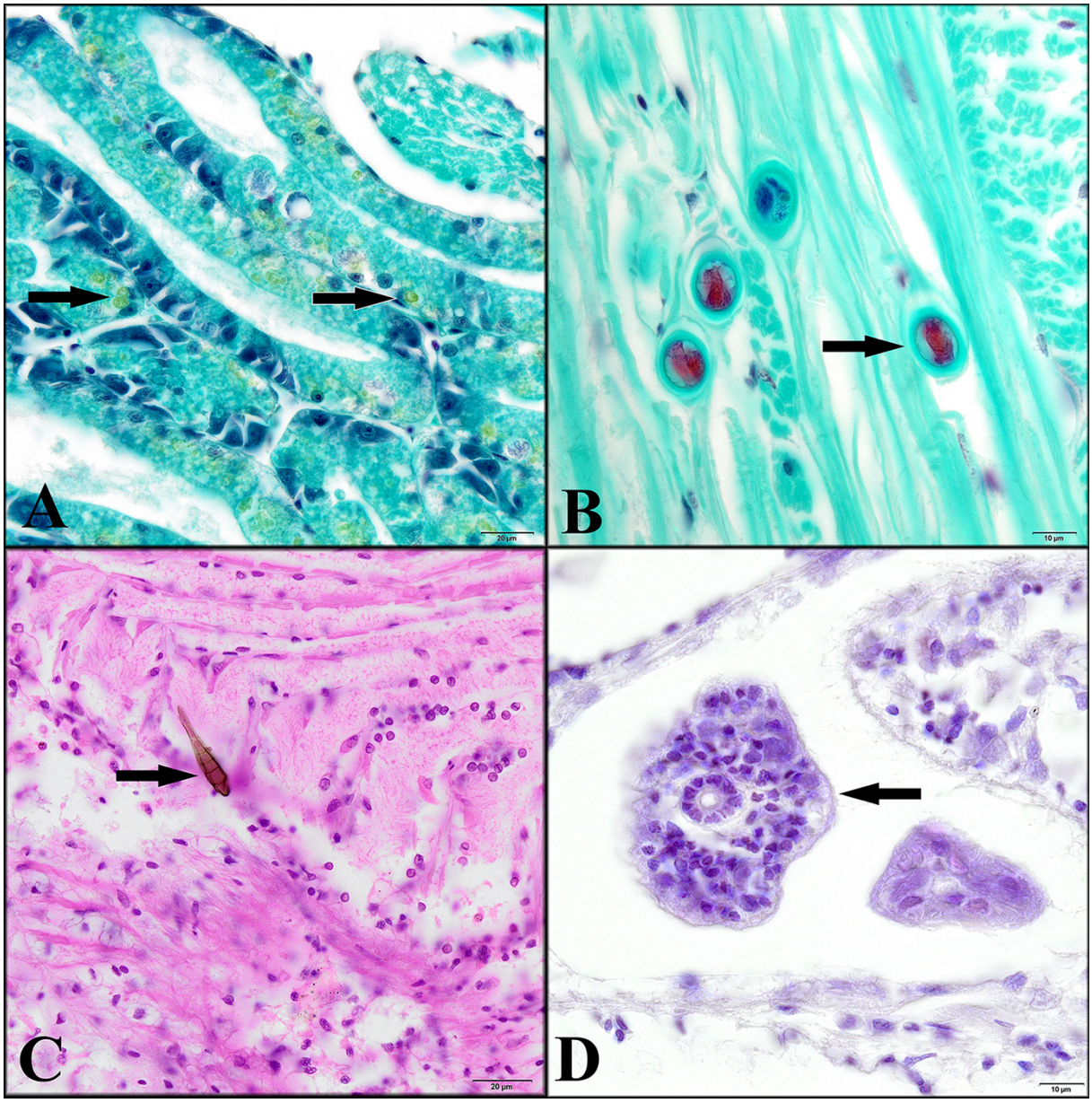

Figure 2: Protists and metazoans from *Donax trunculus*. A. *Marteilia* sp. in digestive gland. B. *Nematopsis* sp. in foot. C. *Porospora* sp. in foot. D. Larval stages of a Digenean Trematode in gonad.

The presence of marteiliasis in bivalves is common (Villalba et al., 1993), although this parasite is not reported in the populations of *Donax trunculus* from the Adriatic Sea (Carella et al., 2019). In the population from Malaga, this parasite has a prevalence of 100% and the survival capacity of the bivalve is surprising, with so much of the digestive gland affected. The highest prevalence in females during spring and autumn of *Nematopsis* sp. and *Porospora* sp. could be related to the fact that females in spring and late summer have a hypertrophied visceral mass due to the development of gonads (Tirado and Salas, 1998), which hinders the closing of the valves. As a consequence, part of the foot may be exposed, which frequently leads to depredation by crabs (Salas et al., 2001) and facilitates the entry of parasites. Also the small spring peak of Digenean Trematodes, which produces castration of the gonad, could be related to a greater number of specimens with full gonads in spring, which provide a source of food for the redia and cercaria larval stages. The prokaryotes show certain seasonality, *Rickettsia*-like organisms have a

higher prevalence in spring and summer, while no *Chlamydia*-like organisms were found in spring, although the reason of that is unknown.

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New records of Bryozoans from the Alboran Sea

Raquel Rodríguez Aporta*[1] , Laís Vieira Ramalho[2]

*Corresponding author: rodriguezaporta10@gmail.com
[1] Universidad de Málaga
[2] Museu Nacional, Universidade Federal do Rio de Janeiro, RJ, Brazil

Keywords: Mediterranean sea, Bryozoans, Faunistic

Introduction

Bryozoans are an important group of aquatic invertebrates which form colonies of different sizes and shapes. They are suspension-feeders characterized by the presence of lophophore (Ryland, 1970) and occur in almost all marine habitats. The presence of hard substrates is very important to this group, because it is a requisite for the settlement of the larvae, but some specimens (Lunulitiforms) can occur on soft bottoms. A few species also inhabit freshwater lakes and rivers (Ryland, 1970). Bryozoan is a very diversified phylum among the marine benthos, with for example, 556 species, 212 genera and 93 families recorded in the Mediterranean Sea (Gofas et al., 2014) but there are still some gaps of knowledge in the taxonomy of this group. Regarding the Alboran Sea, we can mention the Balgim expedition (Harmelin and D'Hondt, 1992a,b) which reported 152 species (115 Cheilostomes collected in the Gulf of Cadiz, the Strait of Gibraltar and the Alboran Sea, five Ctenostomes and 32 Cyclostomes). The INDEMARES project, made representative samplings during 2011 and 2012 between 50-200 meters of depth around the Alboran Island. Most of the important benthic groups such as sponges, cnidarians, polychaete annelids, molluscs, decapod crustaceans and echinoderms have been studied in some detail (Gofas et al., 2014) but bryozoans, although profusely collected, were not addressed. Thus, the aim of this work is to identify the bryozoan species collected during these expeditions and expand the information about the bryozoan fauna from the Alboran Sea, more specifically from the surroundings of the Alboran Island.

Materials and methods

During INDEMARES project (2011 and 2012), three oceanographic campaigns were carried out. The first campaign (September 2011) was used to obtain underwater video information through the remotely operated vehicle (ROV) In the two subsequent campaigns, 44 samples were collected (30 in September 2012 and 14 in July 2012) between 25 and 200 meters depth (Gofas et al., 2014). In the present study 4 samples (BV12, BV13, BV14 and BV15), containing small rocks, debris and complete shells, collected between 95-120 meters depth, were analyzed.

Table 1: Table 1: List of sampling stations of the INDEMARES expeditions (2011-2012).

NAME OF THE SAMPES	CAMPAIGN	DATE	GEOGRAPHICAL COORDINATES	SUBSTRATUM	DEPTH
BV12	INDEMARES Alborán	23/09/2011	35°52.22'N – 03°05.21'W, 35°52.17'N – 03°05.38'W	Bioclastic gravel	112 – 120 m
BV13	INDEMARES Alborán	23/09/2011	35°52.38'N – 03°05.18'W, 35°52.82'N – 03°04.59'W	Bioclastic gravel	95 – 99 m
BV14	INDEMARES Alborán	23/09/2011	35°52.72'N – 03°04.67'W, 35°52.34'N – 03°05.26'W	Bioclastic gravel	96 – 100 m
BV15	INDEMARES Alborán	23/09/2011	35°52.67'N – 03°04.66'W, 35°52.90'N – 03°04.92'W	Bioclastic gravel	96 m

These samples were collected close to each other and here they are considered as replicates. The bryozoan colonies and loose fragments were picked from the samples, cleaned and mounted on stubs using conductive carbon adhesive tape, and imaged using Scanning Electron Microscopy, at Universidad de Malaga. The measurements were made using ImageJ ®.

Results

The four samples yielded until now 67 species, of which 44 have been identified at a specific level, 20 at the genera level and 3 at the family level. They represent both marine classes: Gymnolaemata, the most diverse group (56 taxa) and Stenolaemata represented by the Order Cyclostomatida (11 taxa, but the most abundant in terms of number of individuals). The most diverse families are: Cribrilinidae with six species, following by Celleporidae with five species and Adeonidae, Romanchenidae, Bitectiporidae and Microporellidae (four species each). Of these, 29 species (43.3%) are present in all four replicas, 9 species (13.4%) in three replicas, and 16 species (23.9%) were present in only one of the samples. For the area, 19 new citations were recorded for the Alboran Sea and 42 for the Alboran Island. Besides that 9 new species to science are being proposed (*Cupuladria* sp., *Chorizopora* sp., *Licornia* sp., *Scrupocellaria* sp., *Escharella* sp. 1 and sp. 2, *Microporella* sp., *Escharina* sp. and *Buskea* sp).

Acknowledgments

The authors thank Dr. Serge Gofas and Dr. Carmen Salas (University of Malaga), for organizing the logistics of collecting within the LIFE+ INDEMARES project (LIFE07/NAT/E/000732 INDEMARES, from June 2011 to December 2012), and for handing over the material to us. The scanning electron micrographs were taken by Gregorio Martín Caballero at University of Malaga.

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Stranding records of the loggerhead turtle *Caretta caretta* (Linnaeus, 1758) at the Balearic Islands in the last 25 years

Maria Febrer-Serra*[1] , Emanuela Renga[2] , Gloria Fernández[2] , Antoni Sureda[3] , Guillem Mateu-Vicens[4] , Samuel Pinya[4]

*Corresponding author: maria.febrer@uib.es

[1] Interdisciplinary Ecology Group. University of the Balearic Islands. Palma (Balearic Islands, Spain)

[2] Fundación Palma Aquarium. Marine Fauna Recovery Centre. Balearic Islands, Spain.

[3] Interdisciplinary Ecology Group. University of the Balearic Islands. Palma (Balearic Islands, Spain) / Research Group on Community Nutrition and Oxidative Stress (NUCOX) and CIBEROBN. University of the Balearic Islands. Palma (Balearic Islands, Spain)

[4] Interdisciplinary Ecology Group. University of the Balearic Islands. Palma (Balearic Islands, Spain) / Museu Balear de Ciències Naturals. Balearic Islands, Spain

Keywords: *Caretta caretta*, Entanglement, Longline, Recovery, Strandings

Introduction

Sea turtles (Reptilia, Testudines) are distributed in tropical and subtropical oceans worldwide. Juveniles are mainly found in oceanic waters, while subadults and adults spend most of the time in neritic areas. *Caretta caretta* (Linnaeus, 1758) is listed as Vulnerable in the IUCN Red List, while the Mediterranean subpopulation is classified as Least Concern. Several human factors, such as bycatch and marine pollution have been described as threats to *C. caretta* (Tomás et al., 2008). *Caretta caretta* is the most abundant sea turtle species in the Mediterranean Sea. The western Mediterranean is used as a foraging ground by immature loggerhead turtles from the Northwest Atlantic and Eastern Mediterranean rookeries (Carreras et al., 2006). Despite the wide variety of monitoring methods available, there is still a lack of information concerning the biology and ecology of sea turtles. This fact emphasises the importance of the long-term stranding records (Bellido López et al., 2018). In these studies, the collaboration with Wildlife Recovery Centres is essential, not only for the recovery of the injured turtles but also for the extensive collection of information about the animals admitted at the centre. In this context, the aim of the present work was to analyse the demographic parameters and the main threats of the population of *C. caretta* in the Balearic Islands. For this purpose, the stranding records collected by the Marine Fauna Recovery Centre of Palma Aquarium Foundation between 1993 and 2017 (25 years) were used.

Materials and methods

The staff from the Recovery Centre registered the information concerning the location and the date in each case of stranding during the period 1993-2017. Biometric parameters such as straight carapace length (SCL), weight and sex (when possible) were also taken in the individuals captured. In addition, the initial state (alive or corpse), stranding cause (see Figure 1), time of stay at the Recovery Centre and the final state of the individuals after the recovery period (released, dead or other cases) were registered. Specimens were classified in three age classes determined by their SCL, known as pelagic juveniles (SCL $<$ 42 cm), subadults (42 cm $\leq$ SCL $<$ 70 cm) and adults (SCL $\geq$ 70 cm) according to Orós et al. (2016) and references therein.

Results and discussion

A total of 876 strandings were recorded. A greater number of juveniles (n = 200) and subadults (n = 500) in comparison with adults (n = 45) was obtained, as is also observed in Mediterranean Andalusian waters (Bellido López et al., 2018). It would indicate that the Western Mediterranean and, in particular, the Balearic Sea are foraging areas for immature individuals (Carreras et al., 2006; Báez et al., 2019). Mean and standard deviation (SD) (range) of SCL was 49.97 ± 13.28 cm (13.09 – 92.00 cm). The most frequent causes of stranding were unknown (40.18 %), ingestion of hooks (31.51 %), entanglement (13.70 %) and traumatism (5.48 %). The causes of stranding were similar to those observed in other studies (Tomás et al., 2008; Orós et al., 2016; Bellido López et al., 2018) and reflect the high prevalence of anthropogenic threat factors that affect sea turtles. Regarding the seasonality, up to 54.29 % of strandings occurred in summer as observed in Tomás et al. (2008) and Bellido López et al. (2018), followed by autumn (24.8 %), spring (14.17 %) and winter (6.74 %). In terms of interannual variation, there was an annual average and SD (range) of 35.04 ± 19.42 (4 - 73) turtle strandings. It is worth noting a considerable decrease in the cases of strandings caused by long-line hooks since 2009 (Figure 1). According to Báez et al. (2019), the decrease in the bycatch of *C. caretta* by the Spanish surface fleets is due to the replacement of the Spanish traditional surface longline by a semipelagic longline in 2007. Although these measures were only introduced for improving fishing strategies, they leave the longline inaccessible to turtles (Báez et al., 2019). In reference to the spatial distribution, the greatest number of strandings was observed in the UTM 5 x 5 km squares near the fishing harbours of the Balearic Islands (Figure 2). Finally, the cause entanglement in fishing gears and plastics obtained the highest release rate (87.62 %) in the Rescue Centre, while traumatism got the highest mortality rate (50.00 %). The total release rate (67.31 %) was considerably higher than the total death rate (27.88 %), as observed in Orós et al. (2016). This fact emphasises the relevance of the Recovery Centres in the management and conservation of sea turtles.

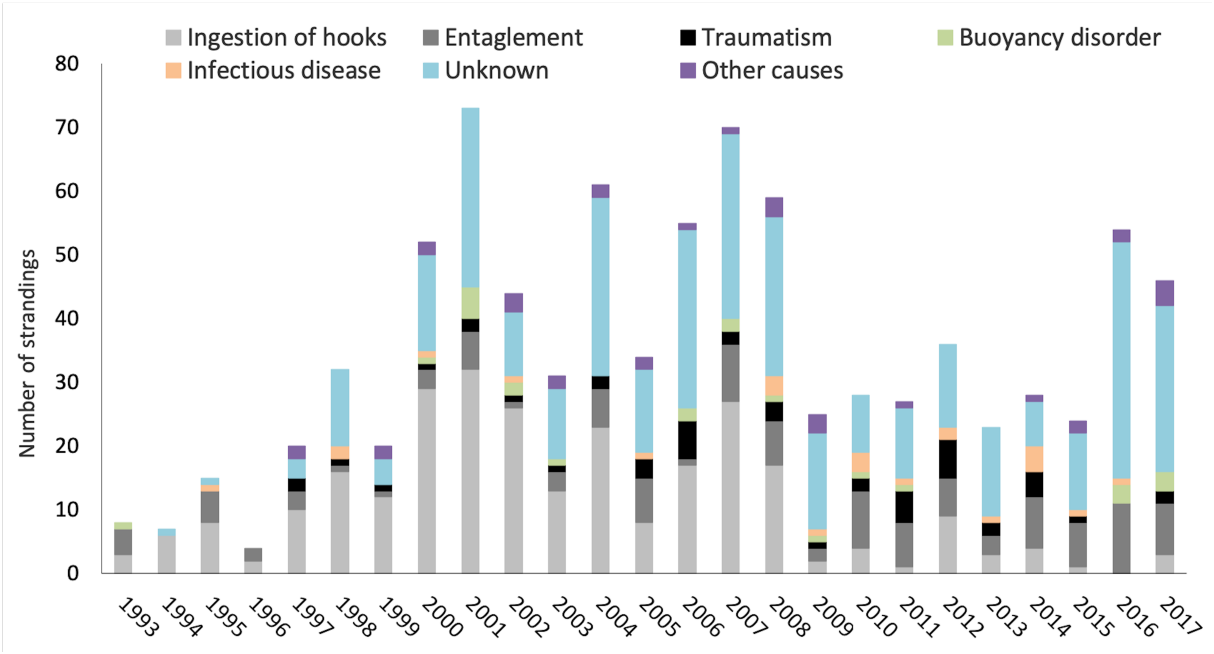

Figure 1: Inter-annual variability in causes of stranding among the period 1993 – 2017.

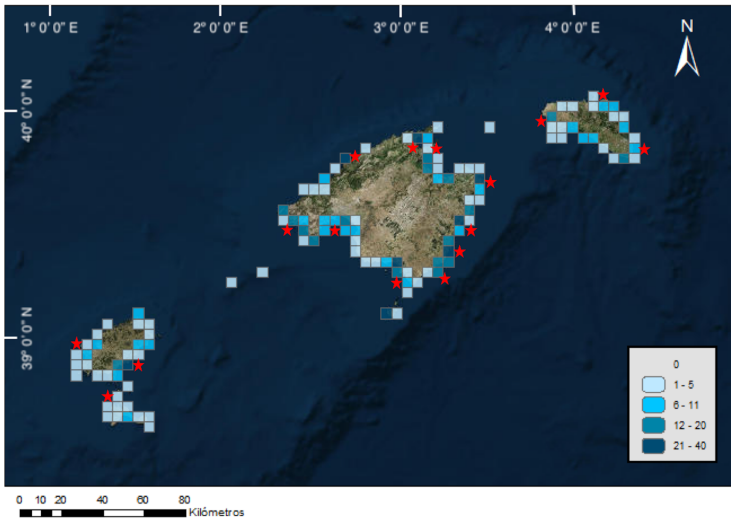

Figure 2: Spatial distribution of *Caretta caretta* strandings in UTM 5 x 5 km squares during the period 1993 – 2017 (ArcGis ©). The colour code indicates the number of strandings. Red marks show the main fishing harbours of the Balearic Islands.

Acknowledgments

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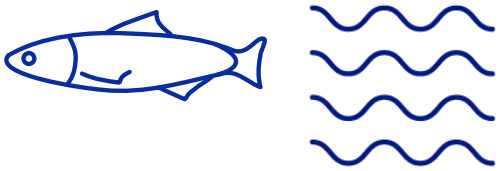
Session 6

The Sea as a Resources Source

Aquaculture



Effect of density on the growth and mortality of the Pacific oyster *Crassostrea gigas* cultured in waters of the Basque coast (SE Bay of Biscay)

Ainhoa Juez *[1] , Izaskun Zorita[1] , Oihana Solaun[1] , Leire Arantzamendi[2] , Juan Bald[1] , Manuel González[1]

*Corresponding author: ajuez@azti.es
[1] AZTI-Tecnalia, Pasaia, Gipuzkoa
[2] AZTI-Tecnalia, Sukarrieta, Bizkaia

Keywords: Basque Country, Density, Growth, Pacific oyster, Mortality

Introduction

An important question in bivalve aquaculture is how many individuals of a certain species can be grown before growth is limited or before mortality becomes intolerable. In addition, the information about stocking density is scarce in the scientific literature and results are not always comparable. Thus, stocking density for oyster culture can be variable according to the type of suspended systems and the life stage of oysters, although it has been observed that it can directly affect growth and finally culture cost-effectiveness. Thus, the aim of the present study was to assess the effect of stocking density on growth, mortality and quality of Pacific oyster (*Crassostrea gigas*) reared on a suspended system in a sheltered environment of the Basque littoral, from spat to juvenile.

Materials and Methods

Oyster spat was obtained from Marinove commercial hatchery (France), with an initial length of 14 mm and an initial body weight of 0.22 g. The oysters were reared in lanterns. The experiment was divided into three cultivation stages (pre-fattening phase I, pre-fattening phase II and fattening phase), in which each stage was further separated into three different stocking densities (low, medium and high) (Figure 1). This study was conducted from March (2017) to April (2018). In each sampling, oysters were weighted and measured to determinate the growth. These measurements were done in 150 oysters per density, corresponding to 3 replicates of 50 individuals extracted by lantern. To determine mortality, 100 oysters were counted per lantern; a total of 300 oysters per density. Once the measurements were made, all the oysters (live and dead) were returned to their corresponding lantern not to affect the stocking density of the experiment.

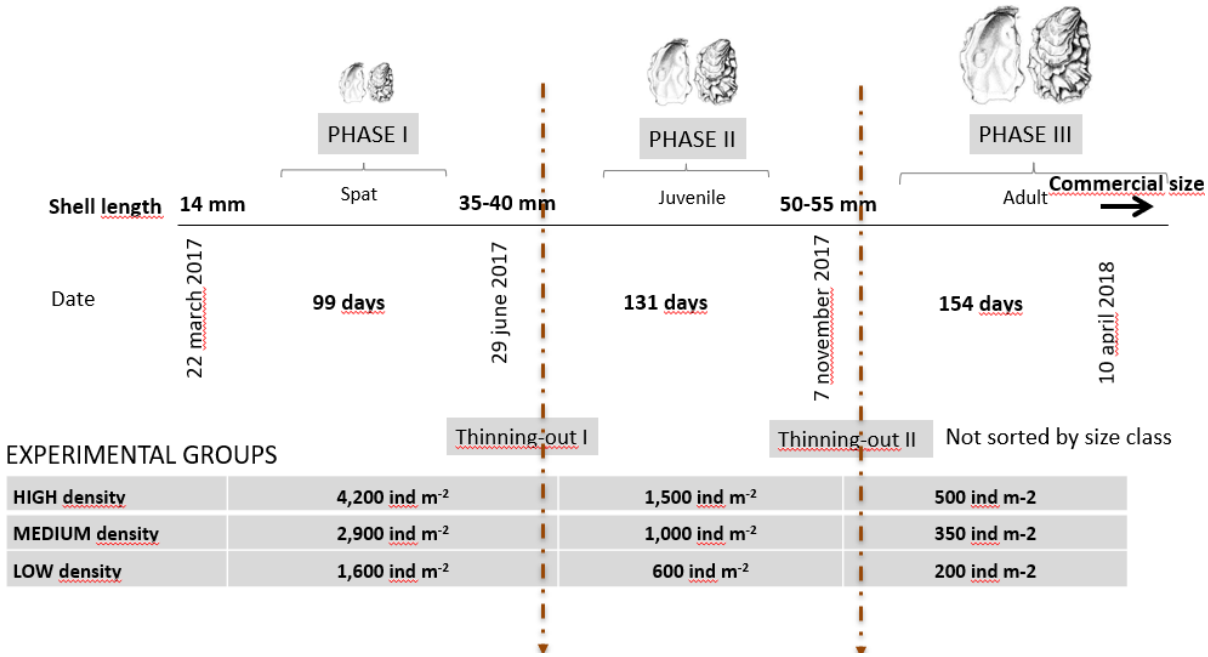


Figure 1: Diagram showing the densities evaluated in each phase of Pacific oyster culture.

Results and discussion

Figure 2 shows the monthly average size of the Pacific oyster cultured in Mutriku from March 2017 to April 2018. In the pre-fattening phase I and II, oysters cultivated at low density showed significantly higher values of length and weight than oysters grown at medium and high density. In the case of weight, low density oysters were the ones that presented the highest weight values, followed by the medium and high density oysters. It has been shown that stocking density has a negative correlation with individual growth in bivalves (Chávez-Villalba et al., 2010). However, after the second thinning-out, in the fattening phase, no significant differences were observed in the size of oysters cultured at different densities, although oysters grown at low density had higher weight values than oysters grown at medium and high density.

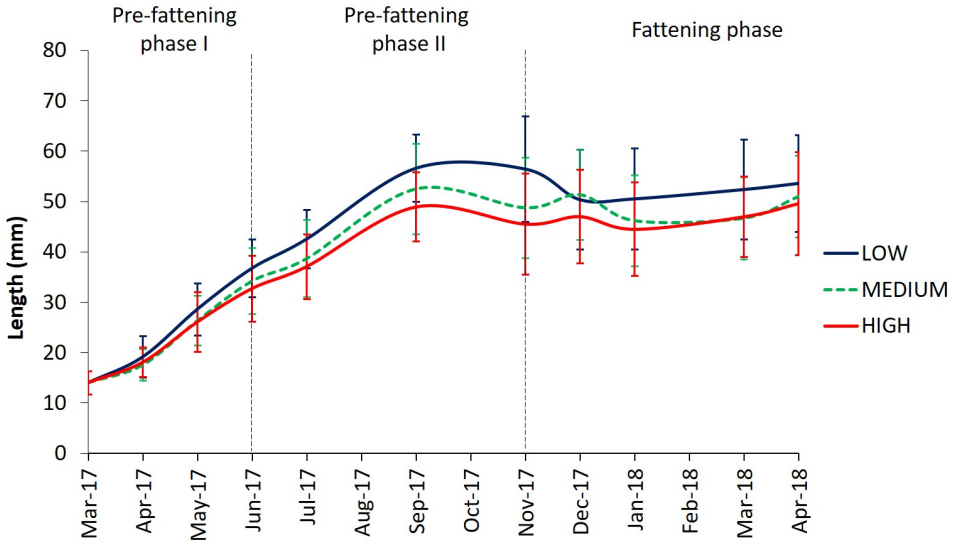


Figure 2: Diagram shows the monthly average size of the Pacific oyster cultured in Mutriku from March 2017 to April 2018.

Mortality values were low for oysters cultured at different densities. Monthly mortality ranged from 0 to 1.56%. The final cumulative mortality was 3.44% in oysters cultured at low densities, 5.61% at medium densities and 4.07% at high densities. In a previous study, no relationship was found between mortality and stocking density (Chávez-Villalba et al., 2010). In conclusion, the density seems to affect negatively the growth of the Pacific oyster reared on a suspended system in the Basque Country, obtaining greater growth rates at low densities. On the other hand, the density had no effect on survival values possibly because mortality is associated with other variables (i.e. pathogens) not measured herein.

Acknowledgments

This study has been funded by the Department of Agriculture, Fisheries and Food Policy, Directorate of Fisheries and Aquaculture of the Basque Government through the OSTRLL, DESOS and MUSSELS projects. A. Juez is a beneficiary of the Basque Government's technologists training scholarship. The authors thank the assistance of the AZTI staff and the Aquaculture School of Mutriku for their help during the sampling campaigns.

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Nutritional evaluation and antioxidant properties of *Anemonia Sulcata* and its symbiont microalgae *Symbiodinium*

Gloria Perazzoli*[1] , Laura Cabeza Montilla[1] , Rosario Martínez Martínez[2] , Cristina Mesas Fernández[1] , Milagros Galisteo Moya[3] , Raúl Ortiz Quesada[1] , Cristina Jiménez Luna[1] , Pedro Álvarez[4] , Jesús María Porres Foulquie[5] , José Carlos Prados Salazar[1] , Consolación Melguizo Alonso[1]

*Corresponding author: gperazzoli@ugr.es

[1] Instituto Biopatología y Medicina Regenerativa, Centro de Investigación Biomédica, Instituto de Investigación Biosanitaria de Granada (ibs. Granada), Dpto. de Anatomía y Embriología Humana, Facultad de Medicina, Universidad de Granada

[2] Departamento de Fisiología, Instituto de Nutrición y Tecnología de los alimentos, Centro de Investigación Deporte y Salud, Universidad de Granada, Granada, España.

[3] Departamento de Farmacología, Facultad de Farmacia, Universidad de Granada, Granada, España

[4] iMare Natural SL

[5] Departamento de Fisiología, Instituto de Nutrición y Tecnología de los alimentos, Centro de Investigación Deporte y Salud, Universidad de Granada, Granada, España

Keywords: Colon cancer, *Anemonia sulcata*, *Symbiodinium*, Antioxidant, Nutritional evaluation

Introduction

Anemonia sulcata has a high nutritional quality as a natural source of proteins and essential fatty acids in the field of health and diet. However, its application in the field of nutrients and the possible antioxidant effects are still not studied. The objective of this work is the use of the anemona and its microalga symbiont of the genus *Symbiodinium* to determine its nutritional value and antioxidant effect in colon cancer cell lines.

Material and methods

The biomass for the study was contributed by the company iMare Natural S.L. Studies of proximal composition and fatty acid profile for both compounds were carried out. An *in vitro* digestibility was performed and the antoxidant activity was measured by DPPH and ABTS. In addition, the antioxidant capacity of these compounds was tested in HT29 colon cancer cell line.

Results and conclusion

The proximal composition of both compounds, *A. sulcata* with and without symbiont, was very similar, obtaining a high content of water and protein with a similar fatty acid profile in which fatty acids of the Omega 3 series were found. In the determination of total polyphenols, a higher content was obtained in the ethanolic extract of the anemone with the symbiont, although when we analyzed its mass spectrometry no bioactive compound was identified. After *in vitro* digestibility, a high percentage was found to be potentially absorbable, in both types of compounds. It was also observed that after this digestibility the antioxidant capacity of both compounds is maintained. The antioxidant capacity tests with hydrogen peroxide were performed *in vitro* on the HT29

colon cancer cell line showing an increase in the percentage of cellular proliferation up to 33 per cent in the treatment with the crude homogenate of *A. sulcata* without the presence of its microalgae *Symbiodinium*. The results obtained indicate that extracts of *Anemonia sulcata* due to its antioxidant capacity, can have a protective effect against colon tumor cells *in vitro*, that supposes a new approach for the prevention of this pathology. However, more studies are still needed to know the potential of *A. sulcata* and its symbiont microalgae *Symbiodinium* in this tumor type.

Acknowledgments

We want to thank to the CEIMAR Foundation for funding this project and the company iMare Natural S.L. for providing the biomass of *Anemonia sulcata*

Impact on the intestinal microbiota of *Sparus aurata* fed with diets supplemented with the microalga *Nannochloropsis gaditana*

Isabel M. Cerezo*[1] , María Isabel Sáez-Casado[2] , Ignacio Ruiz-Járabo[3] , Miguel Angel Moriñigo[1] , Silvana Teresa Tapia-Paniagua[1]

*Corresponding author: b22ceori@uma.es
[1] Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga
[2] Departamento de Biología y Geología, Universidad de Almería
[3] Facultad de Biología y Ciencias del Mar, Universidad de Cádiz

Keywords: Intestinal miscrobiota, DGGE, *Sparus aurata*, *Nannocloropsis gaditana*, Feed

Introduction

Gilthead sea bream (*Sparus aurata*) is an important cultured fish species with great economic interest especially in the Mediterranean countries. Information of the potential benefits derived from the use of different diets on this fish species physiology is of high interest and currently, alternatives for the use of fishmeal are being sought to be used as food for aquatic animals. Vegetable flours represent good alternatives, although sometimes they do not provide all the basic nutrients for the cultured organism. In the other hand, the importance of the diets in the aquaculture is not only in the nutritional level but also can increase the resistance to infection and improve the intestinal permeability. In fact, the intestinal microbiota is a key for excluding potential invaders and maintaining health, stimulating both specific and non-specific components of the immune system and playing an important role for establishment and maintenance of the intestinal barrier. *Nannochloropsis gaditana* is a microalga that can be an alternative ingredient in the feed for juvenile seabream and can be hydrolyzed by enzymatic processes, increasing the bioavailability of components present in the algae, but the effect on intestinal microbiota have not been analyzed. For that, the aim of this work is to study the effect on the intestinal microbiota of sea bream of the microalga *N. gaditana* incorporated to the feed using a molecular method such as denaturing gradient gel electrophoresis (DGGE).

Material and methods

Nannochloropsis gaditana biomass was produced in closed tubular photobioreactors in La Estacion Experimental de las Palmerillas de la Fundacion Cajamar, following a standard procedure. The fresh algae were hydrolysed using commercial enzymes with cellulase activity (Vizcozyme®). Then, an experimental feed was formulated by El Servicio de Piensos Experimentales of the University of Almería. The diets used for this work were: i) CT: control diet; ii) RAB-C: 5% wet crude microalga; iii) RAB-H: 5% wet microalga hydrolysate; iv) FAB-C: 5% freeze-dried crude microalga; v) FAB-H: 5% freeze dried microalga hydrolysate. The feeding assay developed in the facilities of El Servicio Central de Investigacion en Cultivos Marinos of the University of Cadiz, where the juvenile specimens of *Sparus aurata* of 10-15 g of weight were divided into 400L tanks and were fed for 90 days with a commercial diet (control group) and a 5% *N. gaditana* supplemented diets (RAB-C, RAB-H, FAB-C and FAB-H). After 90 days, the fish were anesthetized, and the intestine was removed and divided into the anterior and posterior sections

for each fish. The samples were kept frozen until processed. DNA from the intestinal lumen was extracted following Martínez et al. (1998). DNA was amplified using universals primers for rDNA16S. The amplicons were separated by Denaturing Gradient Gel Electrophoresis (DGGE) (Muyzer et al., 1993).The gels were subsequently stained with $AgNO_3$ (Sanguinetti et al., 1994). DGGE banding patterns were analysed using the FPQuest Software version 4.5 (AppliedMaths BVBA, Sint-Martens-Latem, Belgium). A matrix of similarities for the densitometric curves of the band patterns was calculated using the Bray-Curtis index. Clustering of DGGE patterns was achieved by constructing dendrograms using the Unweighted Pair Groups Method with Arithmetic Averages (UPGMA). Shannon index, Species richness and Range-weighted richness (Rr) of the digestive microbiota also were calculated.

Results

Cluster analysis of DGGE band patterns obtained in all groups of samples showed intragroup similarity percentages $> 40\%$ in the anterior intestinal microbiota and $> 50\%$ in posterior intestinal microbiota. In the anterior microbiota, fish that received a control diet (C) shows a similarity of 10% compared with the rest. 40% is the similarity found between four diets analysed. FAB-C, FAB-H and RAB-C were grouped in the same cluster, but RAB-H was slightly separated. This shows that RAB-H produces a differential effect on the intestinal microbiota compared with the rest of the diets. The posterior intestinal microbiota, RAB-C and C diets showed a major similarity between them that with the rest of the diets. Diets that contained hydrolysed microalgae (FAB-H and RAB-H) showed a similarity greater than 60% between them.

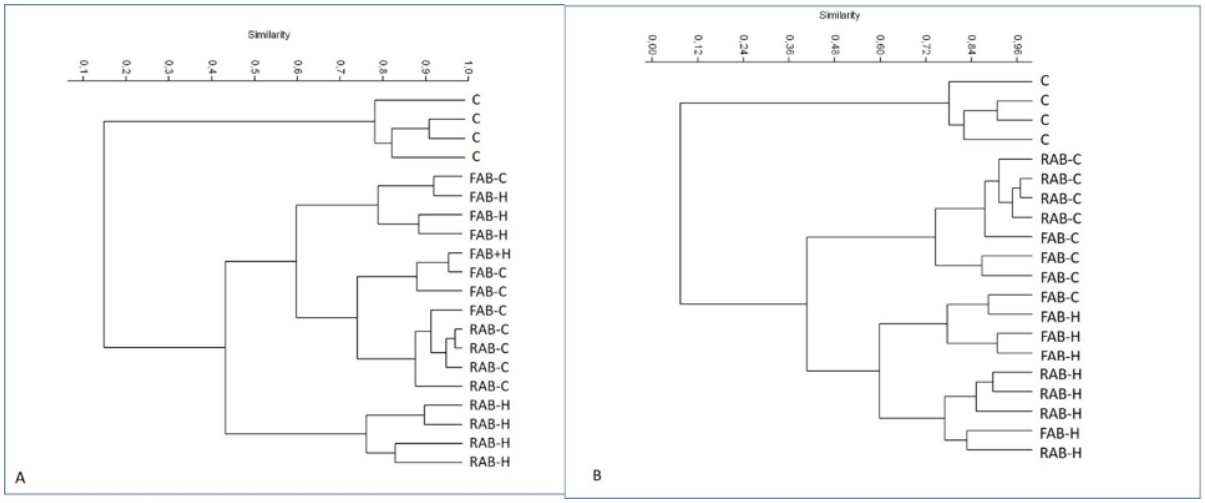

Figure 1: Cluster analysis of DGGE patterns of the intestinal microbiota of *S. aurata* specimens fed for 90 days with commercial diet (C), commercial diet supplemented with RAB-C: 5% wet crude microalga, RAB-H: 5% wet microalga hydrolysate, FAB-C: 5% freeze-dried crude microalga; FAB-H: 5% freeze-dried microalga hydrolysate. A: anterior intestinal microbiota. B: posterior intestinal microbiota.

The ecological index analysed showed that the addition of crude microalga decreased the Shannon diversity, richness and range-weighted richness values. However, diets that contained the microalga hydrolysate increased those values compared with the effect produced by control diet ($p < 0.05$).

Table 1: Species richness (R) (expressed as number of different bands) and Shannon index (H') values of intestinal microbiota of *S. aurata* specimens untreated (C) or RAB-C: 5% wet crude microalga, RAB-H: 5% wet microalga hydrolysate, FAB-C: 5% freeze-dried crude microalga; FAB-H: 5% freeze-dried microalga hydrolysate.

Diet	Shannon index (H')	Richness (R)	Range-weighted richness (Rr)
C	2.65±0.17	19.5±1.29	95.37±12.59
RAB-C	1.87±0.07[a]	13±1.41 [a]	42.62±9.54 [a]
RAB-H	2.87±0.17[b]	23.5±1.29 [b]	138.37±15.17 [b]
FAB-C	2.05±0.11[a]	19.5±1.29 [a]	95.37±12.59 [a]
FAB-H	2.79±0.17 [b]	22.5±1.29	126.87±14.52 [b]

Values are expressed as mean ± SD, (n=4). [a] indicates significant differences ($p<0.05$) respect to the C treatment and [b] indicates significant differences respect to RAB-C and FAB-C treatment, respectively.

All assayed diets showed dierences compared with control diet. Diets that show an increase of diversity index value are recommended because microbial biodiversity is essential in the protection of ecosystems against declines in their functionality (Wittebolle et al., 2008). Diets that contained hidrolysed *N. gaditana* showed an increase of those parameters but those changes should be analysed by NGS technologies for taxonomical characterization and functional approach.

Acknowledgments

This project has been funded for the CEIMAR foundation through the "Proyecto de Jóvenes Investigadores (CEIJ-C05.3)" and with the support of Lifebioencapsulation company.

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Transcription of genes involved in the intestinal integrity and permeability of *Sparus aurata* fed on a diet supplemented with 5% of wet hidrolized *Nannochloropsis gaditana*

Daniel Di Zeo*[1] , María Isabel Sáez-Casado[2] , Ignacio Ruiz-Járabo[3] , María del Carmen Balebona[1] , Miguel Ángel Moriñigo[1] , Silvana Tapia-Paniagua[1]

*Corresponding author: danieldizeo@uma.es
[1] Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga
[2] Departamento de Biología y Geología, Universidad de Almería
[3] Facultad de Biología y Ciencias del Mar

Keywords: Aquaculture, Sparus aurata, Permeability, Tight junctions, Nannochloropsis gaditana

Introduction

Aquaculture represents almost the 50% of the world's fish production for human consumption, and is probably the fastest growing food production sector. In the field of marine aquaculture, the gilthead seabream (*Sparus aurata*) is the most farmed fish in Spain and its culture is widely extended throughout the majority of Mediterranean countries. In order to achieve improved growing conditions, while also reducing the needs of fishmeal of this species and other carnivores, microalgae are being studied as a potential dietary substitute or supplement, as they are an important source of proteins, carbohydrates, and fatty acids of the omega 3 and omega 6 families; and a valuable content of many essential vitamins, minerals and antioxidant substances. However, the uptake of these nutrients will depend on the efficiency with which the fish is able to hydrolyse the cell wall of the algae and absorb them in the intestine (Vizcaíno et al., 2019).
In particular, the microalga used in this study, *Nannochloropsis gaditana*, is well known as a source of different valuable pigments, such as chlorophyll a, zeaxanthin, canthaxanthin and astaxanthin produced at high levels, and is also recognised as a good potential source of EPA, an important polyunsaturated useful in the prevention of several diseases. The bioactive products of this microalgae have led to an increasing interest on developing new functional ingredients with the aim to provide an additional health benefit, better absorption of nutrients and fastest growth (Cerezuela et al., 2012). However, despite the effects of these functional diets have been widely described as immunostimulant in fish, there is little information on how these compounds affect the intestinal integrity and permeability, in spite of its importance in nutrient absorbance, protection and other biological processes (Minghetti et al., 2017). In this study we analyse the expression of some of the most representative tight junctions' proteins and other proteins involved in the integrity of the intestine barrier of *Sparus aurata* specimens fed with a 5% *N. gaditana* diet. The complex of claudins, ocludins, zona-occludens, and cadherins provide one form of cell-cell adhesion in enterocytes and play a critical role in regulating the permeability barrier. Tubulin and vimentin play are an essential component of the cytoskeleton and the integrin 6β participates in epidermal cell-basement membrane adhesion (Pérez-Sánchez et al., 2015).

Materials and methods

The *N. gaditana* biomass was produced in closed tubular photobioreactors in La Estación Experimental de las Palmerillas de la Fundación Cajamar following a standard procedure. The fresh algae were hidrolized using commercial enzymes with cellulase activity (Vizcozyme). Then an experimental feed was formulated by El Servicio de Piensos Experimentales of the University of Almería including a 5% of the hidrolized algae (FAB-H diet). The feeding assay was developed in the facilities of El Servicio Central de Investigación en Cultivos Marinos of the University of Cadiz, where the juvenile specimens of *Sparus aurata* of 10-15g of weight were divided into 400L tanks and were fed for 90 days with a commercial diet (control group) and a diet supplemented with 5% of *N. gaditana* (FAB-H group). After 90 days, the fish were anesthetized and the intestine was removed and divided into the anterior and posterior sections for each individual. The samples were kept frozen until processed.
After extraction of the RNA from each intestine section, reverse transcription was performed adjusting to 500 ng of total RNA. Specific primers were used for the quantification of the relative expression of the following genes involved in the intestinal permeability and integrity: cadherin 1, cadherin 17, claudin 12, claudin 15, vimentin, integrin 6β ocludin, tubulin and zona-occludens 1. For normalization, the samples were analyzed in parallel with two reference genes, elongation factor 1α (EF1α) and ribosomal glyceraldehyde 3-phosphate dehydrogenase (GADPH). Threshold amplification values (Cq) greater than 40 were considered negative. Relative expression of mRNA was calculated using the Livak method 2(-$\Delta\Delta$Cq) normalizing with a geometric average of the two reference genes and in relation to the fish of each control group.

Results and discussion

Despite non statistically differences were detected, the results of the analysis of the relative expression show a tendency on down-regulation of the genes involved in the intestine permeability, especially in the posterior section (Figure 1).

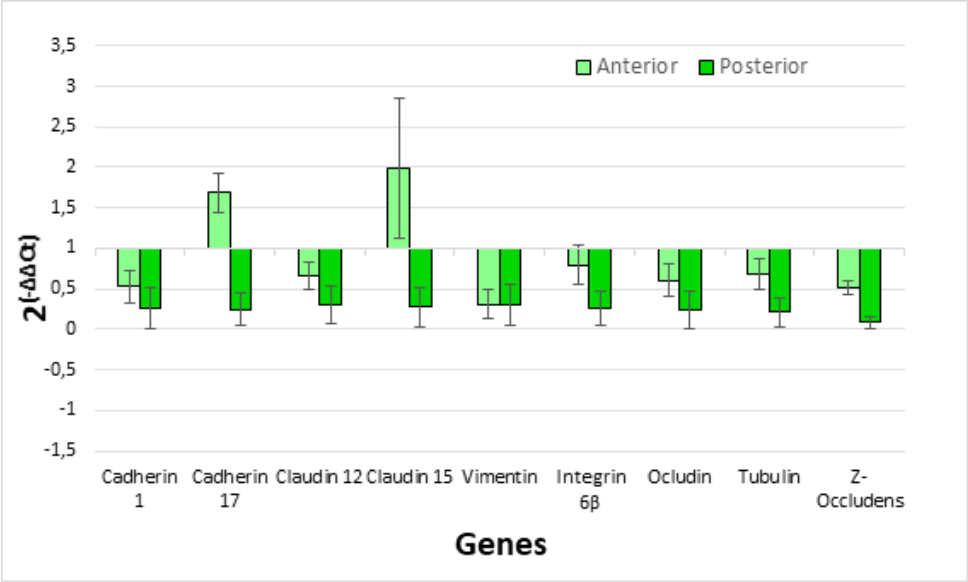

Figure 1: Quantification of the relative expression of genes involved in the intestine permeability of *Sparus aurata* fed with a 5% FAB-H diet.

The FAB-H group did not show significant changes in the expression of genes related to intestinal permeability compared to the control group, indicating that the gut integrity may

remain unaltered after the alga administration. In addition, in histological studies carried out with the same groups of fish, no disruption or symptoms of inflammation in the gut barrier were detected (personal communication). Commonly, an overexpression of the tight junctions' genes is associated with an unhealthy state, on which the epithelial cells up-regulate the expression of those genes to repair the decreased intestinal integrity, mainly due to a pro-inflammatory state. A tendency on a down-regulation of the genes analysed was observed in this study. Slightly less permeability may be related to better nutrient uptake as informed in some studies based on cell lines culture (Yu et al., 2013). To confirm whereas the integrity and permeability are affected or not, further studies analysing the role of the inflammatory response and nutrient absorption are needed.

Acknowledgments

This project has been funded for the CEIMAR foundation through the Proyecto de Jóvenes Investigadores (CEIJ-C05.3) and with the support of Lifebioencapsulation company.

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Intestine fatty acid composition of *Sparus aurata* fed on a diet supplemented with wet hydrolysate microalgae *Nannochloropsis gaditana*

Jorge García-Márquez*[1] , María-Isabel Sáez-Casado[2] , Ignacio Ruiz-Jarabo[3] , María del Carmen Balebona[1] , Miguel Ángel Moriñigo[1] , Silvana Teresa Tapia-Paniagua[1]

*Corresponding author: j.garciamq@gmail.com
[1] Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga
[2] Departamento de Biología y Geología, Universidad de Almería
[3] Facultad de Biología y Ciencias del Mar, Universidad de Cádiz

Keywords: Aquaculture, Sparus aurata, *Nannochloropsis gaditana*, Fatty acids, Intestine

Introduction

The development of strategies to improve the quality and to optimize the costs of production for fish feeding, particularly those related to microalgal supply, are highly desirable. Dietary protein plays a major role in providing a source of essential amino acids for protein accretion in new tissues. New protein ingredients for feeding fish should meet several requirements, the most important of which is providing high-quality protein, in terms of both quantity and bioavailability of well-balanced essential amino acids. Microalgae represent a heterogeneous source of protein, owing to the specific particularities of each strain, a fact that implies that the potential bioavailability of protein for a given microalgae cannot be easily predicted. Several microalgae species might represent an alternative ingredient of interest in fish feed production, due to their high protein and lipid content (Vizcaíno et al., 2019). Studies conducted on mammals have shown that the lipid composition of cell membranes in other tissues (such as intestinal cells) is highly affected by the dietary lipid source and content. The change of some fatty acid incorporated into the membrane phospholipid can alter the physicochemical characteristics of the membrane, which in turn can influence conformation, mobility and function of a wide variety of membrane-bound proteins (Cahu et al., 2000). Desaturation/elongation in enterocytes of PUFAs like 18:3n-3 (linolenic acid, LNA) and 18:2n-6 (linoleic acid, LA) to their PUFA derivates, i.e., 20:5n-3 (eicosapentaenoic acid, EPA) and 22:6n-3 (docosahexaenoic acid, DHA), and 20:4n-6 (arachidonic acid, ARA), respectively can enhance the nutrient absorption (Mourente et al., 2005). For this reason, modifications in fatty acid composition of the intestinal enterocytes of the fish should be considered in evaluating the potential of ingredients for use in the feeding of fish species. The aim of this study was to investigate the long chain fatty acids composition of intestinal tissue of juvenile gilthead seabream (*Sparus aurata*) in responds to formulated diet supplemented with *Nannochloropsis gaditana*.

Materials and methods

Nannochloropsis gaditana biomass was cultivated in a semi-industrial sized (3000-L) outdoor tubular photobioreactor (PBR) in continuous mode at La Estación Experimental "Las Palmerillas" (Fundación Cajamar, Almería, Spain) following standard procedure. The fresh algae were hidrolized as described by Vizcaíno et al. (2019). The experimental microdiet was formulated by El Servicio de Piensos Experimentales of the University of Almeria including 5% of fresh hidrolized algae (RAB-H diet). Gilthead seabream (*Sparus aurata*) juvenile (10-15 g initial body

weight) were provided by Servicios Centrales de Investigación de Cultivos Marinos (SC-ICM, CASEM, University of Cadiz, Cadiz, Spain; Operational Code ES11028000312), and maintained in the fish husbandry facility of the Faculty of Marine and Environmental Sciences (Puerto Real, Cadiz, Spain) until the beginning of the experiments. Fish were divided into 400-L tanks and fed for 90 days with a commercial diet (control group) and with the experimental diet (RAB-H group). After 90 days, intestine was removed and kept frozen until processed. The lipid content of the intestine tissue (IT) and the intestinal content (IC) were measured after extraction with chloroform/metanol (2:1 v/v) according to the method described by Folch et al. (1957). The lipids were dissolved in toluene and the fatty acids methyl esters (FAME) were obtained by transesterification with sulfuric acid (1%) in methanol (Christie, 2003).

Results and discussion

Total lipid (%) dispersion is shown in Figure 1. The total lipid percentage of the intestinal content of both diets (control and RAB-H groups) was higher than in the intestinal tissue. When compared intestinal tissue (IT) and intestinal content (IC) of both diets (control and RAB-H group) no significative differences were pointed.

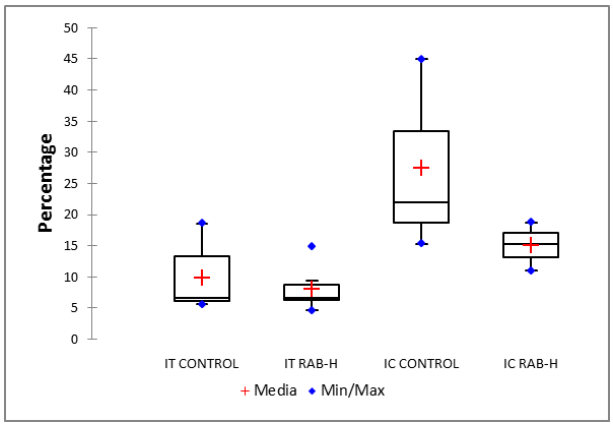

Figure 1: Box plot of total lipid percentage from intestine tissue (IT) and intestinal content (IC) of both diets (control group and RAB-H group).

Although the lipid percentage of the intestinal content of both diets (control and RAB-H groups) was higher than in the intestinal tissue, tendencies shows that the presence in lipid content does not imply a higher present in tissues. Levels of total n-3 PUFA present in the intestine tissue (IT) and content (IC) were higher in the group fed with the fresh hidrolized algae than the control group (Figure 2), mainly due to the increase of EPA and DHA content, but no significative differences were detected when compared.

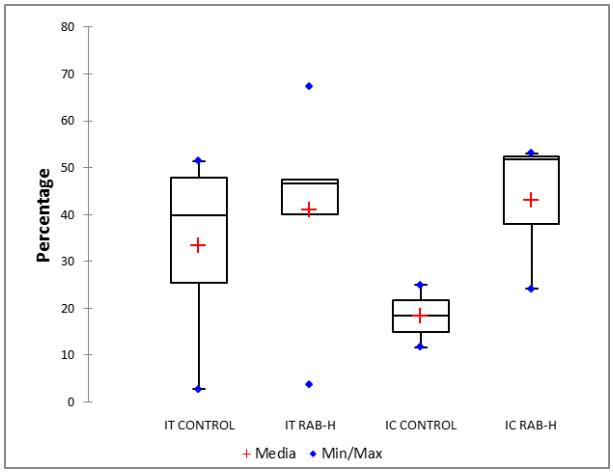

Figure 2: Box plot of total percentage of n-3 Poly Unsaturated Fatty Acids (PUFA) from intestine tissue (IT) and intestinal content (IC) of both diets (control group and RAB-H group).

In general, fatty acids like EPA or DHA increase intestinal permeability, leading into a better absorption of nutrients. Surface active compounds, like oleic (C18:1n-9c) and DHA acids, are related with the integrity of the intestinal epithelium and enhanced the paracellular absorption of poorly absorbed hydrophilic substances (Mourente et al., 2005). We observed a reduction in C18 acids and increase in EPA and DHA, which indicate that an experimental diet with N. gaditana can modify the absorption of nutrients. Despite this, further research on changes associated to fatty acids composition in the gut microbiota related to microbiota composition and intestinal barrier has to be done.

Acknowledgments

This work was funded by “Proyecto de Jóvenes Investigadores" (CEIJ-C05.3) supported by CEIMAR Fundation and also the support of Lifebioencapsulation Company.

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Advances in immunonutrition

Marian Ponce*[1] , Catalina Fernández-Díaz[1]

*Corresponding author: marian.ponce@juntadeandalucia.es
[1] Nutrition Laboratory, IFAPA Centro El Toruño, Junta de Andalucía

Keywords: Immunonutrition, Ulvan, DNA vaccine, Nanoparticles, *Senegalese sole*

Abstract

The losses in aquaculture production due to infectious diseases amount to 10% being the use of antibiotics widespread in order to reduce infectious diseases (Adams, 2019). Senegalese sole (*Solea senegalensis*) is an important species for aquaculture in southern Europe. In 2017, the world production of Senegalese sole was 1,656 tonnes (8.4% higher than in 2016) being the main producers Portugal, France, Iceland and Spain (APROMAR, 2018). However, the production of this species is affected by the appearance of bacterial diseases such as photobacteriosis. The aetiological agent is *Photobacterium damselae* subsp. *piscicida* (*Phdp*), a facultative intracellular, halophilic Gram-negative bacterium that has ubiquitous distribution and widespread antibiotic resistance. In recent years, aquaculture research is focused on the identification of bioactive compounds and/or nutraceuticals in order to limit the use of antibiotics and be able to conduct preventive strategies, thus diminishing the social and economic impact of diseases. Marine-derived polysaccharides (MDPs), isolated from marine macroalgae, microalgae, marine fungi, shellfish and corals, are propose as potential substitutes for antibiotics as they can enhance growth, immune response and disease resistance of aquatic animals. In this sense, ulvan, a MDP from green seaweed of the genus *Ulva*, has been shown to have immunomodulating, antioxidant, anticoagulant, anticancer, antiviral and antihyperlipidemic activities (reviewed in Kidgell et al. (2019)). In addition, disease prevention by vaccination is the most appropriate method to control infectious diseases in aquaculture. DNA vaccines offer a number of advantages over conventional vaccines due to its ability to stimulate cellular and humoral immune response. The use of adjuvants or immunostimulants is often necessary to increase vaccine efficacy (Dalmo et al., 2016). In this context, the project NANOVACSOL: Development of DNA vaccines against *Photobacterium damselae* subsp. *piscicida* and its evaluation in Senegalese sole immune system has arisen in response to the request of the aquaculture sector to obtain stable and effective vaccines. In order to investigate the possible use of ulvan as adjuvant in vaccines against *Phdp*, its cytotoxicity was evaluated in primary culture cells from liver of S. senegalensis as well as its immunomodulatory effects on *S. senegalensis* juveniles. Thereafter, a DNA vaccine against *Phdp* was developed and conjugated with chitosan-ulvan-nanoparticles for non-stressful delivery strategy. The most important advances of the project will be presented and discussed in this presentation.

Acknowledgments

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Synergistic effect of overexpression of genes involved in TAG biosynthesis and substrate supply on the lipid profile of *Chlamydomonas reinhardtii*

Rocío Rengel*[1] , Ana María Molina[1] , Federico García-Maroto[2] , Diego López-Alonso[2] , Javier Vigara[1] , Rosa León[1]

*Corresponding author: rrengel.dominguez@alu.uhu.es
[1] Laboratory of Biochemistry and Molecular Biology, University of Huelva, Avda. de las Fuerzas Armadas s/n, 21071 Huelva
[2] Laboratory of Genetics III, University of Almería, 04120, Almería

Keywords: Microalgae, *Chlamydomonas*, Genetic engineering, TAG

Introduction and objectives

Genetic manipulation of microalgae is one of the most promising approaches to achieve the enhancement of lipid production. Overexpression of key genes of the pathway or regulation of transcription factors have already been used to pursue this aim. We have proposed the synergy between overexpression of acetyl-CoA synthetase (*ACS2*), which, although is not directly involved in tryacylglycerol (TAG) biosynthesis, has been demonstrated to boost neutral lipid synthesis in nitrogen starvation (Rengel et al., 2018) and expression of the heterologous gene dicylglycerol acetyltransferase (*DGAT1*) from the boraginaceae *Echium pitardii* (Mañas-Fernández et al., 2009) that catalyzes the assembly of the last fatty acid chain into the TAG molecule.

Methodology

We obtained *acs2* and *acs2/dgat1 Chlamydomonas* transformants, and performed a screening using Nile Red fluorescence. Selected mutants were grown to determine their lipid profile in different TAG-induction conditions, such as nitrogen starvation or saline stress.

Results

In N-starved cultures, transformant *acs2/dgat1* shows an increase of 23% and 12% in total fatty acids (FAs) regarding the parental strain and *acs2* transformant, respectively. Introduction of *EpDGAT1* leads to a decrease in the total percentage of 16:0 and 18:3 FAs and an increase in 16:3, 18:1 and 18:2, among other variations. Preliminary data from Nile Red assays also indicate a higher neutral lipid ratio per cell number of the double transformant in high salinity conditions and in the simultaneous presence of both stresses. These results suggest that the combination of overexpression of key genes in specific pathways and the genes involved in their feeding substrates could be a good strategy for tailor-made lipid profiles in algae.

Acknowledgments

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Session 7

The Sea Knowledge

Biogeochemistry and Microbial Diversity

Carbon fluxes variability in Oligotrophic mediterranean systems

Natalia González-Benítez*[1] , Lara S. García-Corral[1] , Xosé Anxelu G. Morán[2] , Jack B Middelburg[3] , Marie Dominique Pizay[4] , Jean-Pierre Gattuso[4]

*Corresponding author: natalia.gonzalez@urjc.es

[1] Department of Biology, Geology, Phyisics and Inorganic Chemestry, King Juan Carlos University. C/ Tulipán s/n, 28933 Móstoles, Madrid, Spain

[2] King Abdullah University of Science and Technology (KAUST), Red Sea Research Center, Biological and Environmental Sciences and Engineering Division, 23955-6900 Thuwal, Saudi Arabia

[3] Department of Earth Sciences, Utrecht University, Princetonlaan 8A, 3584 CB Utrecht, The Netherlands.

[4] Sorbonne Université, CNRS, Laboratoire d'Océanographie de Villefranche, 181 chemin du Lazaret, F-06230 Villefranche-sur-mer, France

Keywords: Bacterial respiration, Bacterial production, Oligotrophic bays, Organic carbon metabolism, Northwestern Mediterranean

Abstract

The effect of temporal and spatial variability on the carbon fluxes coupling between heterotrophic bacterioplankton and phytoplankton was studied in two coastal oligotrophic sites (NW Mediterranean). Phytoplankton and bacterial production rates were measured under natural conditions using different methods (^{14}C-particulate and dissolved primary production,^{3}H-leucine bacterial production, light and dark changes in dissolved O_2). In the Bay of Villefranche, temporal variability was studied and revealed net heterotrophy during summer and autumn and net autotrophy during winter and spring. The Bay of Palma showed autotrophic areas at the western stations and heterotrophy at the eastern stations. The bioavailability of dissolved organic carbon (DOC) plays a key role on bacterial metabolism. Temperature increased DOC concentration and therefore, bacterial growth efficiency (BGE) only under autotrophic conditions. Total primary production was potentially able to sustain bacterial carbon demand (BCD) in autotrophic periods, whereas it did not in heterotrophic periods. On average, bacterial respiration (BR) represented 62 % of the total community respiration. BGE values were significantly higher in heterotrophic systems compared to those autotrophic. The results obtained in these oligotrophic bays suggested that shifts in ecosystem trophic status reflect the response of bacterial carbon processing (i.e. BGE) to the bioavailability of dissolved organic carbon and temperature.

Acknowledgments

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Biodiversity of halophilic microorganisms inhabiting Odiel solar salterns in Huelva, Spain

Patricia Gómez Villegas*[1] , Javier Vigara[1] , Rosa León[1]

*Corresponding author: patgomvil@gmail.com
[1] Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias Experimentales, Universidad de Huelva

Keywords: Metagenomics, Halophiles, Carotenoids, Odiel salterns

Introduction

Halophilic microorganisms are those that require high salinity to live. They have been identified in a variety of hypersaline environments, including salty lakes, salt flats and brines (Grant, 2006). In these ecosystems we find representatives of the three domains of life, *Eucarya*, *Bacteria* and *Archaea*, although biodiversity decreases as salinity increases (Ma et al., 2010). Extreme saline habitats are dominated by prokaryotes, bacteria and archaea, although we also find, to a lesser extent, eukaryotic microorganisms such as microalgae, fungus and protists.

Materials and Methods

In this work we present the characterization of the microbial population inhabiting the hypersaline water, 33% of salinity, of the Odiel salterns. Firstly, we analyzed the microbial population by two independent culture methods, the generation of 16S rRNA and 18S rRNA coding genes clone libraries and the massive sequencing of the aforementioned genes. In addition, we combined the use of molecular techniques with microbial culture to isolate and identify several archaeal and microalgal strains attending to their carotenoid production by HPLC (High Performance Liquid Chromatography).

Results

The results showed that the prokaryotic population is formed by only one bacterial species, Salinibacter ruber, and a great variety of archaea belonging to different genera, being *Halorubrum*, *Haloquadratum* and *Halobellus* the most abundant (Gómez-Villegas et al., 2018). Regarding to the eukaryotic microorganisms, we found mainly microalgae of the *Dunaliella* genus and protists belonging to the *Colpodella* genus. Finally, the isolated microorganisms showed ability to produce β-carotene or bacteriorruberin, which are molecules with a high antioxidant power, excellent for many industrial applications.

Acknowledgments

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Nanoplankton dominance in the phytoplankton community of an eutrophic, estuarine ecosystem (Guadalhorce river, Southern Spain)

Jorge Juan Montes-Pérez*[1,2] , Enrique Moreno-Ostos[1,2] , Jaime Rodríguez[1,2] , José María Blanco[1,2] , Valeriano Rodríguez[1,2] , Emilio Marañon[3]

*Corresponding author: jmontesp@uma.es
[1] Departamento de Ecología y Geología - Universidad de Málaga, (Málaga), Spain
[2] Campus de Excelencia Internacional del Mar - CEIMAR, Edifición Constitución de 1812, Avenida Carlos III, 3. Segunda Planta, 11003, Cádiz (Spain)
[3] University of Vigo. Department of Ecology and Animal Biology. 36310. Vigo. Spain

Keywords: Phytoplankton, Size-abundance spectra, Estuary, Lagoon, Guadalhorce

Introduction

Body size is a physical characteristic which is related with many physiological and ecological processes such as metabolic rates, light absorption, nutrient uptake, sinking rates and susceptibility to grazing (Peters, 1983). This individual characteristic emerges at the community level generating patterns related with food web organization and dynamics. Modelling size–abundance spectra (SAS) is a useful ataxonomic approach widely used to study pelagic communities in both freshwater and marine ecosystems (Sprules et al., 2016). In this work, we studied SAS of phytoplankton community in an estuarine ecosystem.

Material and methods

For this study, we selected eight small coastal lagoons located in Gualdahorce river estuary (Málaga, south Spain). Lagoons are distributed along a deep gradient from sea to land. The study period covered a whole year from 2015 to 2016. Physical and chemical variables as temperature, salinity, turbidity and dissolved oxygen concentration were measured *in situ*. Water samples were collected to determine dissolved inorganic nitrogen (DIN), soluble reactive phosphorous (SRP), silica, and chlorophyll *a* concentrations. Phytoplankton community was studied combining two techniques in order to cover the whole size spectrum:

- Picophytoplankton ($<$ 2 um equivalent spherical diameter, ESD) and nanophytoplankton cells (from 2 to 20 μm ESD) were analysed through flow cytometry.
- Microphytoplankton cells ($>$ 20 μm ESD) were analysed through inverted microscopy using the Utermöhl method (Utermöhl, 1958).

Flowcytometry optical signals were calibrated against measurements of cell volume through microscopy and image analysis. SAS were modelled through the correlation analysis between cell volume and abundance.

Results and discussion

All lagoons showed rather good linear SAS with slope values above -1 (Figure 1), indicating a higher proportion of biovolume in larger-size cell categories. This suggests that smaller organisms (picophytoplankton) are in disadvantage with respect to those of bigger cell size. Heterotrophic flagellates grazing, high solar radiation and fluctuation in nutrient availability are important constraints on picophytoplankton cells. However, data point distribution around the statistical model showed that cell size classes within the range of nanophytoplankton frequently reached higher abundances than expected. This was particularity evident in #1, #5, #7 and #8 lagoons, whose mean abundances were above the SAS linear model in the range of nanophytoplankton. Although other SAS (lagoons #2, #3, #4 and #6) did not show this tendency, maximum abundance values (red dashed lines) were also found within this size cathegory. The combination of all lagoons confirms that cells with intermediate size (roughly equivalent to the nanoplankton size range) are the dominant component of cell abundance, biovolume and 'bloom-forming' species in this estuarine ecosystem.

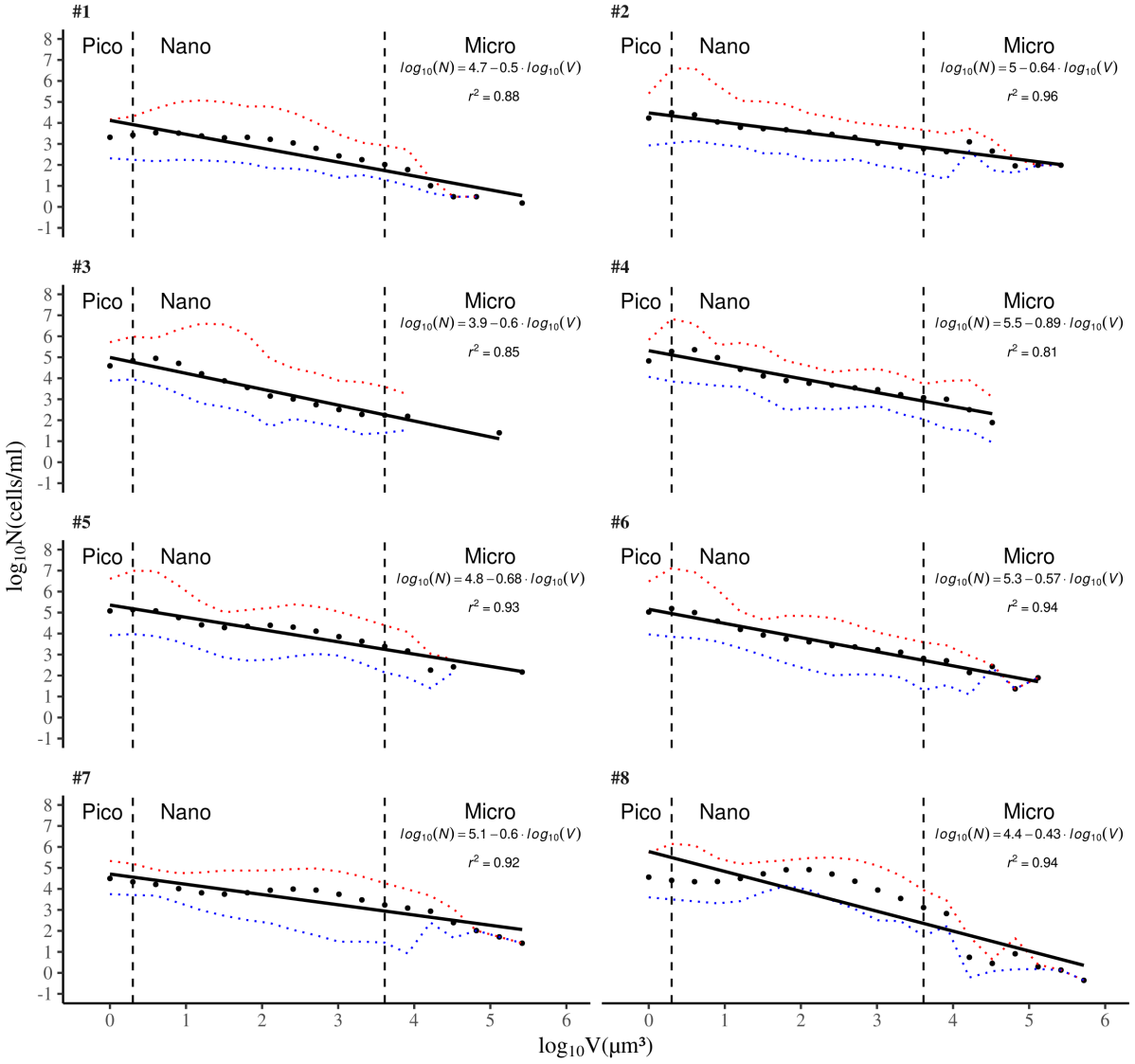

Figure 1: Integrated size-abundance spectra for each estuarine lagoon during the studied period. Black points are mean values, whereas red and blue points correspond to maximum and minimum values, respectively, recorded for each size.

Our findings support the hypothesis of Marañón et al. (2013) about the competitive dominance of intermediate-size cells because of their higher maximum growth rate under conditions of highly fluctuating nutrients inputs.

Acknowledgments

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Optimization of ultraviolet C radiation procedures to eliminate cyanobacteria from ballast waters

Alejandro Ortega Moreno*[1]

*Corresponding author: alejandro.ortegamoreno@alum.uca.es
[1] Universidad de Cadiz

Keywords: Ballast water, Cyanobacteria, Ultraviolet C

Introduction

Water is the most economic and common ballast in ships, but this imply a risk when organisms are transported by this way from a geographic location to other, as those species could become invaders and cause serious impact in ecosystems (Smayda, 2007). The International Maritime Organization (IMO) recently developed strict guidelines to minimize those potential damages. This was reflected in the International Convention for the Control and Management of Ships Ballast Water and Sediments (BWM, 2004), which imply that all ships loading ballast water are obligated to treat that water before release it in destiny locations (BOE, 2016). One of the most used methods to eliminate microorganisms from water is ultraviolet C (UV-C) radiation (Meulemans, 1987), which mainly affects the DNA. In this study, a comparison of the effect of UV-C radiation at different doses in two cyanophyceae species of the same genus (*Anabaena*), one from marine environments and other from freshwater environments, was performed, as ballast waters can be loaded from both environments. Cyanophyceae are known to produce toxins and grow fast, thus they are potential candidates to become a problem if accidental transport of those organisms occurs.

Materials and methods

Two species form the genus *Anabaena* were used: *Anabaena sp.* (CCMM 01/0101), isolated from marine environments, and *Anabaena variabilis* (SAG 1403-4b), a freshwater species. Evolution of cellular density was monitored by chlorophyll fluorescence in a multi-well reader (TECAN Genios 2000), with an excitation wavelength of 360 nm and a fluorescence emission of 670 nm. Cellular density and fluorescence was previously correlated by a calibration. Cyanophyte cultures at 10000 cells $\cdot$ mL^{-1} (initial density) were submitted to different radiation times in a collimation reactor equipped with a low-pressure 10W UV-C lamp. Cultures were radiated for 0 (controls), 1, 3, 5 and 7 minutes, thus implying in UV-C doses of 0, 9.66, 28.98, 48.30 and 67.62 mJ $\cdot$ cm^{-2} for *Anabaena sp.* and 0, 11.57, 34.71, 57.85 and 80.98 mJ $\cdot$ cm^{-2} for *Anabaena variabilis*, respectively. After exposition, Cyanopyceae population was cultured at 20°C and continuous light and monitored for 9 days.

Results and discussion

There is an evident inhibitory effect of UV-C for the marine species *Anabaena sp.*, even at the lowest dose (Figure 1). This effect is not so strong for *A. variabilis*, the freshwater species (Figure 2).

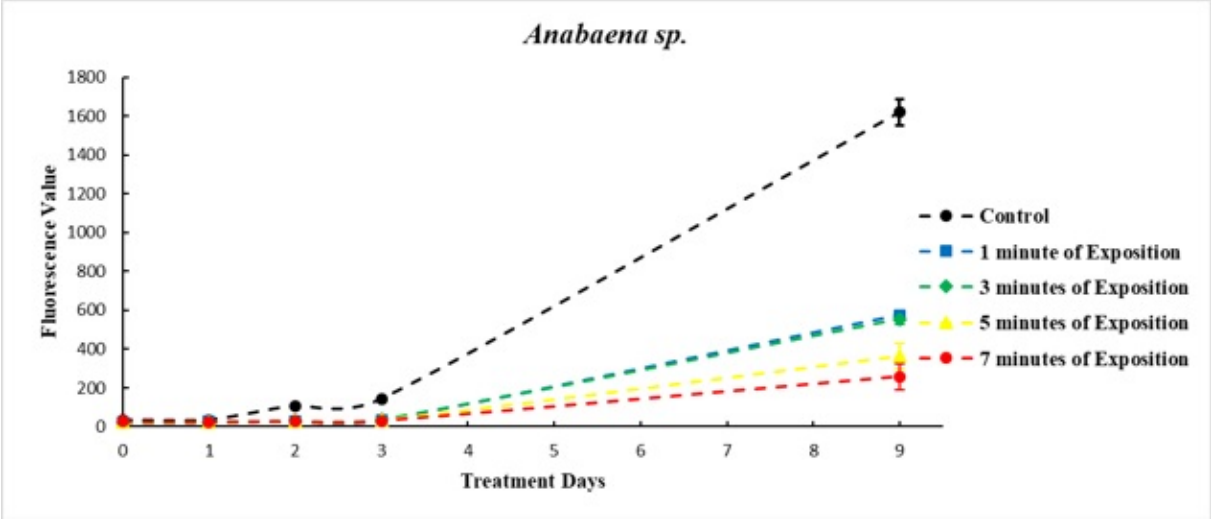

Figure 1: Population growth of *Anabaena* sp. (marine species) for 9 days submitted to different initial UV-C doses

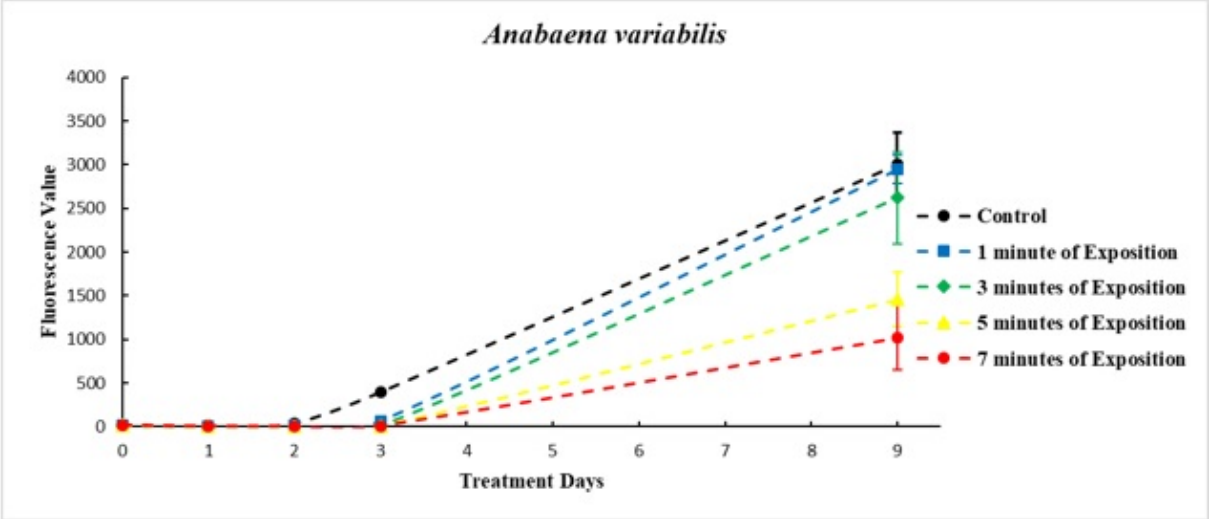

Figure 2: Population growth of *Anabaena variabilis* (freshwater species) for 9 days submitted to different initial UV-C doses

Growth inhibition was calculated with the dates that we measure the 9 day, comparing controls (100% growth, 0% growth inhibition) with treated cultures, and results can be observed in Figure 3. As can be noted, the marine species reach around 60% of growth inhibition after one minute of initial UV-C irradiance, while the freshwater species needs more than 5 minutes of initial UV-C irradiance to reach the same percentage.

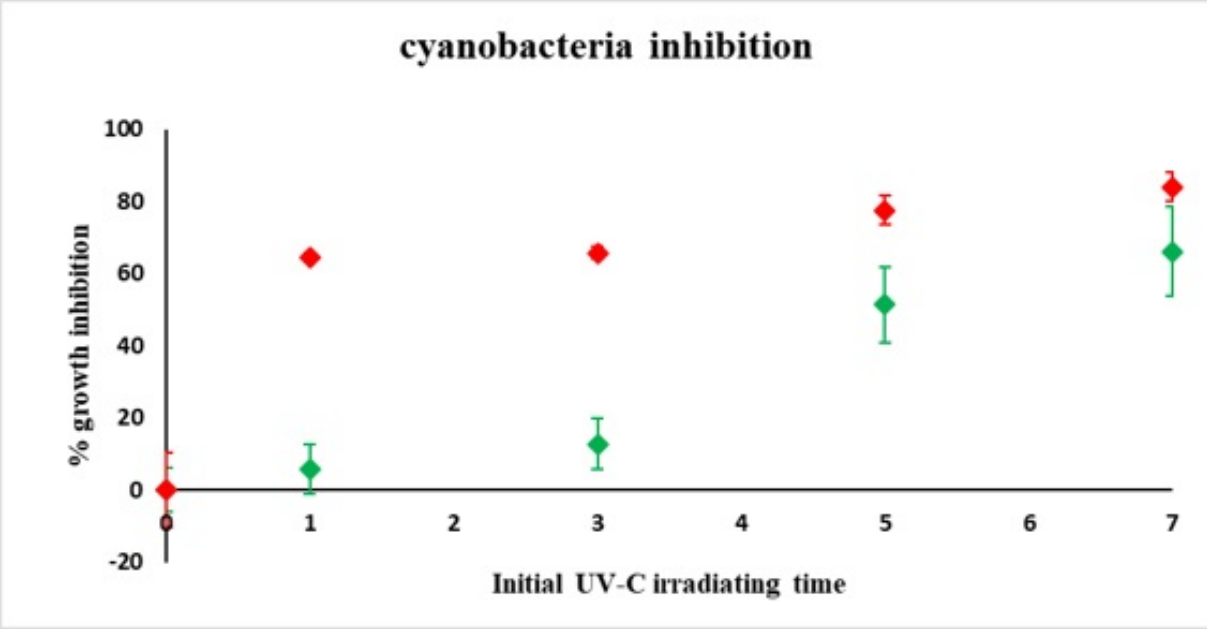

Figure 3: Percentage of growth inhibition vs. UV-C dose for *Anabaena* sp. (red) and *A. variabilis* (green).

Why this difference? Perhaps freshwater species are adapted to a more changing environment, while marine species occupies a more stable ecosystem. Freshwater masses are submitted to desiccation, changes in pH (high by day, low by night), temperature, etc. Those changes do not occur at the same time scale in marine environments. Referred to UV conditions, it is known that Cyanophyceae can develop gas vacuoles to control floating capacity. Thus, in case of naturally high UV conditions, marine species can sink to safer deeps, while freshwater species could not escape from this situation if the water mass where they habit is shallower. In this case, it is expected that freshwater species could have a more developed UV-reparation system for their genetic material.

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A *Mesodinium rubrum* (ciliate) red tide in the Alboran Sea

Teresa Conejo-Orosa*[1] , José Antonio Caballero-Herrera[2] , Juan Diego Castro-Claros[2] , Lidia Pino-de la Torre[2] , Leonardo José Pozzo-Pirotta[1] , Barbara Sellés-Ríos[3] , Lucía Rodríguez-Arias[4] , Simone Sammartino[5] , Candela García-Gómez[6]

*Corresponding author: orosa@uma.es
[1] Ecology and Geology Department, University of Málaga
[2] Animal Biology Department, University of Málaga
[3] Centre for Ecology and Conservation, University of Exeter
[4] Ecology Department, University of Cádiz
[5] Physical Oceanography Group, University of Málaga
[6] Centro Oceanográfico de Málaga, Instituto Español de Oceanografía

Keywords: Red tide, *Mesodinium rubrum*, Cryptophyte, RV UCADIZ, Alboran Sea

Introduction

The Mediterranean Sea is considered a hot spot for marine biodiversity. Within the Mediterranean basin, the Alboran Sea is one of the most productive ecoregions due to exchange between the Atlantic surface current and the Mediterranean deep current through the Strait of Gibraltar. This confluence generates a wide range of physicochemical and biological conditions with important implications for the dynamics of the plankton and benthic ecosystems (Skliris and Beckers, 2009). The current study was developed in the Alboran Sea aboard the RV UCADIZ, during the oceanographic campaign 'Mares de Andalucía'. Specifically, in the section between Málaga and Algeciras, in which seven points were sampled (Figure 1). In this study, we are going to focus in the sampling point CB01 (36° 22.317 N, 4° 39.186 W), where a potential red tide was glimpsed during navigation.

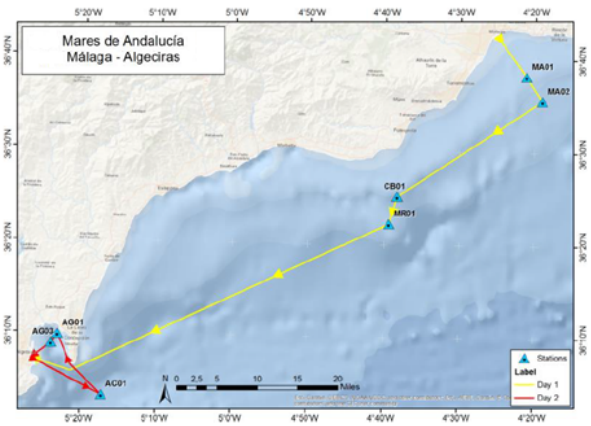

Figure 1: Navigation plan in the section Málaga - Algeciras during the campaign 'Mares de Andalucía' aboard the RV UCADIZ.

Materials and Methods

Two samples were collected from the red tide. They were collected from the surface, and at the depth of 5 meters. Temperature and salinity were measured using the CTD. The chlorophyll-a concentration was calculated by spectrophotometry following the trichromatic method. Microplankton community was observed by inverted microscope. Picophytoplankton, nanophytoplankton and bacteria communities were identified by flow cytometry. In addition, biodiversity was analyzed by metagenomic.

Results and Discussion

In the sampling point where the red tide was found, it was measured a temperature of 18.9°C and a salinity of 37. The chlorophyll-a concentration was 102.87 mg/l, while in the rest of the points sampled at the same deep (5 m) ranged between 0.93 and 13.23 mg/l. Regarding the nutrients, the value of total nitrogen (TN) was not substantially different in this sampling point (574 μg/l); however, the values of total phosphorus (TP) was between two and ten times higher than in the other points sampled (30.03 μg/l). Despite the abundance of cryptophytes represented the 2.7% of the phytoplankton abundance observed by flow cytometry (Table 1), the metagenomic analysis confirmed the presence of cryptophytes (18%). This analysis also revealed the identification of Mesodinium rubrum (45%) (Figure 2).

Table 1: Plankton abundance estimated by flow cytometry.

	Abundance (N° cells x ml^{-1})
Synechococcus	48601.78
Prochlorococcus	84120.55
Picoeukaryotes	3517.79
Nanoeukaryotes	523.72
Cryptophytes	3863.64
Bacteria	2133443.9

We found that the red tide was the result of the obligate mixotroph ciliate *Mesodinium rubrum* bloom. This ciliate is capable of retains cryptophyte organelles (chloroplasts, mitochondria, and nuclei) in the cytoplasm (Hansen and Fenchel, 2006).

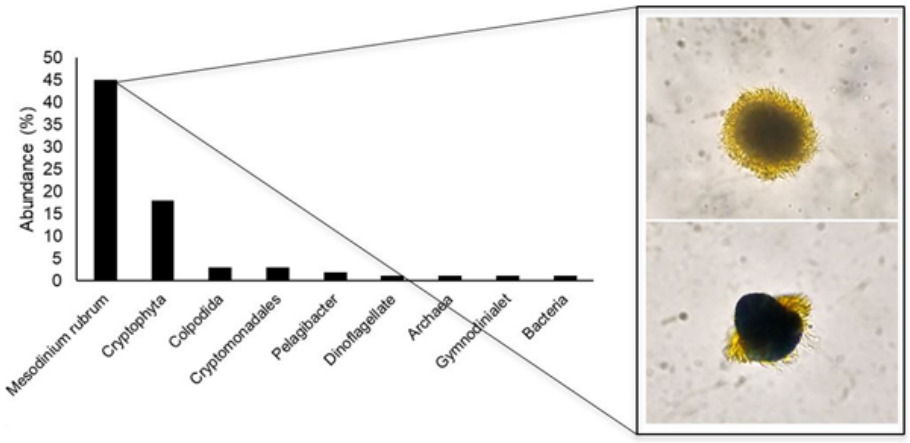

Figure 2: Abundance (%) found by metagenomic analysis. The pictures show two morphotypes of *Mesodinium rubrum* obtained during the campaign.

The environmental conditions needed to develop a *Mesodinium rubrum* bloom are not specific. This species can tolerate temperature and salinity variation, although it tends to grow up in temperature around 15°C. Indeed, Zhang et al. (2018) confirmed that nutrients were not the driving factor for the formation of a red tide event caused by *Mesodinium rubrum*.

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Dynamic of the carbon cycle in the estuary of the Guadalete River

Irene Pérez Guevara*[1]

*Corresponding author: irene95perez@hotmail.es
[1] Universidad de Cádiz

Keywords: Disolved inorganic carbon (DIC), Degree of saturation (Ω), Guadalquivir estuary, Organic alcalinity (A_{Org})

Abstract

Coastal areas are responsible for 20% of the global primary production, in spite of being only the 7% of the planet surface. In addition, unlike ocean waters, they generally act as a source of CO_2 to the atmosphere (Ingroso et al., 2016). These areas have a lower pH than oceanic areas, partly due to the high terrestrial contributions that they receive, since they concentrate 80% of the global population. That is the reason why ocean acidification is stronger in these areas and therefore, its effects on imbalance of precipitation/dissolution of $CaCO_3$ (Bushinsky et al., 2019):

$$CaCO_{3(S)} \overset{K'sp}{\longleftrightarrow} CO_3^{2-} + Ca^{2+} \quad (1)$$

Being K'sp the apparent constant or solubility product, and defined by the following equation. This constant has different expressions depending on whether it belongs to aragonite or calcite, main mineral forms in which $CaCO_3$ precipitates:

$$K'sp = [CO_3^{2-}] \cdot [Ca^{2+}] \quad (2)$$

The subject matter of this study is the characterization of the distribution and behaviour of different species associated with the inorganic carbon cycle in the estuary system of Guadalquivir River during the spring of 2019. This study has been carried out with two different tidal coefficients. For achieving this purpose, two campaigns were carried out aboard the B/O UCADIZ. The first one was in March from 19^{th} to 22^{th} and the second one was in April from 9^{th} to 11^{th}. In both cases, the same transect from Coria del Rio to the mouth of the Guadalquivir River was made, with 20 sampling station. The estuary system of Guadalquivir River, is located in the south-west of the Iberian Peninsula, and the total length of the river is about 657 km from its source to its mouth in the Gulf of Cadiz. It is a completely mixer or vertically homogeneous estuary (González-Ortegón et al., 2019). Besides, the longitudinal distribution of salinity is positive, having its maximum levels in the mouth and decreasing towards the most fluvial zone. The length of the estuary, its navigability and its protected marshes that are part of the Doñana National Park (ZEC Doñana ES0000024), make it one of the most important basins in Spain (Borrego-Marín and Berbel, 2019). Despite this, it is a very anthropized area because of the construction of dams, reservoirs, farmland and the existence of large cities near like Sevilla, Cordoba o Sanlucar de Barrameda. These modifications cause the decrease of the river flow, the changes in tidal dynamics, the salinization of the basin and the increase in the concentration of nutrients, organic matter and heavy metals (González-Ortegón et al., 2019). Total alkalinity (TA) and dissolved inorganic carbon (DIC) increase into the estuary, where they reach maximum concentrations of 4034 and 3760 μmolkg^{-1}, respectively. These high values in the river area are due to processes derived from biological activity such as breathing, and mainly the leaching of

the carbonated basin and the dissolution processes of $CaCO_3$, as indicated by the values above the unit registered for the TA/DIC ratio (Figure 1).

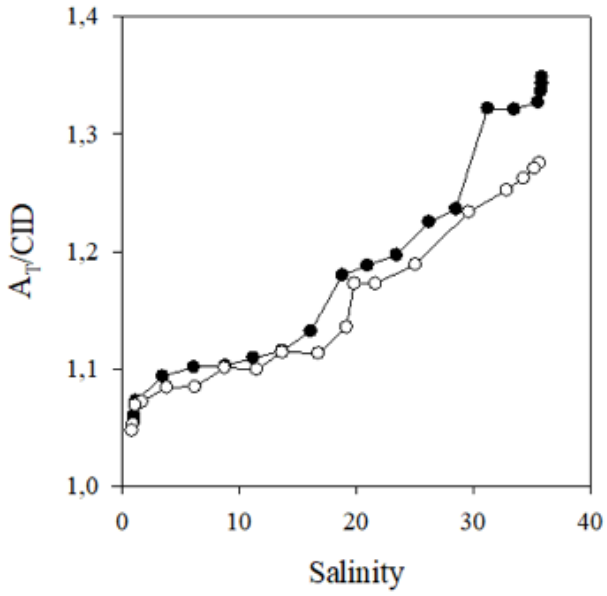

Figure 1: Variation of the relationship between total alkalinity (AT) and disolved inorganic carbon (CID) along the salinity gradient of the Guadalquivir river estuary, in the sampling carried out in March (black) and April (white) of 2019

The contribution of organic bases to the TA (A_{Org}) has maximum values in the most fluvial zone, contributing up to 19% in the TA (Figure 2). The apparent oxygen utilization (AOU) varies between -8,35 and 208,92 μmolkg^{-1}, presenting positive values in most stations. This indicates the predominance of the processes of degradation of organic matter throughout the estuary, especially in the most river area, coinciding with the highest values of DOC (335 μmolkg^{-1}) and nutrients.

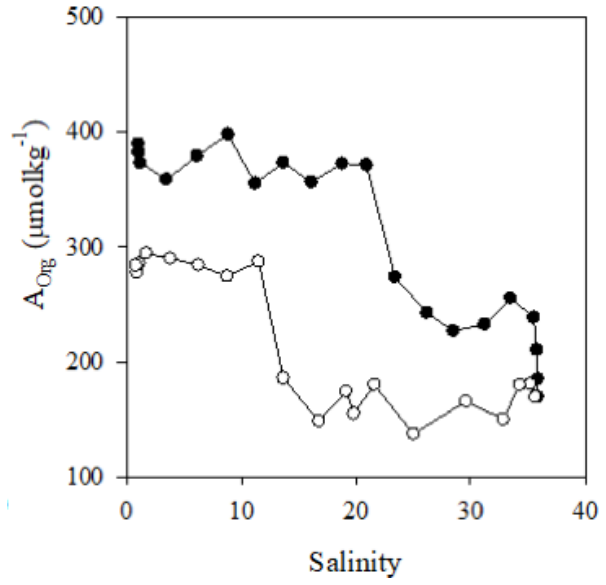

Figure 2: Variation of the concentration of Organic Alkalinity (A_{Org}) (μmolkg^{-1}) along the salinity gradient of the Guadalquivir river estuary, in the sampling carried out in March (black) and April (white) of 2019.

Equally, the high correlations between the DIC and nutrients indicate an intense remineralization of organic matter in the system, especially in the internal zone. The concentration of Ca^{+2} has been measured by potentiometric titration, and its value decreases towards the internal estuary area, with a variation range between 2,54 y 10,59 mmolkg^{-1}. On the other hand, the degree of saturation of $CaCO_3$ (Ω) has values greater than unit in all sampling station (Ω^{Ar}: 2,08-14,56; Ω^{Ca}: 3,23 - 24,99) (Figure 3).

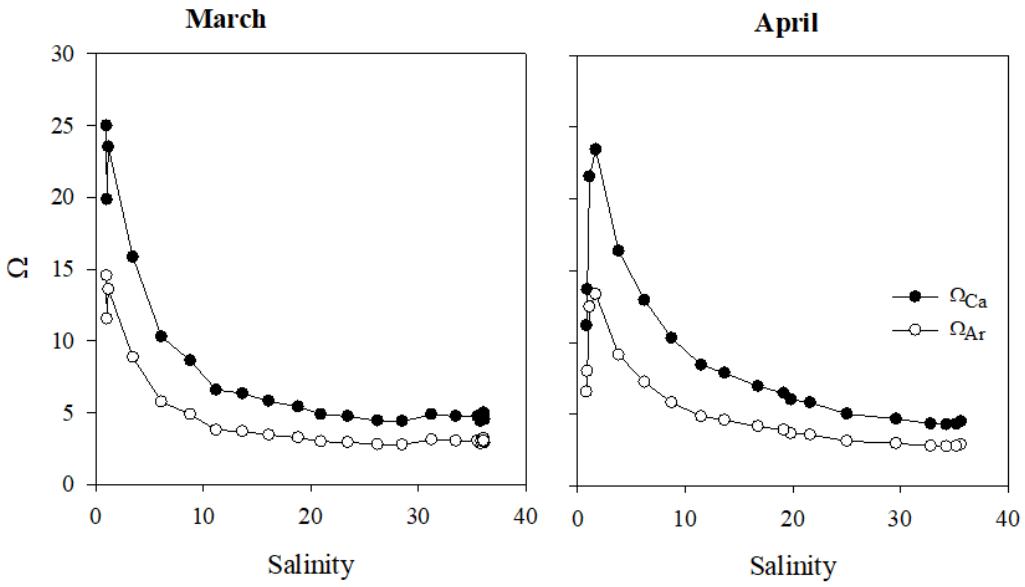

Figure 3: Variation of the degree of saturation of Aragonite (Ω^{Ar}) and Calcite (Ω^{Ca}) along the salinity gradient of the Guadalquivir river estuary, in the sampling carried out in March and April of 2019.

Traditionally, calcium concentration has been estimated using the Culkin and A. (1966) equation. This equation estimates calcium from its relationship with salinity. In this case, this equation assumes errors less than 1% at salinities greater than 30. On the other hand, at low salinities the error made is up to 90% (Figure 4).

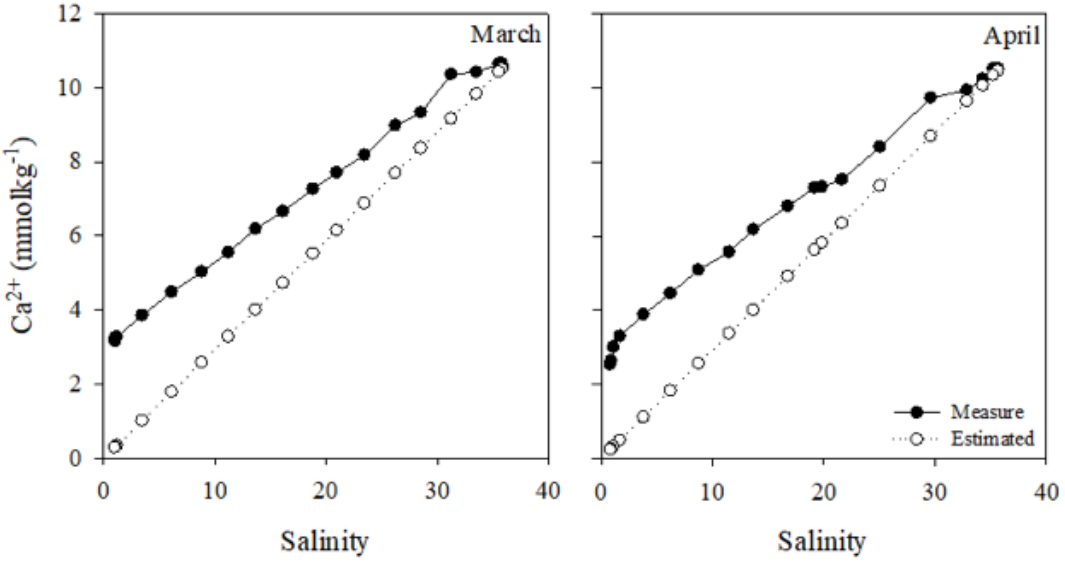

Figure 4: Variation of the measured and estimated calcium concentration along the salinity gradient of the Guadalquivir river estuary, in the sampling carried out in March and April of 2019.

From the relationship of the concentration of Ca^{2+} with salinity (R_2=0,994) the following expression has been obtained. This expression could be used for the estimation of calcium in the Guadalquivir estuary for this sampling:

$[Ca^{2+}]$ = 3,0176 + 0,2157 * S (mmolkg^{-1}) R_2 = 0,994 If more seasonal measurements of calcium were available over different years and seasons, such an expression could help estimate the concentration of Ca^{2+} in this system, instead of the Culkin and Cox equation as usual.

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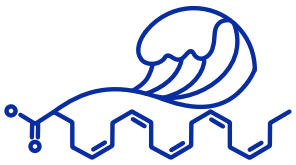
Session 8

Health and the Sea



Antitumor activity of functional extracts from *Anemonia sulcata* and its symbiont microalga *Symbiodinium*

Laura Cabeza Montilla*[1] , Rosario Martınez[2] , Cristina Mesas[1] , Milagros Galisteo[3] , Gloria Perazzoli[1] , Ana Rosa Rama[4] , Cristina Jimenez-Luna[1] , Pedro Alvarez[5] , Consolación Melguizo Alonso[1] , Jesus Maria Porres Foulquie[2] , Jose Carlos Prados Salazar[1]

*Corresponding author: lautea@ugr.es

[1] Institute of Biopathology and Regenerative Medicine, Biomedical Research Center, Institute of Biosanitary Research of Granada, SAS-University of Granada, Dept. of Anatomy and Human Embryology, Faculty of Medicine, University of Granada

[2] Department of Physiology, Institute of Nutrition and Food Technology, Sports and Health Research Center, University of Granada

[3] Department of Pharmacology, Faculty of Pharmacy, University of Granada

[4] Institute Biopatology and Regenerative Medicine, Biomedical Research Center, Institute of Biosanitary Research of Granada, SAS-University of Granada, Dept. of Health Sciences, University of Jaen

[5] iMare Natural S.L.

Keywords: Colorrectal cancer, *Anemonia sulcata*, *Symbiodinium*, Antitumor effect

Abstract

Half of the world's biodiversity is represented by marine organisms, being marine invertebrates one of the largest deposits of bioactive molecules. Anemones, included within this large group of marine invertebrates, have been the source for the obtainment of certain compounds with antihypertensive, antimicrobial, immunomodulatory and antithrombotic properties, but also with antioxidant and antitumor activities. Colorectal cancer is the third most common type of cancer worldwide, being the second with the highest mortality rate in 2018, behind lung cancer. Despite the recent advances in its treatment, the advanced stage in which there is metastasis, the only feasible treatment is surgery or the administration of chemotherapy with the drug 5-fluorouracil (5FU) associated with other chemotherapeutic agents (irinotecan, capecitabine or oxaliplatin) and/or monoclonal antibodies (cetuximab and bevacizumab). Despite the existence of these treatments, the therapeutic results are very limited. The improvement of its prognosis, therefore, requires the development of new strategies that add to the therapeutic activity, the preventive action. For this purpose, in this work we evaluated the antitumor activity in *in vitro* models of colon cancer of the marine invertebrate *Anemonia sulcata* that may or may not be associated with its symbiont microalga (*Symbiodinium*), which are cultivated by the company iMare Natural S.L. From this biomass, some extracts have been obtained that have allowed to determine the chemical composition, nutritional value and antitumor capacity of this marine organism. After carrying out different cell proliferation experiments using the MTT technique with colon cancer tumor cell lines treated with the different extracts of *A. sulcata*, very positive results have been obtained, highlighting the high antitumor activity shown with the homogenized treatments crude and of etaolic extracts of *A. sulcata* with and without the presence of its symbiont microalga *Symbiodinium*. Specifically, we have obtained values of IC50 (50% inhibitory concentration) of up to 14.1 and 16 μg/mL in the T84 human colon cancer cell line with the crude homogenate treatments of *A. sulcata* with and without the presence of its symbiont microalga *Symbiodinium*,

respectively, and of 112.5 and 59.1 μg/mL in the human colon cancer cell line HCT15, as well as 43.4 and 16.8 μg/mL in the mouse colon cancer cell line (from mice strain C57BL/6) MC38. Therefore, from these results we can conclude that *A. sulcata* both with and without the presence of its symbiont microalga *Symbiodinium* presents a good antitumor capacity in *in vitro* colon cancer models, which supposes a new approach for the treatment of this pathology. However, more studies are still needed to fully evaluate the excellent potential of this marine organism.

Acknowledgments

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Evidence of mycobacterial presence in *Pinna nobilis* infected by *Haplosporidium pinnae* maintained under quarantine conditions

Monserrat Lopez-Sanmartín*[1] , José Ramón López Fernández[1] , Roberto de la Herrán[2] , José Rafael García-March[3] , José Ignacio Navas[1]

*Corresponding author: monserrat.lopez.s@juntadeandalucia.es; monserratls@yahoo.es
[1] IFAPA Agua del Pino, Cartaya, Huelva, Spain
[2] Departamento de Genética, Universidad de Granada, Spain
[3] IMEDMAR-UCV, Universidad Católica de Valencia SVM, Calpe, Spain

Keywords: Pen shell, Pathology, Extintion, Mediterranean Sea

Introduction

Pinna nobilis Linnaeus (1758), also known as noble pen shell or fan mussel, is protected by European Union laws since 1992 and was incorporated into the Spanish Catalogue of Threatened Species in 2011 (Category: Vulnerable, Real Decreto 139/2011). Recently, the Spanish Government has catalogued it as endangered with extinction in Spanish waters (Orden TEC/596/2019, de 8 de abril). Emergency actions are being carried out by several research institutes from all over the country. The parasite, *Haplosporidum pinnae* is considered as the main responsible for the mass mortalities event (MME) of *Pinna nobilis* occurring since late 2016 (Darriba, 2017; Catanese et al., 2018), having been detected over all target animals of this study (López-Sanmartín et al., 2019) as well as other regions of Mediterranean Sea (Catanese et al., 2018; Carella et al., 2019). On the other hand, a mycobacterial disease has recently been associated with the silent mass mortality of the pen shell from Tyrrhenian coastline of Italy (Carella et al., 2019). The aim of the present work was to evaluate the presence of *Mycobacterium* sp. in dying specimens of *P. nobilis* infected by *H. pinnae* and maintained under quarantine conditions.

Material and methods

Pen shells used in this study were collected in Portlligat (Girona) and transported to IFAPA Agua del Pino (Huelva) facilities, where they were maintained under quarantine conditions. A total of 9 sick pen shells were analyzed. 7 of them were sampled twice, first when the symptoms of the disease were obvious (haemolymph and mantle) and another time when they were dying (all tissues). The remaining two specimens were only sampled in moribund gaping condition. In this way, biopsies from mantle, gills, labial palps, digestive gland, haemolymph, nephridium, gonad, adductor muscle and rectum were obtained.
The pen shell tissues were processed according to López-Sanmartín et al. (2019) for DNA extraction and to Catanese et al. (2018) and Carella et al. (2019) for histological analysis. Histological sections were stained with haematoxylin-eosin, Ziehl-Neelsen and Gram stain.
The 16S rDNA region was amplified by PCR using primer sequences derived from mycobacteria T39 (5′ -GCGAACGGGTGAGTAACACG-3′) and T13 (5′-TGCACACAGGCCACAAGGGA-3′) as described by Buller (2004). The PCR fragments were directly purified using Exo-Sap restriction enzymes (GE Healthcare) and sequenced by BigDye® Terminator technology (Applied Biosystems).

BLAST algorism was applied in 16S nucleotide sequences obtained in the present study. The overall mean distance was calculated among these sequences and sequences with highest BLASTN score from different species of *Mycobacterium* gender included *Mycobacterium* sp. described in Italian pen shells (Carella et al., 2019). ClustalW software was used to align the sequence and neighbour-joining tree was made by MEGA 6 software, using Tamura 3-parameter method with 1000 bootstrap replicates.

Results and discussion

The molecular analysis confirmed the presence of *Mycobacterium* sp. in the 9 pen shell analysed. In a previous study, *H. pinnae* presence was quantified by qPCR in this samples (López-Sanmartín et al., 2019). The histological analysis confirmed the presence of *H. pinnae* in connective and digestive epithelium of all fan mussels and also identified Ziehl-Neelsen-positive Gram-positive mycobacteria-like structures, mainly within the hemocytes in peripheral connective tissue around digestive gland.
A total of 33 sequences showed to be similar (upper to 99%) to 16S subunit of *Mycobacterium* sp available in NCBI database. The sequences obtained showed an average distance of 0.022. Blast analysis revealed that the sequences from this study were grouped together with *Mycobacterium* sp. sequence previously characterized such as from pen shell (Carella et al., 2019) 2019) and human mycobacterium (*M. tuberculosis*, *M. florentinum*, *M. shigaense*, *M. somatepiae*, *M. sherrisii*, *M. genavense*, *M. simiae*, *M. triplex* and *M. lentiflavum*) and moray eels (*M. montefiorense*). The phylogenetic analysis showed that at least two different groups of sequences were detected in the pen shell tree.
Mycobacteria group is ubiquitous in the marine environment, their DNA detection in this study would not have to be synonymous with disease. However, histological analysis confirmed the presence of mycobacteria-like structures in tissues. Mycobacterial infections have been associated with silent mass mortality of *P. nobilis* from Italy Carella et al. (2019). However, in this study, *H. pinnae* was also detected in each of pen shell, while in Italy, it was detected in only one of the fifteen specimens examined.
The present study confirms that *P. nobilis* may be infected both by *H. pinnae* and mycobacteria at the time of its death during the mass mortality event. Therefore, these results support the hypothesis that the mycobacterial disease and *H. pinnae* infection are associated with the mortality episodes of *P. nobilis*. Anyway, it is necessary to carry out more studies to determine the role of both infections in the MME of fan mussel and thus optimize rescue efforts.

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Incidence and control of *Neobenedenia melleni* in greater amberjack *Seriola dumerili* in breeding facilities in Tenerife

Andrea Villena*[1] , Salvador Jerez[2] , Beatriz Concepción Felipe[2] , Amador Misol[2] , Flor Correa-Fiz[3] , Roberto Dorta[4] , Covandona Rodríguez[1] , Jose Antonio Pérez[1]

*Corresponding author: anviro93@gmail.com

[1] Departamento de Biología Animal, Edafología y Geología. Universidad de La Laguna, Santa Cruz de Tenerife, España.

[2] Instituto Español de Oceanografía. Centro Oceanográfico de Canarias. Vía Espaldón, Dársena Pesquera, Parcela nº 8, 38180 Santa Cruz de Tenerife, España.

[3] IRTA Institute of Agrifood Research and Technology. Barcelona, España.

[4] Departamento de Matemáticas, Estadística e Investigación Operativa. Universidad de La Laguna, Santa Cruz de Tenerife, España

Keywords: *Neobenedenia melleni*, Monogenean parasites, Plasma parameters, *Seriola dumerili*, Transfection (fecal microbiota transplantation, FMT)

Introduction

The greater amberjack (*Seriola dumerili*) is one of the species of greatest interest for the future development of aquaculture. However, high incidence of pathologies especially those caused by monogenean parasites, as the genus *Neobenedenia*, are an important limitation for the commercial production of this and other cultivated *Seriola* species around the world. Juvenile and adult parasites attach to the skin, fins and eyes of fish, they migrate through the epithelial surface feeding and causing in turn hemorrhage, inflammation and hyperproduction of mucus which can cause secondary infections and massive mortalities in culture conditions, mainly due to their direct life cycles and the difficulty of detecting them sufficiently in advance. The aim of the present study was to evaluate and develop alternative control methods against the monogenean parasite *Neobenedenia melleni* in greater amberjack (*S. dumerili*) farming facilities. Some were based on the mechanisms responsible for the specificity of the binding between the parasite and the host fish, and others, focused on testing reinforcement methods of the immune system of *S. dumerili*, through the modification of the intestinal bacterial flora by fecal microbiota transplants (FMT), a technique recently tested on fish (Smith et al., 2017).

Material and methods

96 specimens of greater amberjack juveniles (*S. dumerili*) (210,0 ± 68,6 g) where was detected *N. melleni* infection, were randomly divided into 12 indoor cylindrical 1 m^3 tanks. Fish were maintained under natural conditions of photoperiod, water salinity and temperature (19,0 ± 0,3 ^oC). The level of parasitation by monogenean parasites was monitored by dish traps placed in the tanks to collect monogenean eggs released by adult parasites (Cejas et al., 2014). In the immersion treatment or "bath" in mannose solutions, the fish, divided into 4 homogenous group in triplicate, were treated with a concentration of 0 (Control), 30, 50 and 80 mM and their effect was examined for 12 days later. In the fecal microbiota transplantation (FMT) were applied 4 treatments by triplicate: Control (C); transfection with pig faeces (*Phacochoerus*

africanus) (TF) and transfection with faeces of gilthead seabream (*Sparus aurata*) with (TSA) and without (TS) previous dose of antibiotic (sulphanilamide), analyzing the effect for 34 days post-transfection. The specimens of gilthead seabream came from the same facilities, and even though they were parasitized by *N. melleni*, did not show symptoms or mortality associated. The objective of antibiotic was to eliminate native bacterial flora and trying to make the exogenous flora (donor) were installed more easily. The FMT was carried out orally by syringe for 3 consecutive days. The dose was 0.3 mL of stool suspension/ fish /day. All the fish were weighed and measured by taking blood samples from the caudal vein using a heparinized syringe on days 0, 7, 15, 21 and 34 post-transfection. With the samples, hematological parameters were analyzed (hematocrit, red and white blood cells, and mean corpuscular volume, MCV), and plasmatic (glucose, triglycerides, total cholesterol, lactate, chloride, sodium and potassium), as potential indicators of the physiological state of fish.

Results and discussion

The tested methods did not negatively affect the growth, condition and survival of the specimens. Fish treated with 30 mM of mannose showed a 62% reduction in the number of eggs emitted daily in the tank, 5 days after treatment, while in the control treatments, 50 mM and 80 mM the reduction was 15, 39 and 2% respectively. The lower effect of the higher concentrations of mannose could be due to the fact that it is not the only carbohydrate of *N. melleni* involved in the binding to the lectin (mannose binding lectin, MBL) of the host or that in the union of the parasite to the host participate, in a combined way, more than one MBL (Ohashi et al., 2007). The treatments of FMT did not show an immediate effect on the number of *N. melleni* eggs released, although some hematological and plasmatic parameters showed different behavior. For example, MCV decreased over time in the control (C) and transfection treatments with pig faeces (TF), while in the transfection treatments of gilthead seabream with and without previous dose of antibiotic (TSA and TS) the trend was opposite, being the TSA treatment the one that showed a remarkable increase (310,0 μm^3 final compared to the initial 241,5 μm^3), which could be indicative of a reduction of the anemic state of the parasitized fish. The levels of sodium, potassium and chloride were similar in the different treatments. The high levels of lactate (35.2-195.4 mg dL^{-1}), triglycerides (83.5-1261.5 mg dL^{-1}) and plasmatic cholesterol (183.3-360.4 mg dL^{-1}) got, independent of the treatment used, suggests a possible alteration of the lipid metabolism of the specimens as a consequence of the stress associated with parasitization by *N. melleni*. The significant reduction of plasma triglycerides and cholesterol on the seventh day post-transfection in the TSA treatment could be related to the improvement in the regulation of lipid metabolism, an effect that could be lost in time due to the recovery of the initial levels observed from day 15 (Figure 1).

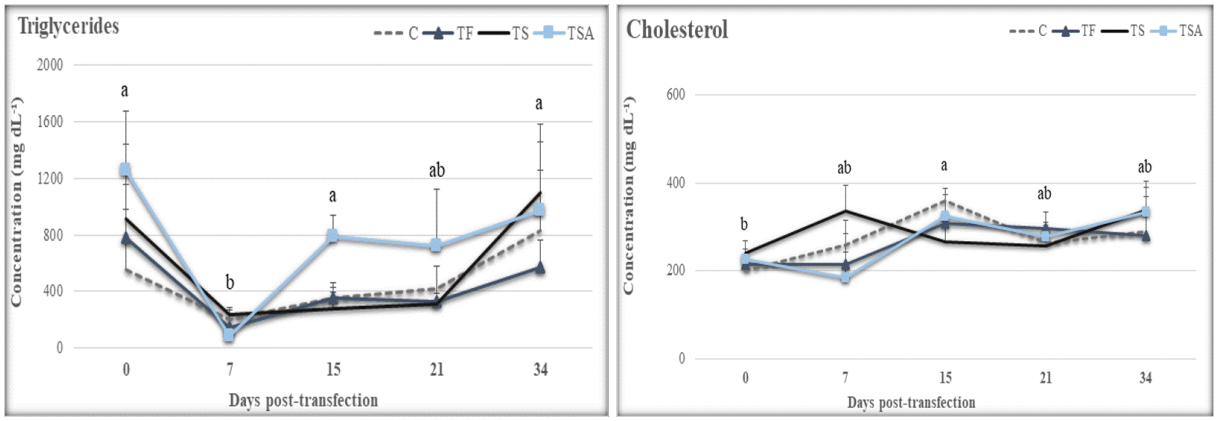

Figure 1: Evolution of plasma levels of triglycerides and cholesterol ($mg \cdot dL^{-1}$) during the post-transfection experimental period. Different letters indicate significant differences ($p < 0.05$).

Future experiments are expected to apply a second dose of reinforcement that has reached higher success rates in applications in humans infected with the bacterium *Clostridium difficile* (CDI) (Borody et al., 2013).

Acknowledgments

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Screening for UV absorbing compounds in red macroalgae: Cosmeceutical applications

Julia Vega*[1] , Félix L. Figueroa[1]

*Corresponding author: juliavs90@gmail.com
[1] University of Málaga

Keywords: Antioxidant activity, MAAs, Macroalgae, Photoprotection, Polyphenols

Introduction

In the last decades, the increase of the ultraviolet (UV) radiation due to the reduction of the ozone layer and the changes in the lifestyle with higher sunlight exposure, have increase the concern about the negative effects of these type of radiations in the human health and the environment. The cosmetic industry is constantly looking for innovations like new sources of active principles, especially natural ones due to its good photo and thermostability and the biodegradable character. Marine organism, specially algae, have acquired a great biotechnological interest in this area due to its high content of bioactive compounds with antioxidant, antimicrobial, inmuno-stimulant and photoprotective properties (Kim and Chojnacka, 2015) that can be beneficial for the skin health. This work was focused on two different molecules: Mycosporine like aminoacids (MAAs), small molecules implicate in the photoprotection against UV radiation (Carreto and Carignan, 2011) and predominantly found in red algae. And polyphenols, secondary metabolites mainly involved in the chemical defense of algae, with the capability of absorbed UV radiation (Pavia et al., 1997) and predominantly found in brown algae. Eight red macroalgae from diverse genus (seven collected from coastal areas of Málaga and Cádiz, and one from culture), extracted in four different solvents (H_2O, ethanol 50, 80 and 100%) were screened analyzing the content of UV absorbing compounds. From the extracts were determine the UV spectrum, the concentration of phenolic compounds and MAAs, and the antioxidant activity.

Materials and methods

The UV-spectrum were measured using a UV-Spectrophotometer. Phenolic compounds were quantified according to the Folin-Ciocalteau method using phloroglucinol as standard. Mycosporine like aminoacids (MAAs) content were calculated through HPLC. The antioxidant activity was determined using the ABTS method based on the free radical scavenging effect and a standard solution of trolox was used as reference. All analysis was made according to Álvarez Gómez (2017).

Results and discussion

The highest content of polyphenols was observed in *Osmundea pinnatifida* and *Porphyra umbilicalis* (aprox. 12 mg g^{-1} DW), although some differences appeared depending on the solvent used, i.e. in *P. umbilicalis* the best solvent was H_2O whereas in *O. pinnatifida* was ethanol 80% (Figure 1). *Ceramium rubrum* and *Crassa cornea* also showed a high content (aprox. 8 mg g^{-1} DW). In general, the best solvent for the extraction of these molecules was H_2O and the worst ethanol 100%.

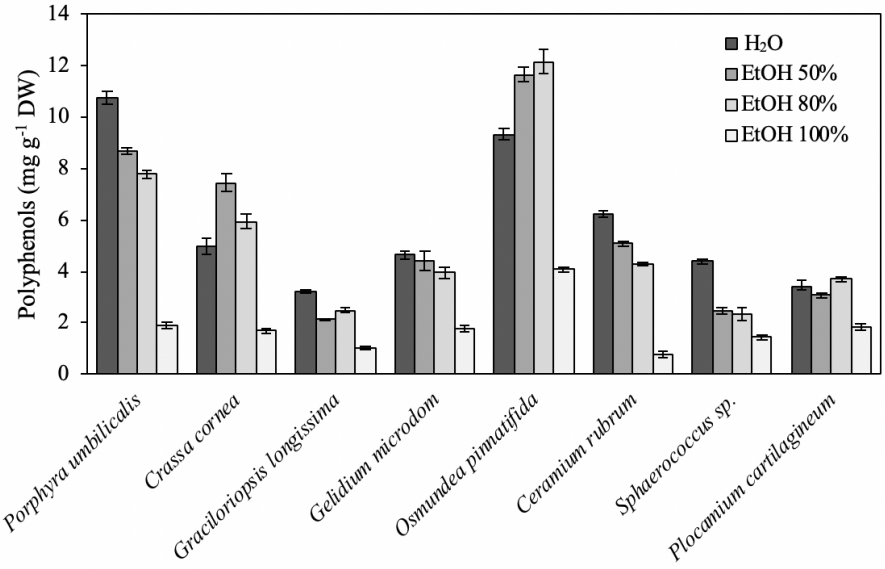

Figure 1: Phenolic compounds (mg g^{-1} DW) extracted in the different solvents, expressed as average ± standard deviation.

P. umbilicalis showed the highest MAAs content with differences (aprox. 4.5 mg g^{-1} DW) being porphyra-334 the one with the highest percentage (80%). *Gelidium microdom*, *O. pinnatifida* and *C. rubrum* also showed a high MAAs content (approx. 1 mg g^{-1} DW) but much lower than *P. umbilicalis* (Table 1). In *G. microdom* the predominant MAAs was shinorine, in *O. pinnatifida* asterine-330 and in *C. rubrum* also shinorine. In *C. rubrum* also appeared an unknown UV absorbing compound with a high concentration, also observed in other species from the family Ceramiaceae (non-published results). In all the cases, the best extraction solvent was ethanol 80% and the worst was ethanol 100%. In the UV-spectrum, *P. umbilicalis* presented the highest peak with difference at 332 nm demonstrating the highest content of MAAs in this specie.

Table 1: Total MAAs (mg g^{-1} DW) extracted in the different solvents, expressed as average ± standard deviation. n.f.: not found.

Species	H_2O	EtOH 50%	EtOH 80%	EtOH 100%
Porphyra umbilicalis	1.67 ± 0.09	1.49 ± 0.09	4.53 ± 0.11	0.68 ± 0.04
Crassa cornea	0.11 ± 0.01	0.12 ± 0.01	0.36 ± 0.02	0.03 ± 0.01
Graciloriopsis longissima	0.05 ± 0.00	0.06 ± 0.01	0.29 ± 0.03	0.01 ± 0.00
Gelidium microdom	0.21 ± 0.02	0.16 ± 0.01	1.16 ± 0.05	0.02 ± 0.00
Osmundea pinnatifida	0.10 ± 0.01	0.24 ± 0.03	1.32 ± 0.02	0.57 ± 0.15
Ceramium rubrum	0.12 ± 0.01	0.11 ± 0.01	1.03 ± 0.12	0.02 ± 0.00
Sphaerococcus sp.	n.f.	n.f.	n.f.	n.f.
Plocamium cargilagineum	0.05 ± 0.00	0.21 ± 0.03	0.83 ± 0.12	0.06 ± 0.00

The highest antioxidant activity was observed in *P. umbilicalis* and *O. pinnatifida* showing a positive correlation with phenolic compounds and MAAs content (Figure 2), as has been reported by other studies (De la Coba et al., 2009).

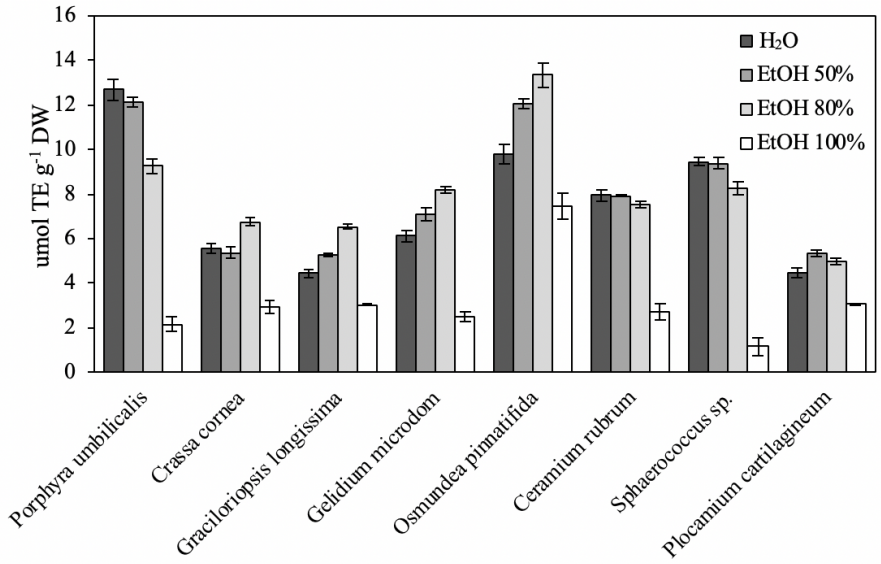

Figure 2: Antioxidant activity (μmol Trolox Equivalent (TE) g^{-1} DW) extracted in the different solvents, expressed as average $\pm$ standard deviation.

The diverse biochemical content in the different extractions depend on the polarity of the solvent, being H_2O the most and ethanol 100% the least polar. In all cases, ethanol 100% was the worst solvent obtaining the lowest content of all molecules of analyzed. The results of this study point out that *P. umbilicalis* can be a potential source of antioxidant and photoprotective compounds. Other species like *C. rubrum*, *O. pinnatifida* or species from the Gracilariales family are also good options due to its culture possibilities as it has been previously demonstrated in several works. The determination of the sun photoprotection factor (SPF) of the different species will be necessary in order to decide its use as part of a sunscreen, it is expected that those species with higher polyphenols and MAAs content will obtained the higher SPF.

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Seagulls as a reservoirs of ARG (antibiotic resistance genes)

Dayana Ayelen Jarma*[1] , Carles Borrego[2] , Alexandre Sánchez-Melsió[3] , Francisco Hortas[4] , Andy J. Green[5] , Marta Isabel Sánchez Ordoñez[1]

*Corresponding author: dayana.ayelen@uca.es
[1] INMAR (Instituto Universitario de Investigación Marina), Universidad de Cádiz, Cádiz, Spain.
[2] ICRA (Instituto Catalán de Investigación del Agua), Girona, Spain.
[3] ICRA (Instituto Catalán de Investigación del Agua), Girona, Spain
[4] INMAR (Instituto Universitario de Investigación Marina), Universidad de Cádiz, Cádiz, Spain
[5] EBD-CSIC (Estación Biológica de Doñana - Consejo Superior de Investigaciones Científicas), Sevilla, Spain.

Keywords: Antibiotic resistance, Dissemination, Seagulls

Abstract

Antibiotic resistance is a global health concern due to the worldwide dissemination of antibiotic resistant bacteria (ARB) and their resistance genes (ARGs) by different mechanisms. Among them, birds can disperse ARBs and ARGs thanks to their ability to migrate long distances. Seagulls occupy a wide variety of environments, including highly anthropized habitats, where they can acquire ARB, thus acting as reservoirs. Using *Larus fuscus* as a model, we collected feces from 15 individuals that feed on landfills. After extraction of DNA from these fecal samples we measured the abundance of ARGs conferring resistance to beta lactams, sulfonamides, tetracyclines and fluoroquinolones by qPCR. We compared these data with values from other aquatic species (*Anser anser* and *Grus grus*) that feed on non-impacted wetlands. We found that the concentration of ARGS in seagull feces were significantly higher than that from other waterbird species. Our results suggest that the use of habitat by seagulls might contribute to the high prevalence of ARGs in their feces compared to other migratory waterbird species, which are less prone to feed in anthropized sites.

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Fucoidan extracted from brown seaweed *Sargassum muticum* using green technologies and potential applications in the biomedical field

Noelia Flórez-Fernández*[1,2,3] , María Dolores Torres[1,2] , Ana M. Grenha[3] , Herminia Domínguez[1,2]

*Corresponding author: noelia.florez@uvigo.es

[1] Centre for Marine Sciences, Campus Gambelas, University of Algarve, 8005-139 Faro, Portugal

[2] Department of Chemical Engineering, Science Faculty, University of Vigo, Campus Ourense, 32004 Ourense, Spain

[3] Centre for Biomedical Research, Campus Gambelas, University of Algarve, 8005-139 Faro, Portugal; Centre for Marine Sciences, Campus Gambelas, University of Algarve, 8005-139 Faro, Portugal

Keywords: Fucoidan, Bioactive compounds, Extraction technologies, Biomedical applications

Abstract

Sargassum muticum is a brown seaweed native to the Pacific Northwest coast in Japan. This seaweed is also present in the Atlantic coast of Europe being considered invasive. The existence of non-native species causes a negative impact due to both species competing for light and nutrients. These factors could alter the biodiversity and other parameters related to the marine ecosystem. For these reasons, the revalorisation of the brown seaweed *Sargassum muticum* was suggested. Potential compounds with possibilities in the cosmetic and pharmaceutical industry have been widely studied (Menshova et al., 2015). Fucoidan, only present in brown seaweeds, is a sulphated polysaccharide mainly constituted by fucose and sulphate groups. Its location, collecting season and extraction technology, among other factors, are directly related with the resulting structure. Several biological activities have been also associated with this compound (Morya et al., 2012). Different technologies were proposed to extract the fucoidan fraction from *Sargassum muticum*. Ultrasound assisted extraction (UAE) and subcritical water extraction (SWE) are interesting eco-friendly technologies. Both were proposed to extract bioactive compounds from brown seaweeds. UAE was carried out in an ultrasonic bath at room temperature with a liquid:solid ratio 20:1 (w/w, dry base) from 5 to 30 minutes of reaction time (Flórez-Fernández et al., 2017). SWE was proposed at a liquid:solid ratio 30:1 (w/w, dry bases) and heating until 170 °C in a pressurised stainless-steel reactor followed by cooling step (González-López et al., 2012). In both cases, liquid and solid phases were separated and analysed. Antioxidant activity, using spectrophotometric methods, and cytotoxicity activity in four human cell lines (A549, HCT-116, PSN1 and T98G) were also evaluated. The use of natural polymers has been widely described in the scientific literature. Marine biopolymers frequently exhibit properties as biocompatibility, low toxicity, cell adhesion and others, that foster potential applications in biomedical areas, including drug delivery, wound healing and tissue engineering (Cardoso et al., 2016).

Acknowledgments

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N.F.F. thanks Xunta de Galicia for her postdoctoral grant (ED481B 2018/071) and M.D.T. thanks Spanish Ministry of Economy and Competitiveness for her postdoctoral grant (IJCI-2016-27535).

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Synthesis and evaluation of the antioxidant activity of marine analogue phthalides.

Belén Parra-Torrejón*[1] , María J. Ortega[1] , Eva Zubía[1]

*Corresponding author: belen.parra@uca.es
[1] Department of Organic Chemistry, Faculty of Marine and Environmental Sciences, University of Cadiz

Keywords: Marine Natural Products, Phthalide derivatives, Antiischemic activity, Antioxidant activity, Synthetic Methodology

Abstract

Natural products are considered one of the essentials pillars of Organic Chemistry because of their structural diversity and their pharmacological properties. In that way, studies about plants and other organisms supported by biological assays have led researchers to get a vast variety of natural products, some of them used as lead compounds in the development of new drugs against some infections and diseases (Newman and Gordon, 2016). Due to the difficulty in the accesibility of marine organisms, and thus to marine natural products, it is usual to understand why most of the natural products derived drugs proceed from plants, microorganisms and terrestrial fungi. However, the improvement in technical issues, equipments and more efficient spectroscopy methods, resulted from the technological development, have allowed marine enviroment to take an important role in the new bioactive natural products search and development. Although marine environment is still scarcely known, oceans cover about 70% of Earth's surface and the potential of marine biodiversity allows us to estimate that marine organisms are able to generate a huge number of secondary metabolites with interesting structures and biological activities. In fact, if we compare them with the terrestrial natural products, marine compounds usually show more biological activities due to their diversity and their high structural complexity (Altmann, 2017). Phthalides represent an important class of natural products mainly isolated from terrestrial plants and also from marine organisms. Structurally, phthalides contain a benzene ring fused to a γ-lactone. These compounds have been widely studied because of their pharmacological properties, including antiinflamatory, antiviral, antifungal, modulation of the central nervous system, protection against brain ischemia and citotoxic activities. According to bibliography, one of the main characteristics responsible for the bioactivity seems to be related to the substitution at C-3 position of the phthalide system, being the most reknown compound in the phthalide family the 3-*n*-buthylphthalide (NBP), (Figure 1). This compound have shown interesting results as antiischemic drug and is used in China for the treatment of patients who suffered an ischemic stroke. Between the multiple mechanisms involved in the process of ischemia injury, the oxidative stress is considered the most important cause of delay in the recovery of the surrounding tissue affected by the stroke. To overcome this drawback, NBP is usually administered to patients in combined therapy with antioxidant agents. Thus, investigation in this field is focused on potential compounds combining both activities.

Figure 1: 3-n-butylphthalide (NBP).

Phthalides from marine origin usually have shown a substituent at C-3 position and a substitution pattern in the aromatic ring that could provide them with antioxidant activity (Figure 2). For that reason, in this study, we propose to develop a synthetic methodology for the synthesis of marine derived phthalides which combine in their structure both pharmacologycal activities, mainly antiischemic and antioxidant. Taking into account the antioxidant activity of phenolic compounds, our main objective is to synthesize phthalide derivatives containing the NBP structural frame bearing at C-3 a phenolic residue (Almeida et al., 2011; Liu et al., 2011). More recently, phthalide derivatives containing S have improved their antioxidant activity (Wu et al., 2012).

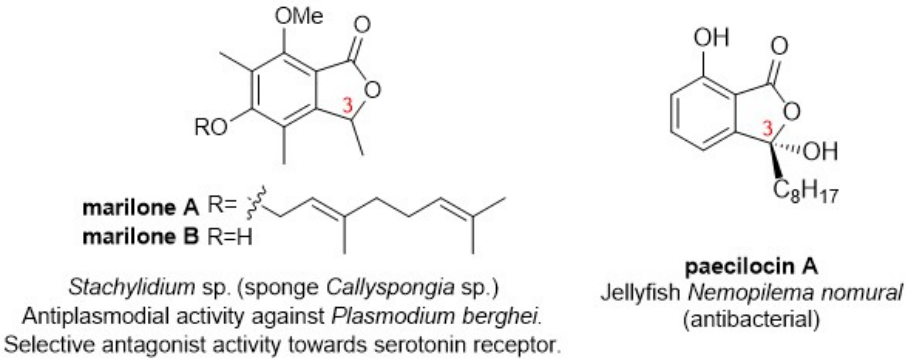

Figure 2: Phthalides isolated from marine environment and their activity.

Our synthetic metodology implies the following procedures: 1) The study of the viability of the aldol reaction for the synthesis of phthalides functionalized at C-3 with an alkylphenolic group. 2) The study of the viability of the Grignard reaction for the introduction of an alkyl phenolic group at C-3 of the phthalide or thiophthalide unit. 3) The study of the viability of the Friedel-Crafts reaction for the introduction of a phenolic ring at C-3 of the phthalide unit. In Figure 3 is remarked a summary of the proposed analogues synthesized:

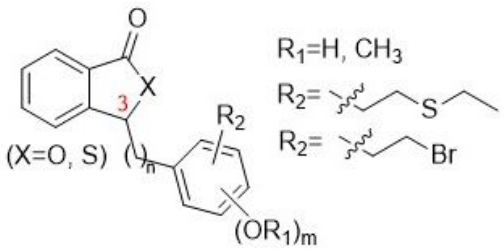

Figure 3: Marine phthalides analogues proposed and synthesized.

Finally, the new synthetic compounds have shown good results in the antioxidant activity evaluated in the ABTS assay.

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Clonation of one Toxin-Antitoxin System from a Cyanobacteria *Halothece* sp. PCC 7418

Carlos Enrique Payá[1] , Victor Fernández Juárez[1] , Nona Sheila Agawin [2] , Toni Bennasar Figueras[2]

*Corresponding author: carlospaya94@gmail.com
[1] Universidad de Las Islas Baleares
[2] victor.fernandez@uib.es

Keywords: Cyanobacteria, Molecular biology, Toxin-Antitoxin System, VapBC

Introduction

Cyanobacteria are probably the most important type of marine bacteria for its big contribution as primary producers in the oceans. These organisms dating from the precambrian period are ones of the responsibles to changee the inital conditions of the Earth and transform the primitive atmosphere. Interest in this group of bacteria has increased in the last two decades, thanks to its great genetic polyvalence and the different biotechnological applications which are being developed. They are the main primary producers of the oceans and are the largest fixators of diatomic nitrogen in the open ocean. They have the capacity to synthesize large amounts of fatty acids (studied to obtain biofuel). Furthermore, these microorganisms are subject to different environmental pressures and need the activation of systems or mechanisms by which to adapt and survive the changing environment. An ecological niche in which they are subject to different factors are the meadows of Posidonia oceanica. It is an ecological niche that presents a high diversity of nitrogen fixers (Archaea, Firmicutes, Bacteriodetes, Proteobacteria and Cyanobacteria). One of these bacteria is *Halothece* sp. PCC 7418, identified by the gene *nifH*. There are some small genetic modules called antitoxin toxin systems, which could be involved in survival. These TA systems have many functions assigned, from the maintenance of plasmids to the formation of persistence cells. These systems are widely diversified among prokaryotes, the latest studies suggest that these TA systems help in certain cells of a population to survive in certain adverse conditions that persist in the environment. Both the toxin and the antitoxin are continuously secreted, finding themselves in equilibrium, however, before a stimulus such as the loss of plasmids or environmental stress interrupts the segregation of the antitoxin breaking this balance. This triggers the response of the toxin and the consequent cellular stasis. TA systems are classified into six types, according to the molecular nature of the antitoxins and the mechanisms by which they counteract the related toxins. Thus, we found type I and type III that are characterized by a small non-coding RNA (sRNA) carrying out the role of antitoxin, while types II, IV, V and VI are antitoxin proteins. Type II systems are the best characterized having identified thousands of loci.

Materials and methods

To carry out the isolation of each of the TA systems, the toxin and the antitoxin were cloned into Escherichia coli B21 DE3 plyss. First two pairs of primers were designed, in order to amplify each of the genes, respecting the codon that marks the beginning of transcription (ATG) and

that the reading frame was in phase. The designed primers contained in their sequences specific restriction targets that were used to bind to two expression vectors. The plasmids used were pRSFDuet-1 and pBAD, the first for the toxins and the second for the antitoxins. These plasmids were transformed by heat shock into *Escherichia coli* B21 DE3 plyss, and the clones were selected for their resistance to antibiotics. With these transformants carrying both plasmids, different functional experiments will be applied to verify the effect of the toxin, the antitoxin and both, inducing these elements with different inducers, IPTG.

Conclusion

In the present work we focus on the isolation of a TA system belonging to the cyanobacterium *Halothece* sp. PCC 7418. This system is formed by the VapC toxin and the VapB antitoxin. In conclusion, we present the construction of an *Escherichia coli* B21 DE3 plyss carrier of a TA VapBC system with which to carry out studies of these systems, and see the role they can play in cyanobacteria.

Acknowledgments

This work was supported by funding to NSRA trough the Agencia Estatal de Investigación and the European Regional Development Funds project (CTM2016-75457-P). We also thank Pere Ferriol Buñola.

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Determination of ultraviolet filters and stabilisers in market fish

Sandra Gimeno-Monforte*[1] , Sarah Montesdeoca-Esponda[1] , Zoraida Sosa-Ferrera[1] , Jose Juan Santana-Rodríguez[1]

*Corresponding author: sandra32gm@gmail.com
[1] Instituto Universitario de Estudios Ambientales y Recursos Naturales (i-UNAT), Universidad de Las Palmas de Gran Canaria, 35017, Las Palmas de Gran Canaria, Spain

Keywords: Benzotriazole UV stabilizers, Ultraviolet filters, Ultra-High Performance Liquid Chromatography, Fish, Bioaccumulation

Introduction

The use of ultraviolet (UV) filters and stabilisers in personal care products (PCPs), plastics and textiles is rising due to the necessity of protection that our skin and the different objects exposed to sun light required against the UV radiation (Cantwell et al., 2015). This increase of their use and the poor elimination at the wastewater treatment plants (WWTPs) generate a continuous discharge of these compounds to rivers, lakes and seas (Montes-Grajales et al., 2017) that could pollute the environment and result in health problems in organisms (Montesdeoca-Esponda et al., 2018). The negative effect in fishes has been demonstrated; they exhibit endocrine disrupting properties, induced morphological abnormalities during embryonic development (Quintaneiro et al., 2019) and have inflammatory and immunotoxic properties (Li et al., 2019). Because of the high persistence of these compounds in tissues and the effect of bioaccumulation and biomagnification in the trophic chain, it is necessary to know their presence in marine organisms. We have developed a methodology to determine the level concentration of 7 UV filters and 6 UV stabilisers in different species of fishes bought at the market in two different locations, Tarragona and Las Palmas de Gran Canaria (Spain).

Material and methods

Target compounds were 7 UV filters (4-Methylbenzylidene camphor (4MBC), Benzophenone 3 (BP-3), Homosalate (HMS), Drometrizole trisiloxane (DTS), Octocrylene (OC), Avobenzone (BM-DBM), Amiloxate (IMC)) and 6 UV stabilisers belonged to benzotriazole family (UV-P, UV-326, UV-327, UV-328, UV-329 and UV-360). They were obtained from Sigma-Aldrich (Madrid, Spain) and stock solution (250 $\mu g \cdot mL^{-1}$) were prepared in acetone and stored in glass stoppered bottle at 2-5 °C prior to use. A microwave-assisted extraction (MAE) was optimized to determine the 13 target compounds in fish. The parameters that can affect the MAE procedure were evaluated to determine which conditions (temperature, time, sample amount and type of dissolvent) are the best to extract all the compounds. The optimized MAE procedure, coupled with Ultra-High Performance Liquid Chromatography with tandem mass spectrometry detection (UHPLC-MS/MS) methodology described in Figure 1, was applied to determine the target analytes in 10 different species of fishes.

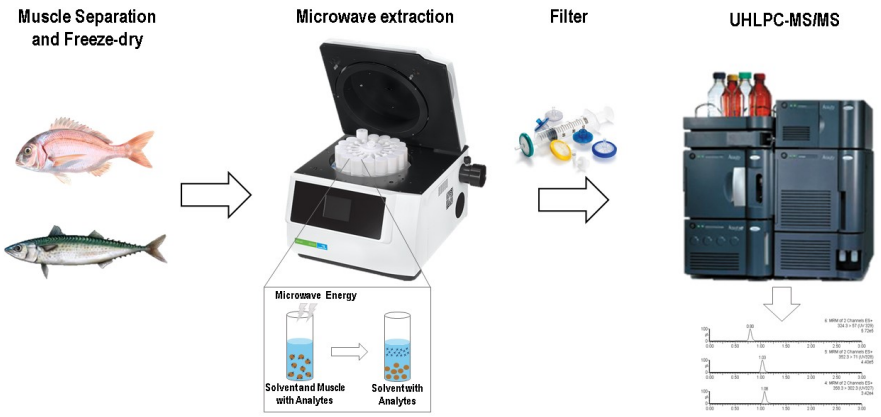

Figure 1: Whole analytical procedure.

Results

For the MAE optimization we utilized a 2^3 experimental design for each dissolvent. The levels tested for each parameter were; temperature (60-80 °C), time (5-10 minutes), sample amount (100-300 mg) and type of dissolvent (methanol, acetonitrile and the mix 1:1 of them). Figure 2 shows the influence of each factor in the extraction yield for UV-P extracted with acetonitrile. The same behaviour was observed for the others target compounds.

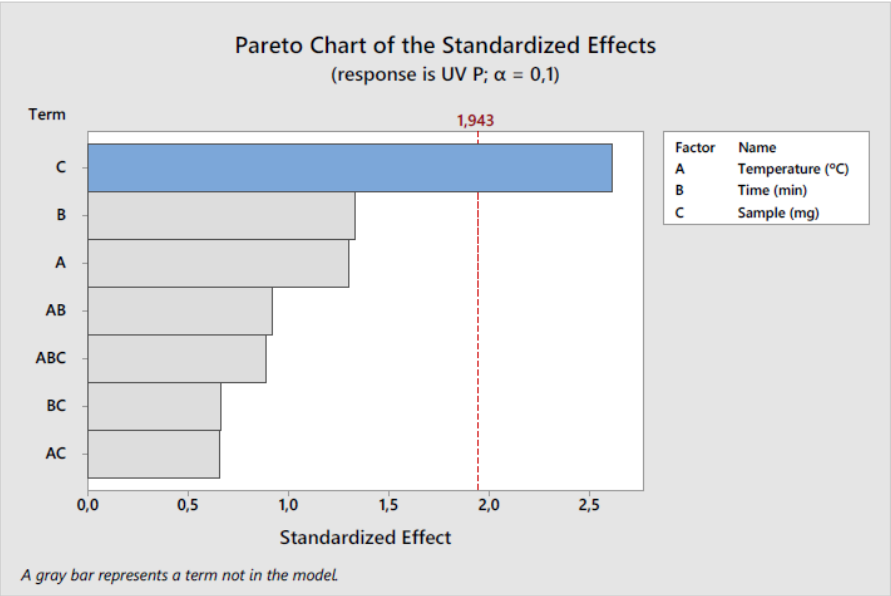

Figure 2: Pareto chart for the influence of the three tested factors (A= Temperature; B=Time; C=Sample) for the compound UV-P extracted with acetonitrile.

The best results were obtained using acetonitrile as extractant and 100 mg as sample amount. Then we created another experiment where we fixed the conditions cited below developing a 3^2 design to continue the study of temperature (testing 60, 70 and 80 °C) and time (testing 7, 10 and 12 minutes). The best recovery percentages were obtained at 60 °C during 10 minutes. These conditions were validated for different species of market fishes from Gran Canaria (*Pagellus*

bellotii bellotii, *Serranus cabrilla*, *Comber colias*, *Katsuwonus pelamis*) and Tarragona (*Merluccius merluccius*, *Sardina pilchardus*, *Solea solea*, *Thunnus alalunga*). After that we have applied the developed method to study the presence of each compound in fishes from three different levels in the trophic chain to determine their bioaccumulation and biomagnification and their possible ingestion by population.

Acknowledgments

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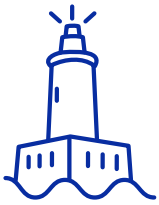
Session 9

Engineering and the Sea

The Sea Cultural Value



Numerical simulation of stationary current shock and entropy

Santiago Pavón Quintana*[1] , Daniel José Coronil Huertas[1] , María José Legaz Almansa[1] , Juan Manuel Vidal Pérez[1] , José Juan Alonso del Rosario[2]

*Corresponding author: santiago.pavon@uca.es

[1] Navigation Sciences and Techniques and Ship-Building Department, School of Naval and Oceanic Engineering, University of Cádiz, Avda. República Saharaui s/n, Puerto Real, 11510, Cádiz, España.

[2] Applied Physics Department, Faculty of Marine and Environmental Sciences, University of Cádiz, Avda. República Saharaui s/n, Puerto Real, 11510, Cádiz, España.

Keywords: Finite elements, Navier-Stokes, Turbulence, Entropy

Abstract

There are many areas in the ocean where two marine currents meet. An example is where the Gulf Stream meets with the Labrador current. These areas use to be well located and the currents have relatively low variations in speed. It is well known the eddies generation, *cum solem* and *contra solem*, up to the character of the gradient of pressure and the hemisphere (Talley et al., 2011). We present the results of two numerical simulations for the meet of two theoretical and constant currents to see how the entropy of the resulting velocity field evolves. The currents are defined to be perpendicular and the Navier-Stokes equations are numerically solved. In any case, the numerical tools have been the open source finite/volume elements solver for multiphysics problems FreeFem++ (Hecht et al., 2012). The results can be briefly summarized as the time evolution of entropy, increasing in time even with constant currents meeting.

Material and methods

The evolutive Navier-Stokes system of equations are (Kundu and Cohen, 2015)

$$\rho\frac{D\underline{u}}{Dt} = \rho\underline{F} - \underline{\nabla}\cdot p + \mu\cdot(\frac{1}{3}\underline{\nabla}(\underline{\nabla}\cdot\underline{u}) + \nabla^2\underline{u}) \tag{1}$$

$$\underline{\nabla}\cdot\underline{u} = 0 \tag{2}$$

Where $\underline{u}$ is the velocity vector, p is the pressure, F is an external force, ρ is the density and μ the viscosity. Equation (1) is the law of conservation of momentum and Equation (2) is the mass conservation.
They are written in variational form or weak formulation, Equation (3), as:

$$\int_\Omega((\underline{u}\cdot\underline{\nabla})\underline{u})\cdot\underline{v} + \mu\underline{\nabla}\cdot\underline{u} : \underline{\nabla}\underline{v}; p\underline{\nabla}\cdot\underline{v}; q\underline{\nabla}\cdot\underline{u} = 0 \tag{3}$$

Where v is the vector test function for the velocity and q is the test function for the pressure.
After applying the Galerking, and with the no consideration of turbulence models, the resulting

equations has been solved using the open source finite elements program FreeFem++ (Hecht et al., 2012) on a i7 linux platform. The domain is a square of 1000 km of side and the currents are considered perpendicular: from South to North and from West to East, of about 200 km width (Figure 1).

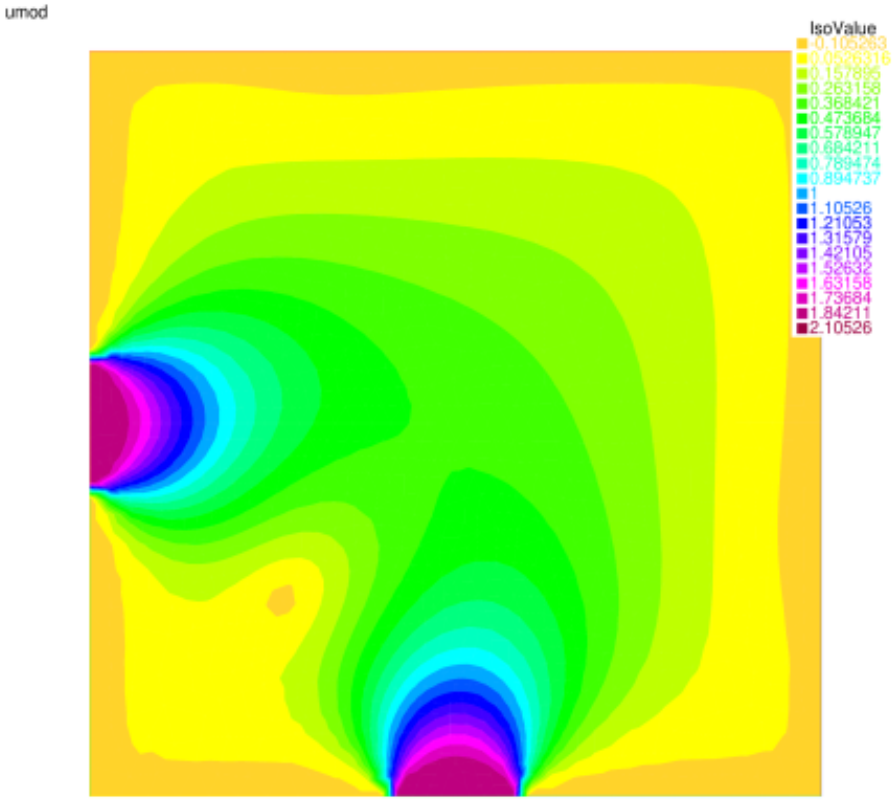


Figure 1: Velocity module for the initial situation.

Results

Two simulations have been carried out: without and with Coriolis force, considering the f-plane. The velocity of the currents is constant. Each simulation spent about five hours of computing time. Figure 2 shows the velocity field for the simulation without Coriolis force for different times.

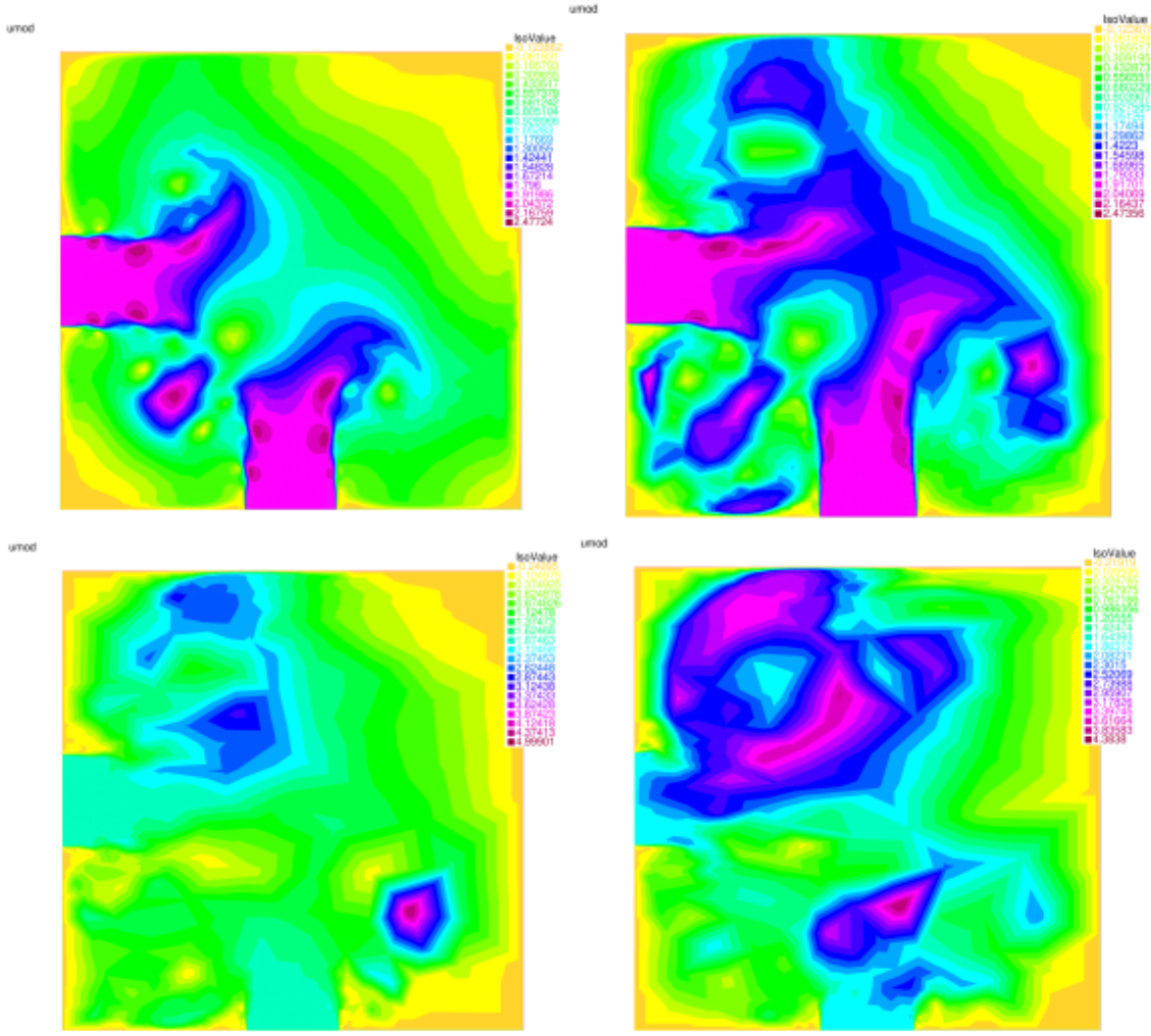


Figure 2: Velocity fields module at different times.

The entropy has been computed at each iteration from the definitions of Burg and Frieden (Starck and Murtagh, 2006):

$$S = \sum_{\Omega} ln(X_i) \tag{4}$$

$$S = \sum_{\Omega} X_i \cdot ln(X_i) \tag{5}$$

Where Equation (4) is the Burg's entropy definition and Equation (5) is for Frieden. Both formulations born from the Shannon's Mathematical Theory of Information (Shannon, 1948).

Figure 3 show the time evolution of the entropy following the Burg and the Frieden formulations. In any case, a turbulent state is established followed by a stabilization. After that, a new more complex state happens. The result is an increasing entropy.

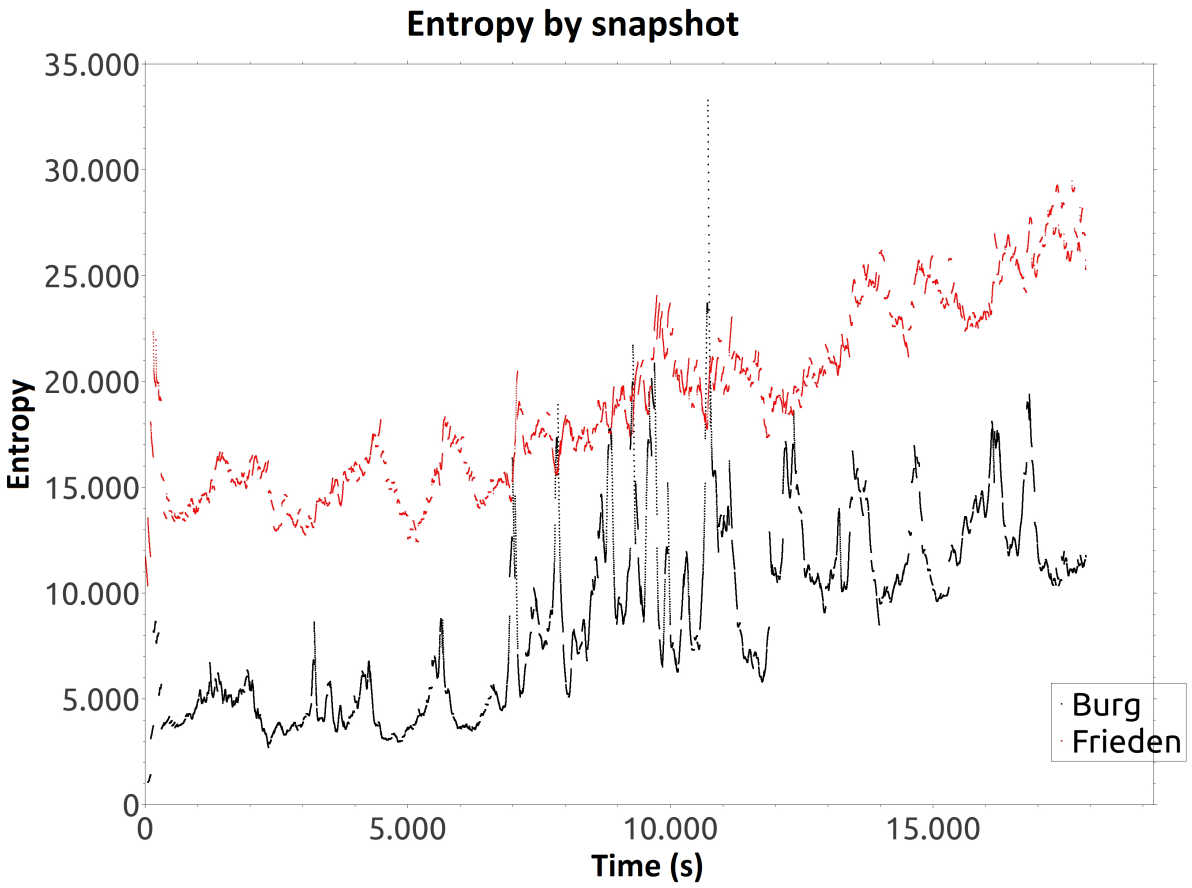


Figure 3: Evolution of entropy in the domain of numerical simulation. Burg in black and Frieden in red.

The results of this work are the basis for a more complex work on several areas of the ocean where currents meet.

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Simulation of water flow and contaminant dispersion at coastal areas: application to the Seaport of Malaga

Javier Fernández-Pato*[1] , Mario Morales-Hernández[2] , Geovanny Gordillo[3] , Pilar García-Navarro[3]

*Corresponding author: jfpato@unizar.es
[1] University of Zaragoza, Hydronia-Europe
[2] Computational Science and Engineering Division, Oak Ridge National Laboratory, Oak Ridge, TN (USA)
[3] University of Zaragoza

Keywords: Numerical simulation, Shallow-water equations, Coastal flow, Contaminant dispersion, Malaga seaport

Introduction

Hydrologic modeling in coastal environments is becoming a relevant tool to predict and evaluate the effects of several phenomena of interest, including tidal and tsunami waves, beach geomorphological changes or contaminant transport and dispersion. The use of 2D distributed models for the surface flow simulation provides a more detailed computation of the spatial variations of the hydraulic variables, such as water depth of velocities. In order to take advantage of all the capabilities of a distributed simulation model, detailed input topographic and bathymetric data are mandatory.
One of the most relevant issues when dealing with shallow flows is the correct treatment of wet/dry fronts, which is an inherent feature of the coastal flows at the coastline. This is a nontrivial challenge for the development of an accurate numerical scheme, as these situations frequently lead to extreme and non-physical velocities in the wet/dry front, causing instabilities and a drastic reduction in the numerical time step (Burguete et al., 2008; Murillo et al., 2007). In Medeiros and Hagen (2013) a few techniques used in the last years are classified in four categories: thin film, element removal, depth extrapolation and negative depth. Each group has benefits and drawbacks regarding, for instance, the correct capture of the wet/dry interface or the local and global mass conservation. More recently, Murillo and GarcÃa-Navarro (2010) presented a novel procedure to deal with wet/dry fronts consisting of the water positivity preserving and the friction losses limitation. This is the strategy considered in the present work.
Regarding the contaminant transport simulation, additional scalar equations is added to the mathematical model in order to calculate the temporal evolution of one or several solute concentration, associated to the water flow (Petersen et al., 2001).

Mathematical and numerical models

Surface flow is modeled by means of the 2D full shallow-water equations, which can be expressed as follows:

$$\frac{\partial \mathbf{U}}{\partial t} + \frac{\partial \mathbf{F}(\mathbf{U})}{\partial x} + \frac{\partial \mathbf{G}(\mathbf{U})}{\partial y} = \mathbf{S} + \mathbf{H} \tag{1}$$

where

$$\mathbf{U} = (h, q_x, q_y)^T \tag{2}$$

are the vector of conserved variables, in where h represent the water depth and $q_x = hu$ and $q_y = hv$ are the unit discharges, with u and v the depth averaged components of the velocity

vector $\mathbf{u}$ along the x and y coordinates respectively. The fluxes of the conserved variables can be written as

$$\mathbf{F} = \left(q_x, \frac{q_x^2}{h} + \frac{1}{2}gh^2, \frac{q_x q_y}{h}\right)^T, \qquad \mathbf{G} = \left(q_y, \frac{q_x q_y}{h}, \frac{q_y^2}{h} + \frac{1}{2}gh^2\right)^T \tag{3}$$

where g is the acceleration due to gravity. The sources of (1) are split into three terms. The term $\mathbf{S}$ represents the friction losses and it is defined as

$$\mathbf{S} = (0, -ghS_{fx}, -ghS_{fy})^T \tag{4}$$

where S_{fx}, S_{fy} are the friction slopes in the x and y direction respectively, traditionally written in terms of the Manning's roughness coefficient n:

$$S_{fx} = \frac{n^2 u\sqrt{u^2+v^2}}{h^{4/3}}, \qquad S_{fy} = \frac{n^2 v\sqrt{u^2+v^2}}{h^{4/3}} \tag{5}$$

The term $\mathbf{H}$ accounts for the pressure force variation along the bottom in x and y directions and can be formulated in terms of the bottom level z bed slopes:

$$\mathbf{H} = \left(0, -gh\frac{\partial z}{\partial x}, -gh\frac{\partial z}{\partial y}\right)^T \tag{6}$$

Equations (1) to (6) deal with the hydrodynamic component of the flow. In order to consider the solute or contaminant transport and dispersion, an additional equation should be taken take into consideration, as follows:

$$\frac{\partial(h\phi_i)}{\partial t} + \frac{\partial(hu\phi_i)}{\partial x} + \frac{\partial(hv\phi_i)}{\partial y} = E\frac{\partial}{\partial x}\left(h\frac{\partial\phi_i}{\partial x}\right) + E\frac{\partial}{\partial y}\left(h\frac{\partial\phi_i}{\partial y}\right) \pm hR_i \pm f_i \tag{7}$$

where ϕ_i is the average concentration for each pollutant, E is the longitudinal diffusion coefficient, R_i represents the generation or consumption of each constituent and f_i stands for the point sources.

The term R_i is established according to the Petersen matrix. The matrix is composed of processes (rows) and state variables (columns), with elements within the matrix that include stoichiometric coefficients that establish the relationships between the components in the individual processes (Petersen et al., 2001).

Model conceptualization and numerical results

The Port of Malaga is an international seaport located in the city of Malaga in southern Spain, on the Costa del Sol coast of the Mediterranean. It is the oldest continuously-operated port in Spain and one of the oldest in the Mediterranean. Its complex geometry together with the proximity to residential areas and the intense port activity represent the ideal choice for testing the capabilities of the full (hydrodynamic and contaminant) model.

The input information for the model is based in the combination of a $5m \times 5m$ Digital Elevation Model (DEM) and several bathymetric points, interpolated by means of the inverse distance weighting method, in order to obtain a continuous representation of the sea bed level. All these elevation data have been used to generate a flexible triangular mesh of 41350 elements. The use of this kind of meshes allows to get a better adaptation to irregular terrains and complex geometries as in this seaport, where multiple breaklines have been used to delimit the shape of the moorings and breakwater. Figure 1 shows a 3D representation of the discretized geometry of the seaport together with the considered initial condition, assumed as a uniform water level at $z = 0m$ with no velocity. A tidal wave with an amplitude of $2m$ and a period of $4h$ is imposed offshore while the coast and port boundaries remain closed.

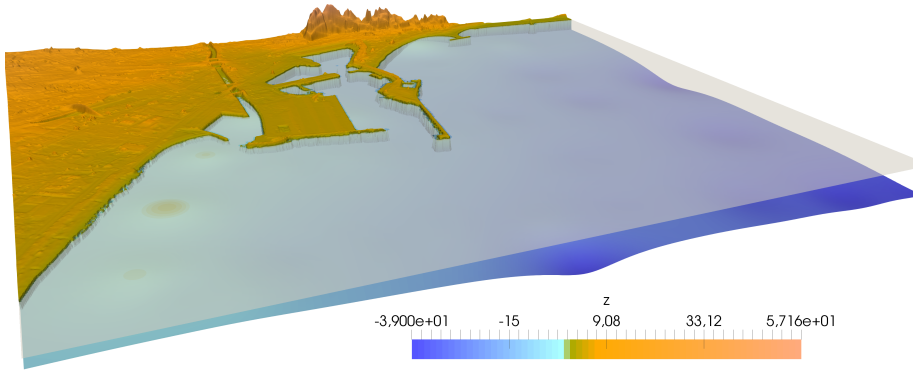

Figure 1: 3D view of the computational triangular mesh. Bed elevations and initial water level have been exaggerated.

Figure 2 shows illustrative examples of the numerical results obtained from the model. Figure 2 (left) shows the effect of the breakwater reducing the intensity of wave action in inshore waters and thereby providing safe harbourage. On the other hand, Figure 2 (right) shows the water velocity modulus and vectors at $t = 180s$ from the beginning of the simulation. It can be seen how the maximum velocity values are reached at the breakwater and in the Guadalmedina river mouth. The local mesh refinement at these points allows to determine this velocity values and vectors more accurately.

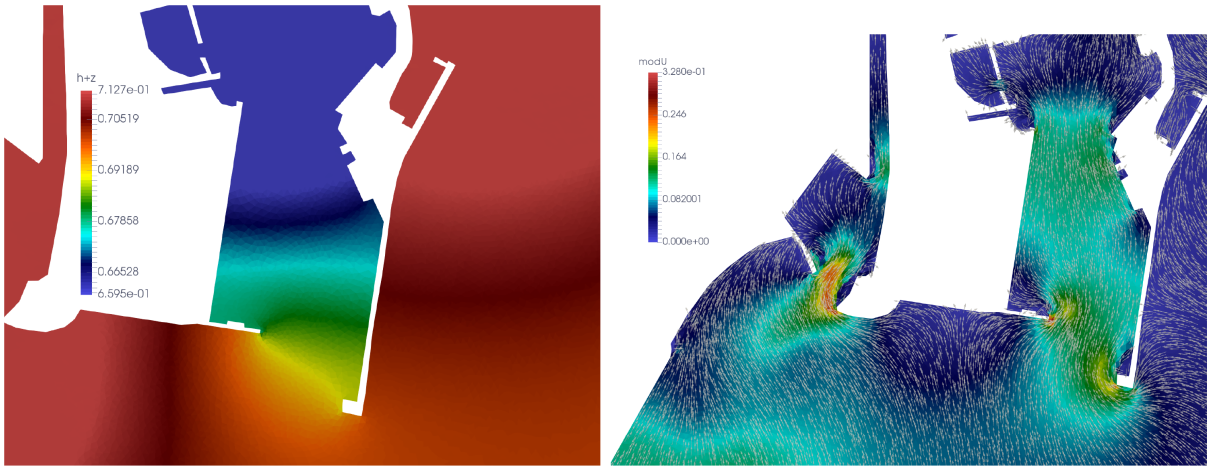

Figure 2: Numerical results for water level modulus and vectors at $t = 1200s$ (left) and for water velocity modulus and vectors at $t = 180s$ (right) from the beginning of the simulation.

Acknowledgments

The present work was partially funded by the Aragón Government. This research has also been supported by the Research Project PGC2018-094341-B-I00 funded by the Spanish Ministry of Economy and Competitiveness (MINECO). The corresponding author also wants to thank to the MINECO and Hydronia-Europe S.L. for his Research Grant DI-14-06987.

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Multihop based Scalable Routing Protocol (MSRP) for Underwater Acoustic Sensor Networks

Sateesh Kumar Hindu*[1] , Jitander Kumar Pabani[1] , Miguel-Angel Luque-Nieto[1] , Javier Poncela[1] , Pablo Otero[1]

*Corresponding author: thesateeshkumar@gmail.com
[1] Department of Ingeniería de Comunicaciones, University of Malaga, Spain;

Keywords: Multihop routing, Transmission range, End to end delay, Self-organization, Energy consumption

Introduction

Underwater Acoustic Sensor Networks (UASN) are composed of large number of fixed or mobile nodes, deployed, collaboratively monitoring a specific area and forwarding data to one or more base stations. The nodes are connected through wireless links which are either manually setup prior to node placement or centrally assigned after deployment, also it is necessary to reconfigure the links whenever node is lost or added which requires considerable efforts. Each node has capabilities of sensing, storing, processing and wireless communication which enable it to sense, gather application specific information and send the data to sink node. Depending on the various parameters and network considerations such as network size, distance between sink and source node, routing protocol etc., single or multihop communication is implemented. The use of acoustic communication proves to be efficient communication technology because of underwater medium properties and composition. Furthermore, various studies have shown that RF and optical communication experience high losses due to higher frequency and scattering, respectively. Routing is the process of finding the path that can forward the messages from source and destination in a network. Mostly network layer is used to execute the routing of messages in sensor networks (Dipobagio, 2009). Depending on the application requirement, the routing type is implemented. The two important routing models are centralized and decentralized routing. Considering the hostile environment of undersea and ad-hoc arrangement of nodes in the target, decentralized model is an appropriate choice for such networks where nodes make the decision for route selection based on the defined criteria of choosing the next node and autonomously organize themselves (Mansouri and Ioualalen, 2016). However, in centralized approach, central node is responsible for making the decision which may lead to network disintegration in case of central node failure. Moreover, the sensor nodes are powered through batteries. It is inconvenient to replace or recharge the batteries of the depleted nodes, considering the underwater environment, cost and time required for such operations (Xia et al., 2012). The propagation environment also has substantial effect on energy consumption; therefore energy efficiency is one of major concern in designing the protocol for UASN. Various routing techniques are proposed by researchers for UASNs using clustering, direct, channel aware and multi-hop communication. The purpose of this study is to design a protocol to achieve the stability as network size increases, providing efficient paths to the sink node and fault tolerance through self-organizing the nodes in the network. We have named this protocol as Multihop based Scalable Routing protocol (MSRP). The performance of the protocol is measured against end to end delay, energy consumption and number of hops in the path by varying network size and transmission range for optimal and pragmatic behavior of MSRP.

Methodology

Considering the above-mentioned challenges, harsh ocean current and environment, a self-organizing routing protocol is proposed for UASNs named as "Multihop based Scalable Routing protocol (MSRP)". The protocol enables the deployed node to form a network based on the broadcast information, forming a connectivity matrix with neighbors. Initially, the nodes are deployed one by one at random location and depths with no prior information of network and sink at the surface of sea with zero depth. The protocol is simulated in MATLAB in 3D cube (200*200*200 (m)), the energy and propagation model are also implemented, contemplating the undersea space and conditions (Zhen et al., 2018; Padmavathy et al., 2012). After node deployment, a "HELLO" packet is transmitted in the defined radius by sink node, carrying base station ID and hop count from node to sink, respectively. After receiving the packet, the node increments the value, stores the hop count if it is not already present or is smaller than the stored hop count and relays the message with the updated value. In case of the hop count is equal or larger than the current value the node will discard the message. This process continues until the message reaches every node in the network. In neighbor discovery phase, two control packets (Request and INFO packets) are considered to form the local connectivity between the nodes. In this phase, neighbor discovery and creation of neighbor table is performed by each node based on the information contained in broadcasted request packet (4 bytes), and INFO packet (6 bytes) sent by nodes in response, respectively. The information stored in INFO packet is sender/neighbor ID, timestamp, hop count and accumulated hop-count distance to the sink. The Time of Arrival technique is considered for calculating the distance between two nodes. In order to conserve the energy, the neighbor discovery phase is only initiated when a change in topology is detected, such as nodes addition or losses. The path selection criterion for proposed protocol is based on hop count and distance between source and destination. The protocol selects the shortest path between source and sink. On sensing the event, the path formulation begins with the selection of next hop by source node based on smallest hop count in the neighbor table. If two neighboring nodes have same hop count value in neighbor table, the node with the shortest hop count distance will be selected as next node. In Packet transmission phase, on acquiring the data, the source node checks the local routing table for the selection of next hop. The selection of node is performed using path selection criteria where hop count and distance are compared among all the entries stored in the table and the packet is transmitted from source node to sink using multihop communication. The process repeats at each node for the selection of next intermediate node in multi-hop path until the packet reaches the base station. If a route failure is detected, the algorithm selects an alternate path to transmit the data.

3. Results and conclusion

The performance of the proposed protocol is assessed with different network sizes (from 50 to 100 nodes). For each network size, a new ad-hoc topology is deployed where each node is assigned new random location to observe the effect of newly formed paths. Moreover, the transmission range was also swept (from 70 m to 100 m) to evaluate the effects on the end to end delay, number of hop count in total generated paths and total energy consumption of the network. The results obtained are shown in Figures 1 and 2 for a network area of 200 * 200 * 200 (m), where a stable operation is kept in the network with ad-hoc topology for each network size. In general, increasing the network size tends to reduce or keep the end to end delay. This means the new routes are converging to the sink in an efficient way.

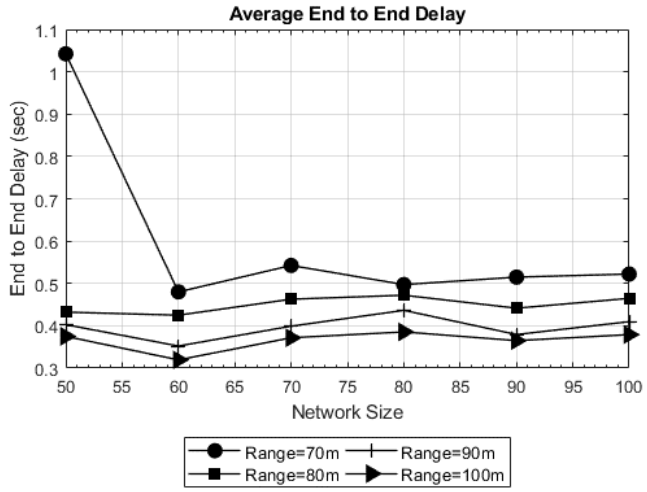

Figure 1: Average delay in network of 200 * 200 * 200 (m)

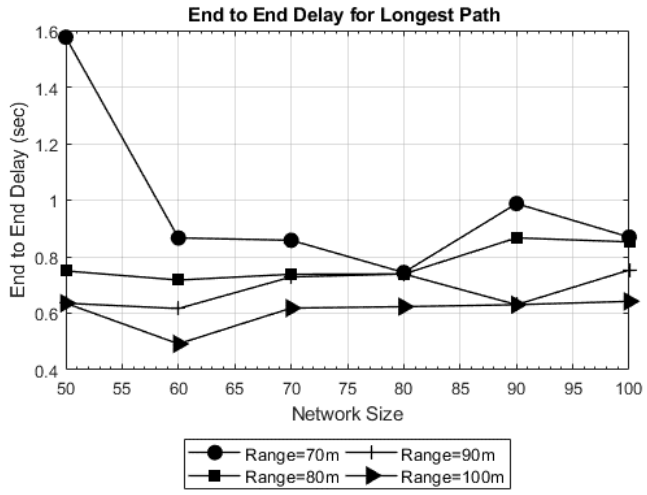

Figure 2: End to end delay for longest path in network of 200 * 200 * 200 (m)

It is also observed when only the transmission range is increased: the delay decreases. The reason is that the coverage area of the nodes is increased which in return decreases the hop count between the source and destination. This effect can be seen in Figure 2, where the delay for the longest path is as high as 1.6 seconds for a 50 nodes network and the shortest transmission range (70 meters). The delay relative to the MAC layer depends on the specific technique employed and it is not included in the end to end delay. The results obtained for energy consumption are shown in Figure 3. It is evaluated from the results that as the network size grows, the energy consumption increases because of more nodes participating in sensing and transmitting data. Furthermore, it can also be seen that as the transmission range increases, the energy consumption reduces because of fewer hops in paths leading to sink due to the nodes are not too far from each other. This fact leads us to reduce the energy employed in small network area such as 200 * 200 * 200 (m).

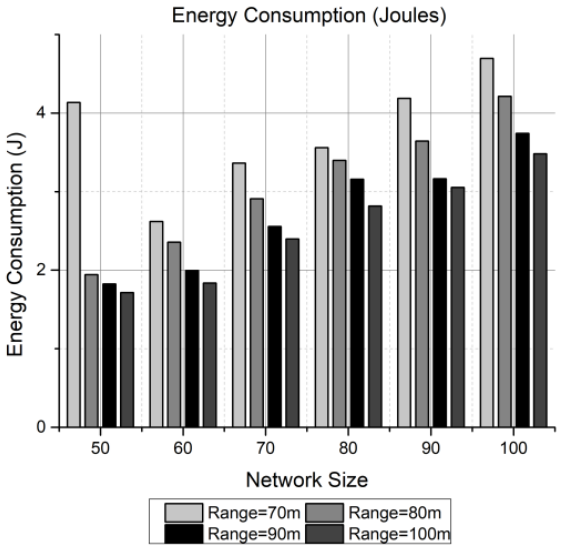

Figure 3: Total energy consumption in 50 Rounds (200 * 200 * 200 (m)

In this paper, a decentralized protocol Multihop based Scalable Routing Protocol (MSRP) is proposed, and has been simulated in MATLAB. The protocol utilizes multihop communication technique to transmit the sensed data to the sink node using routing table. The table is generated from information acquired during initial operations. Simulation results show that an optimal transmission range and network size can improve the performance of protocol by decreasing the number of hops in the generated path. The results show that MSRP provides stable operation, scalability, and fault tolerance and isolation detection for UASNs.

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Wave energy farms as coastal defence elements under global warming

Rafael J. Bergillos*[1] , Cristóbal Rodríguez-Delgado[2] , Gregorio Iglesias[3]

*Corresponding author: rafael.bergillos@uco.es
[1] Hydraulic Engineering Area, Department of Agronomy, University of Córdoba, Campus Rabanales, Leonardo Da Vinci Building, 14071, Córdoba, Spain
[2] PROES Consultores, Calle San Germán 39, 28020, Madrid, Spain
[3] MaREI, Environmental Research Institute & School of Engineering, University College Cork, College Road, Cork, Ireland

Keywords: Wave energy, Sea level rise, Coastal protection, Erosion, Flooding

Introduction

The development of renewable energy is one of the most relevant targets confronting society in the coming decades, due to the finite nature of fossil fuels, their high costs and, last but not least, the environmental impacts of their exploration and use, including climate change and the subsequent sea-level rise. Wave energy is, among the renewable energy sources, one of the most promising due to its comparatively huge potential and low impacts on the environment. This work analyzes the efficiency of wave energy farms as coastal protection elements against erosion and flooding under sea-level rise. The study site (Playa Granada, southern Spain, Figure 1) is a deltaic beach which has been experiencing strong erosion and flooding issues in the past two decades, mainly induced by human interventions in the Guadalfeo River basin.

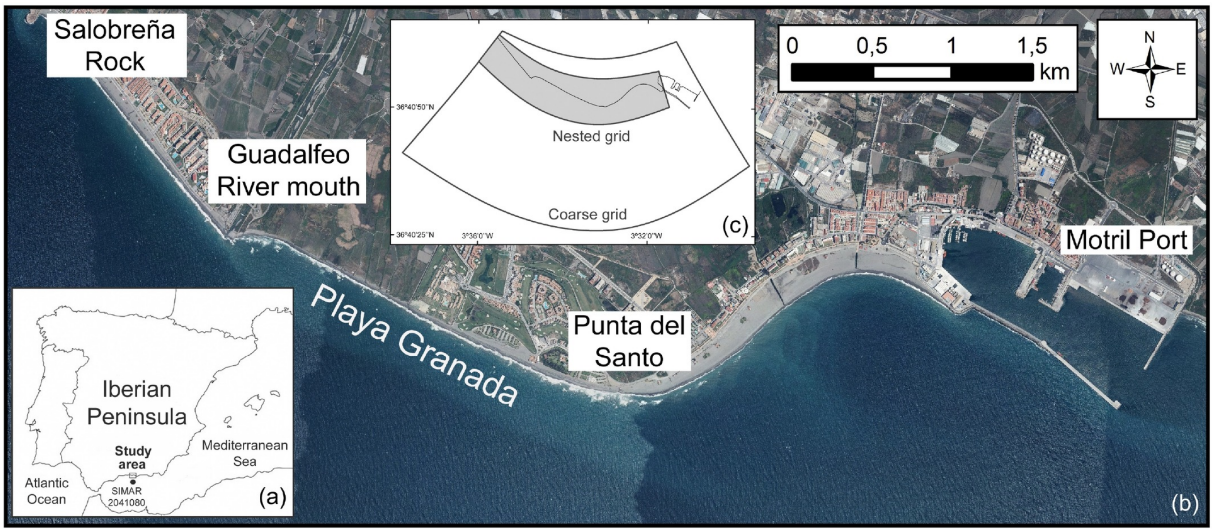

Figure 1: (a) Location of the study area in southern Spain, (b) plan view of the deltaic coast, indicating the studied stretch of beach (Playa Granada), (c) contours of the numerical grids used in the SWAN model. [Source: Bergillos et al. (2019). Reproduced with permission of Elsevier].

Methodology

In order to analyze the effects of a wave farm on wave propagation and coastal flooding, we selected the wave farm location indicated in Figure 1, with the geometrical center situated at 30 m water depth. This position was found to be optimum in terms of both wave energy availability (Rodriguez-Delgado et al., 2018b) and coastline protection (Rodriguez-Delgado et al., 2018a). The wave farm layout consisted of eleven WaveCat devices, distributed in two rows and with an inter-device spacing equal to 180 m (Figure 2).

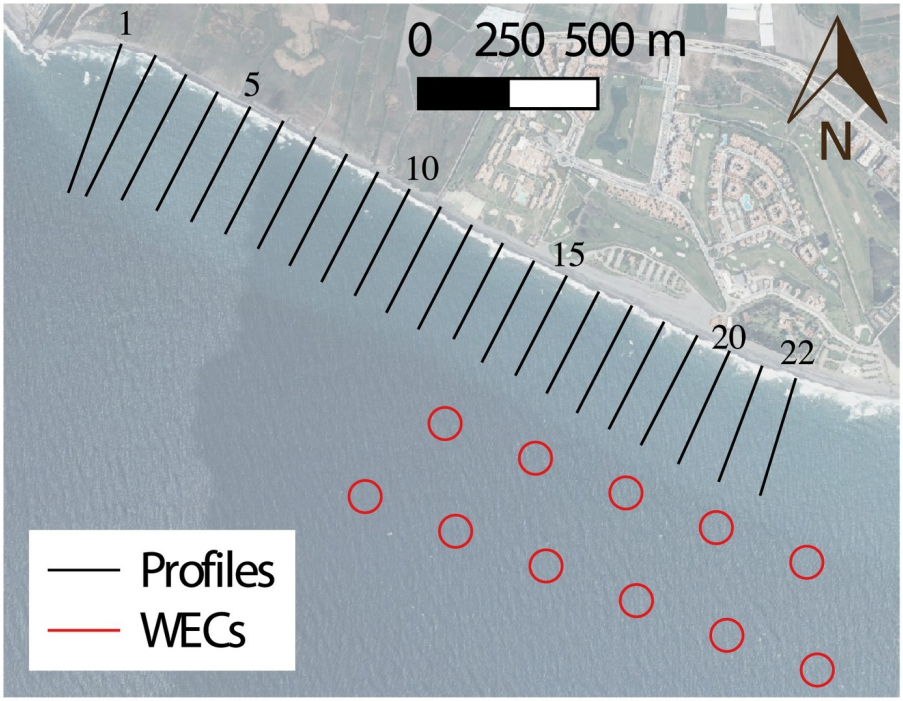

Figure 2: Location of the studied beach profiles (1–22, in black) and wave energy converter farm (in red). [Source: Bergillos et al. (2019). Reproduced with permission of Elsevier].

WaveCat is a type of overtopping WEC composed by two hulls connected by the stern, with a distance between them commonly equal to 90 m. The efficiency of WaveCat and the wave farm layout selected for coastal defence purposes has been widely demonstrated in previous works (Rodriguez-Delgado et al., 2019). The performance of wave farms for coastal defense against erosion was evaluated through the joint application of a wave propagation model (Delft3D-Wave), a LST formulation and a one-line model under three sea-level rise scenarios: the present situation (SLR0), and the optimistic (SLR1) and pessimistic (SLR2) projections proposed by the Intergovernmental Panel on Climate Change in 2014. This allowed computing the final position of the shoreline and final dry beach areas for the three scenarios under storm conditions for the prevailing wave directions at the study site (southwest and southeast). On the other hand, to evaluate the efficiency of the farm for coastal protection against flooding, the Delft3D-Wave and XBeach-G models, previously validated for the study site, were coupled and applied to 22 beach profiles in order to assess wave propagation patterns, total run-up values (including water level), flooded cross-shore distances and total flooded area for the prevailing storm directions and the three SLR scenarios. In both cases (protection against erosion and flooding), the results were compared to the baseline (no farm) case study to properly quantify the effects of the wave energy farm.

Results and conclusions

The results indicate that the absorption of wave power by the wave farm affects wave propagation in its lee and, in particular, wave heights, with alongshore-averaged reductions in breaking wave heights about 10% (25%) under westerly (easterly) storms. These lower significant wave heights, in turn, induce variations in the shoreline evolution and flooded dry beach area. In the case of protection against shoreline erosion, under westerly waves the wave farm reverses the behavior of the coast from an erosive to an accretionary response in every sea-level rise scenario. Whereas the subaerial beach area differences without the wave farm are 90.15 m^2, -42.83 m^2 and -51.66 m^2 for scenarios SLR0, SLR1 and SLR2, respectively; with the wave farm these differences are 2.31 m^2, 28.76 m^2 and 8.14 m^2, respectively. Under the easterly storm, the coastal response is accretionary, and this behavior is strengthened by the wave farm. On the other hand, in terms of coastal flooding mitigation, the presence of the farm leads to alongshore-averaged run-up reductions for the three sea-level rise scenarios and for both wave directions. The flooded cross-shore distances are also reduced by the farm along the studied coastline section for both wave directions and the three sea-level rise scenarios. Importantly, the dry beach area flooded under westerly (easterly) storms is reduced by 5.7% (3.2%), 3.3% (4.9%) and 1.99% (4.5%) in scenarios SLR0, SLR1 and SLR2, respectively. Thus, the results indicate that the wave farm reduces erosion and promotes accretion regardless of the sea-level rise scenario considered. The run-up and flooded dry beach area are also reduced by the wave farm for the three scenarios. In general, sea- level rise strengthens the effectiveness of the wave farm as a coastal protection mechanism. This fact enhances the competitiveness of wave farms as coastal defense elements compared to traditional hard engineering solutions such as groynes or detached breakwaters, whose effectiveness tends to weaken under sea-level rise.

Acknowledgments

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Methodologies for the detection and study of submarine groundwater discharges (SGD) in the Gulf of Cadiz

Alejandro Román Vázquez*[1]

*Corresponding author: alejandro23rv@gmail.com
[1] Universidad de Cádiz, Departamento de Ciencias de la Tierra

Keywords: SGD, ASTER Images, UAV, Gulf of Cadiz, Radium Isotopes

Introduction and Objectives

Submarine groundwater discharge (SGD) is defined as any flow of water through the bottom of the sea without taking into account its composition, its origin or its drivers (Figure 1). It is an important mechanism which transfers nutrients, trace metals, carbon and other dissolved constituents to the ocean (Burnett et al., 2006). These excesses of nutrients not only lead to the eutrophication of coastal areas, but also produce blooms of algae and red tides (Moore, 2010). These discharges are also important from a hydrological point of view since they are source of drinking water, for hygiene, for agriculture or fishing, among other uses. The scientific interest not only tries to estimate and quantify the behavior of these discharges, but also their effect on the coastal ecosystems (Moosdorf and Oehler, 2017). In this project, different methodologies are applied for the detection, and later study, of these discharges in the Gulf of Cadiz. On the one hand, the temperature of these discharges is generally different from the water that receives them, so infrared thermography is used via satellite or UAV for the detection of SGDs. On the other hand, the radio isotope concentration is three times higher than that in the ocean, therefore they can be used as biogeochemical tracers for the study of SGDs.

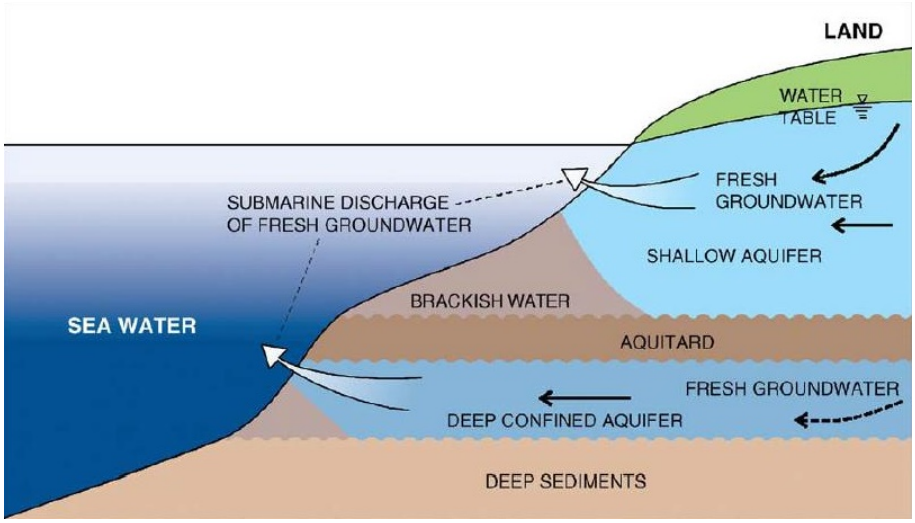

Figure 1: General scheme of the SGD. Arrows indicate fluid movement.

Methodology and Results

For the detection of SGDs, we have taken advantage of the temperature contrasts between the groundwater discharge and seawater. On the one hand in the Bay of Cadiz, we have used ASTER satellite images showing sea surface temperature (Figure 2), with a spectral resolution between 8 and 12 um. On the other hand, in the San Pedro river, we have used TIR images obtained via UAV with a spectral resolution between 7 and 14 um. These thermal anomalies have been related to other variables, such as the level of the tide, the accumulated precipitacion, or the wind.

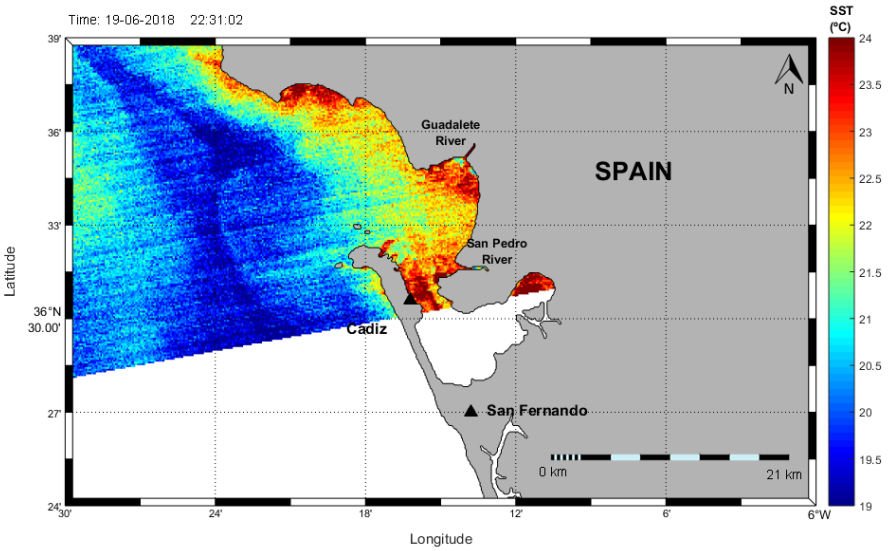

Figure 2: ASTER satellite image showing sea surface temperature in the Bay of Cadiz. The image was taken on the 19-06-2018 at 22:31:02.

SGDs have very high radium concentrations due to the decay of the uranium in continental rocks. The radium dissolves in the seawater, and that explains these high concentrations of radium in the discharge plume. For the characterization of the SGD, four samples were taken in the Bay of Cadiz, which have been analyzed with the RaDeCC methodology, obtaining data of the concentration of 223Ra, 224Ra, 228Th and 224Ra in excess (Table 1).

Table 1: Activities of 223Ra, 224Ra, 228Th and 224Ra in excess (DPM/100L), salinity, sampling coordinates and sampling time (UTC), of each of the samples taken during the MIO2019 campaign of the Master in Oceanography (19-02-2019).

	Coordinates	Hour (UTC)	S (PSU)	^{223}Ra (DPM/ 100L)	^{224}Ra (DPM/ 100L)	^{228}Th (DPM/ 100L)	Ex ^{224}Ra (DPM/100L)
M1	36°23'096 N 6°39'865 W	13:04	36.3	0.54	18.55	3.40	9.71
M2	36°29'131 N 6°26'767 W	15:46	36.2	0.17	85.14	8.84	84.85
M3	36°32'755 N 6°16'546 W	17:20	36.3	0.57	9.94	0.29	9.86
M4	36°34'837 N 6°18'688 W	10:05	36.0	0.76	43.11	0.08	43.11

Conclusions

We can demonstrate the great variety of coastal processes that can benefit from the surface temperature maps obtained from aerial thermography. The advantage of obtaining TIR images with UAV compared to those obtained via satellite is that they have a higher spatial resolution. In addition, the use of UAV is cheaper, simpler, faster, safer and cleaner. The results of the RaDeCC methodology seem to indicate that in sample 2 there are higher concentrations of Ra isotopes. We can associate it with a possible SGD.

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Assessing littoral dynamics by drone aerophotogrammetry

Alejandro Delgado*[1] , Simone Sammartino[2] , Jorge Rey Díaz de Rada[3]

*Corresponding author: alexdepy@hotmail.com
[1] School of Industrial Engineering, University of Málaga
[2] Physical Oceanography Group, University of Málaga.
[3] ESGEMAR,S.A., Local M5/L2-05. Puerto de Málaga, 29001, Málaga

Keywords: Aerophotogrammetry, Drone, Pix4D

Introduction

This study describes the methodology of applying aerial photogrammetric survey to obtain high precision three-dimensional reconstructions of rocky-sandy beaches, and so assess the natural evolution of the littoral caused by the dynamic action of waves. Further than the own aerial images, ground control points (GCPs), whose location is determined by professional topographic equipment with a high level of accuracy, are also used during the processing stage, with the aim of increasing the accuracy of the results. Two flights of the same zone, deployed in two different dates, are processed similarly in order to obtain two accurate models of the beach before and after the occurrence of strong wave events, and hence compare them to quantify the volumes of sand lost or gained.

Methodology

Photogrammetry is the science of making reconstructions of the form, dimensions and volumes of an object using photographs (Orellana, 2006). The conical perspective of the object is transformed into a projection orthogonal to the focal plane. Given two or more images pointing at the same target, their three-dimensional coordinates are inferred by correlating the geometry of the camera with the shape and orientation of the footprint. This method is called restitution. In aerophotogrammetry the pictures are taken by a flying device. The main advantage of aerial photogrammetry over classic photogrammetry stands on the fact that coordinates and angles of the shooting point are measured. The procedure to transform a terrain into a 3D model using drone aerophotogrammetry follows the steps detailed below. Once identified the coastal zone to be surveyed, the flight parameters have to be defined: region of interest, flight mesh, speed and height, and degree of overlap. With these in mind the flight plan is drawn and uploaded into the drone. Figure 1 show the two flight plans made to obtain the models. The study zone is the beach "La Mezquitilla" at the east coast of Málaga. In the first flight (left one) two partially overlapping flights have been deployed. The aim was to study the possibility of carrying out high precision aerophotogrammetry surveys in large areas, fragmenting it into contiguous flights and then overlapping them using GCPs.

Figure 1: Flight plans used in this study. Left: first survey (two flights). Right: second survey (one flight).

For both surveys an overlap of 85 % , aimed at improving the post-processing stage, a flight height of 15 m, aimed at maximizing ground resolution, and lowest speed, aimed at attenuating the effect of rolling shutter (Otero et al., 2008), have been chosen as flight plan parameters. Once the flight has been planned, 6 physical targets (a rock, a marker or a particular object) have been identified to be used as control ground points. Their position has been measured with sub-centimetric accuracy using a professional topographic equipment, while their locations have been clearly marked to be easily recognized in the aerial images. The software used to execute the flights was Pix4D Capture, installed on a tablet connected to the drone controller. Once collected the pictures, they were processed with the software Pix4D Mapper, integrating the GCPs such to constraint the terrain model to adjust to their accurate positions. Points clouds has been created and a digital terrain model and textured mesh have been obtained. On these models, a series of reference areas have been defined and discrete volumes have been determined for both flights (Figure 2), to identify the erosive (where beach volume diminished from flight 1 to flight 2) and sedimentary (where new sediment has been gained from flight 1 to flight 2) regions.

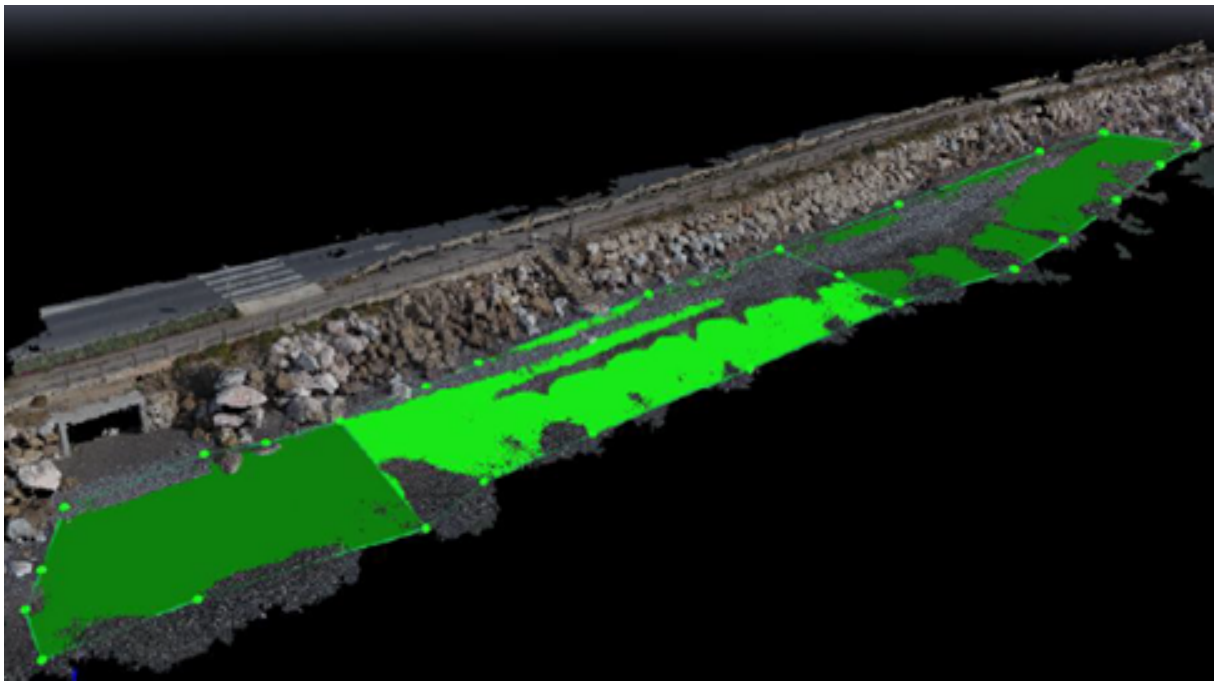

Figure 2: Areas used for calculating volumes. (Left to right: Area 1, 2, 3).

The software Pix4D Mapper has been used to make volumes computation.

Results and conclusions

Results obtained by the comparison of volumes between the first and the second survey prescribes a net loss of 376.34 m^2, identifying at least two different behaviour in terms of erosive or sedimentary dynamics (Table 1).

Table 1: Volume comparisons in the three studied zones.

	Volume calculated		
	in model 1 (m^3)	in model 2 (m^3)	Difference (m^3)
Area 1	-13,31	-3,04	10,27
Area 2	-21,32	-156,84	-135,52
Area 3	-43,35	-294,44	-251,09
		Total	-376,34

The study presents an example of the use of aerophotogrammetry to the precise characterization of the coastal dynamics. The method allows to quantify with high accuracy the variation in shape, volume and distribution of sediments in the coast analysed. Special regard has to be given to the use of the GCPs, which allowed both models to be accurately referenced, obtaining high-precision volume computations. Moreover, the possibility to obtain accurate overlap of two contiguous flights has been proven, setting out the chance of mapping large areas using multiple flights, so coupling high resolution and low operational costs.

Acknowledgments

The authors are very grateful to Real Aeroclub de Málaga for their consultancy on flight permissions.

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AS DE GUIA: Inclusive science and oceanic culture outreach for youth in social exclusion risk

Francesca Iuculano*[1] , Marta Fernández Clemente[1] , Lara Garcia Corral[1,2] , Carla Huete Stauffer[1] , Josep Lluis Oliver[3]

*Corresponding author: noctiluca.asociacion@gmail.com
[1] Noctiluca Association
[2] Universidad Rey Juan Carlos
[3] Universitat de les Illes Balears

Keywords: Oceanic culture, Inclusive citizen science, Outreach and horizontal dissemination, Marine conservation, Biodiversity and heritage, Informal education

Introduction

"As de guía" is an inclusive science project, founded by Spanish Foundation for Science and Technology (FECYT). It is framed as an extension of the pilot project "Science at home", developed during 2018, founded by the call for Innovative Scientific dissemination projects "Innovative Divulgation also for other publics" of FZCC. With this local project we approach sea and science to all, in order to achieve and contribute to the encouragement and awareness of oceanic culture, or oceanographic literacy, promoted by UNESCO and extremely necessary for the conservation of the oceans for our planet and life.

Objectives

1. To approach science to collectives of young people traditionally far away or excluded from culture and scientific outreach and education.
2. To realize a pilot experience with collective with less access to scientific outreach to identify barriers, methodologies and indicators that influence the isolation of informal education in scientific areas, improving this access for future research lines.
3. To improve and promote scientific education, culture and ocean literacy to young people in social exclusion risk.
4. To promote the diversity of scientific vocations, making them explore the research work field and the centres where scientists develop their research.
5. To raise awareness of these youth about the importance of marine biodiversity and conservation through the knowledge of the natural marine and littoral heritage of the Balearic Sea, where they live.
6. To help their inclusion through the participation in science, collaborating in citizen science initiatives.
7. To create an active collective of scientific dissemination, providing them tools in order to be themselves the main characters who explain to the society the importance of the conservation of the marine environment and science.
8. To promote in this youth collective the interest for scientific culture in order to improve their confidence related with STEM subjects.

Methodology

"As de guía" project is divided in seven blocks of work:
Block 1: Coordination, technical and educational design.
Block 2: Scientific method on the beach.
Block 3: Oceanographic cruise and scientific experience in research centres.
Block 4: Horizontal divulgation in radio capsules on board of Noctiluca boat.
Block 5: No Plastic Festival 2 Event.
Block 6: Evaluation of the project and proposal of diagnosis for inclusive activities.
Block 7: Communication and dissemination of the project.
We use citizen science as an effective tool to learn the scientific method. We address an informal education, promoting the horizontal dissemination as a new way of transmitting science to society.

Preliminary results

We approach the scientific method and the knowledge of the sea to around 100 youths belonging to 7 foster home of Mallorca and 1 of Ibiza. We sampled 4 beaches by collecting data of micro and meso-plastics (Seawatchers platform) and beach characterization (Reefcheck platform). We sample 4 on-shore transects (5gyres platform) around Mallorca island. We disseminate 8 radio sessions. We provide a diagnosis guide to identify barrier, limitations and indicators on working with this sector of youth.

Conclusions

"As de guía" project is an important and innovative contribution and an effort to inclusive science to create an active collective of scientific communicators, providing scientific and educational marine tools to aware the society about the importance of the conservation of the marine environment.

Figure 1: Sampling protocol "Plastic0" in Can Pere Antoni beach, Mallorca.

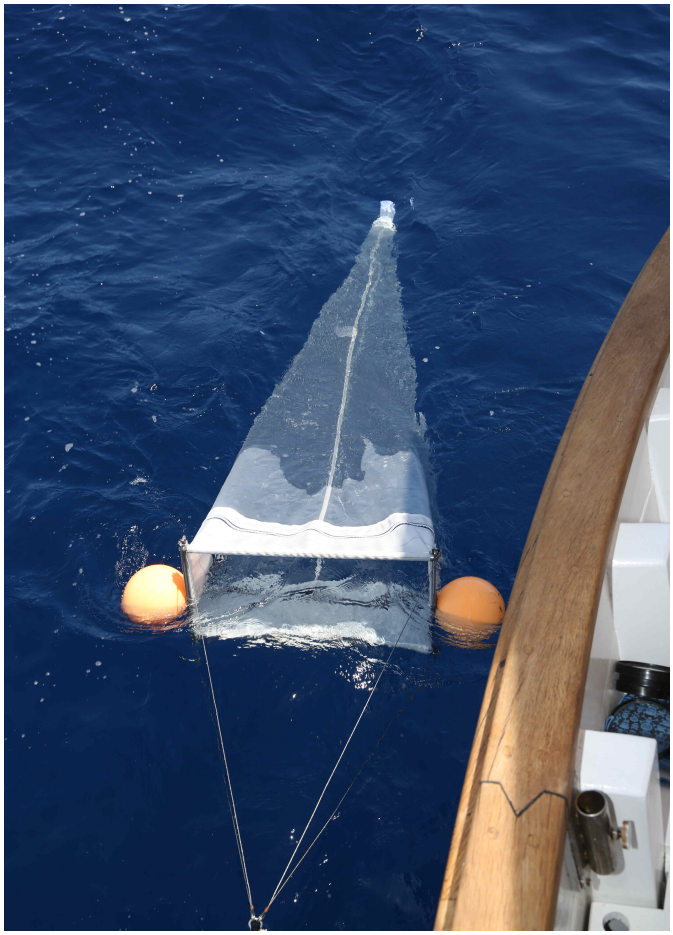

Figure 2: "Manta-trawl" sampling strategy to collect and quantify micro-plastics in the surface waters, on board of La Balear vessel, Mallorca.

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Use of aquatic resources as possible sound artefacts or musical instruments and their symbolism during the prehistory

Marina Zafra Granados*[1]

*Corresponding author: marinazfg@gmail.com
[1] University of Cádiz

Keywords: Prehistory, Aquatic, Music, Resources

Research goals

This dissertation provides an interdisciplinary study of Underwater Archaeology and Music Archaeology to the understanding of the phenomenon of music during prehistory, through the study of aquatic resources as possible sound artefacts or musical instruments. This study offers an approach to the problem of the study of aquatic resources, as well as its relationship with the environment and its subsequent symbolic involvement with these communities during prehistory. Meanwhile, we will try to create a methodological framework for the study of these possible sound artefacts or musical instruments and their evolution.

Abstract and other aspect

The phenomenon of music has accompanied us since our origins, and it is currently an essential part of our day. Bearing in mind that nowadays music generates different types of behaviours and feelings for individuals of the same culture, for many, thinking about the meaning or development that it had during prehistory is an idea that will always be far from our understanding. It was even inaccessible to create an approach, archaeologically speaking. During these last years the relationship between music and human evolution has been explored from different perspectives, arguing in general that this is a mechanism capable of promoting group identity, survival a very powerful cultural communication mechanism that acted on the group to unify the community, coordinate and achieve common goals (Salius i Gumá, 2010). The studies that were carried out until very recently on the music in the prehistory often considered only the direct archaeological evidence of the Upper Paleolithic, and have unfounded these studies and results like synonyms of the origin of the music. Considering that theoretically, they would dominate the percussion instruments to the melodic instruments during the prehistory. This idea is apparently contrary to the results that we have obtained in the database, since 94% of those who have recorded are aerophones, and between they highlight triton horns with Neolithic chronology. Today although it is true that there is no irrefutable evidence of musical instrumentation prior to the Upper Palaeolithic in Europe, (Morley, 2003) also most of the research has focused on the study of aerophones, leaving aside other types of instruments during prehistory, since these have been better preserved, are more visible within the archaeological record, and have aroused greater interest on the part of numerous researchers due to the complexity in the technical design of some of these. But this great sophistication in the technological design suggests that said instruments must have had certain previous conceptual stages. According to several researchers and observing ethnographic examples, we can surely consider "rattles" as one of the first sound artefacts (Brade, 1975). There are a great diversity of designs and these are almost universally found throughout the

world. The pendants suspended from the neck, arms, legs or clothes shook during the movements and produced rattle sounds. This sound is often used to highlight dance or group coordination in certain modern ethnographic groups. We can find evidence of this type of ornaments even in the Neandertals themselves. This has been interpreted as a test of symbolic thought, and there are also indications to think that the origin of these was prior to the Middle-Upper Paleolithic transition in Europe. In this sense, archaeology has not become aware of the methodological tools necessary to face the study of what might be sound artefacts of these characteristics during the prehistory and protohistory. When carrying out any type of study on the prehistory, and in particular perhaps of Archeomusicology and on aquatic resources we are before some singularities. To the fact that we can only find material remains and the poor conservation of these, we must add the difficulty of interpreting the registry, and the lack of a good work methodology (Garcia, 2014). We have always used different aquatic resources for a variety of purposes since prehistory. These created deep signs of identity in certain societies, and even today these resources continue to have great symbology and importance for many cultures. We have used them to produce dyes, food sustenance, decorative elements or even sound artefacts among other uses. These have had fundamental importance at the time of being able to understand our past and the different contexts, but they have not always been given the importance that they should have. It has been defended that it was the anatomically and cognitively modern man who started the exploitation of aquatic resources around 50 and 40 ka. It is said that this exploitation began with the appearance of the so-called modern behaviour (Cuadrado, 2016). It is this idea of the cultural revolution that has impeded any other evolutionary version of the cognitive abilities of hominids and has distorted our view of their way of life and their abilities. In this way, it has lately become more and more inescapable to ask whether other hominids, even before the Neanderthal, could also possess certain modern cognitive characteristics. In addition, artefacts made with these resources or materials gained cultural importance through their modification in these populations. We will try to create a methodological frame of reference when will we try to deal with certain artefacts of this nature. Meanwhile, we try to find in the archaeological record since until very recently there was no interest in the study of these, and many of these resources have been documented as simple food support for these populations. We must come to understand that other conceptual forms came to adopt these aquatic resources. Although these have had a fundamental importance in archaeology, at the time of being studied in different contexts, these have not been as studied or proposed as other terrestrial resources have been. In Spain, Archaeomusicology has been developed little by little, and even today, this is still very unknown by the general public and by the scientific community. In addition, especially the knowledge of music in prehistory, is very scarce in the Iberian Peninsula. In this context, it is necessary to study the role of everything that encompassed the sound phenomenon since it is completely linked to many aspects and issues of our day to day in the past and our origins, which we will not be able to solve or understand if not we approach it from other perspectives. In this case from the sound-musical perspective. Finally, through the presentation of this work within the framework of the II Congreso de Jóvenes Investigadores del Mar, we aspire to create a debate among the different scientific disciplines that meet in this, in order to enrich ourselves from the experience and perspective of the attending researchers, to the time to give a little more knowledge about Archaeomusicology among the general public.

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Landscapes of salt in Huelva

Laura del Valle Mesa*[1]

*Corresponding author: laura.delvalle@dhga.uhu.es
[1] Universidad de Huelva

Keywords: Salt pans, Cultural landscapes, Cultural heritage, Heritage management, Natural heritage

Introduction

UNESCO defines the Cultural Landscape as the "joint works of man and nature... that illustrate the evolution of human society and its settlements over time, conditioned by the limitations and/or physical opportunities presented by its environment natural and by the successive social, economic and cultural forces, both external and internal". Framed in this definition is the trace of the salt activity developed on the coast of the Gulf of Cádiz due to the morphological, climatological and saline conditions that the territory and the Atlantic Ocean proffer (Ojeda Zújar, 2004); activity protagonist since the first human occupations of this territory and that has lasted until the present. The saline activity, in its traditional origins, but now also industrial, has been a way of taking advantage of the natural resources that is decisive in the development of societies, in the improvement of their quality of life and, of course, in this territorial area, in the generation of economies linked to fishing, the canning industry and its commercialization. Its importance as an economic activity meant a specific design of the work in the salt pans that is based on the Phoenician occupation, which expands and regulates with the Roman organization (Alonso et al., 2003) and remains without undergoing significant transformations until it begins to industrialize from the second half of the twentieth century. Thanks to this, the coast of Huelva is drawn by the proliferation of saltworks linked to the marshes and estuaries that, active or obsolete, have shaped the cultural landscape and the collective imagination of the local population, determining that the symbolism of part of the coast can not currently be understood without the transformation of the land for the occupation of the salt pans, the huge salt mountains and the traditional or industrial architectures which adhere to such activity.

Methodology

To study the saline landscape on the coast of Huelva, we have used a methodology that is mainly qualitative, and in some cases it is necessary to go to the quantitative one for the grouping and analysis of numerical information. In the first place, we have delimited the contemporary salt mining units of traditional and industrial character and identified the natural landscape with which it is related, since they are those that are currently printed in the territory together with their architectures and manufacturing centers that still remain erected. Next, we have made a compilation and bibliographic and documentary analysis to know the significance of this activity in Huelva society, economy and culture and the magnitude of the salt footprint in the province. Finally, we have developed a fieldwork in the Huelva salt pans through different methods: Genius Loci, which consists on the knowledge of the sites at present to know their past history; sensory analysis, to know the connotations of the territory and understand the relationship of people with the physical environment; and the documentation of the elements constructed in relation to their geographical context. All this, has been collected in a database with which it has been able to group the information in order to draw conclusions and to prepare maps in which to

georeference the landscapes of salt in the province of Huelva. It is important to point out that to know the patrimonial and cultural relevance of these landscapes we have gone to the databases of the administration and competent organizations in the matter of Cultural Heritage.

Results

The landscapes of the salt are distributed throughout the coastal section of Huelva, from the mouth of the Guadiana River, to the mouth of the Guadalquivir River, there being a greater concentration of these landscapes on the west coast and in the estuary of the Tinto and Odiel rivers (Román, 2014). It is a characteristically coastal and flat landscape, although anthropised, linked to the marshes, in which the prominence is shown by the unevenness of the saline rafts systems, the marine and fluvial waters that conquer them; the occupation of the soils by the plants of families such as the Plumbagináceas and Quenopodiáceas, Tamarix and Atriplex and Salicornias, without forgetting, although it is not recognizable in the landscape of the salt, of the halophytes that extend through the marshes and rafts (Clares, 2004). In this landscape also other human works stand out, such as tidal mills (Rubio and Serveto, 2004), closely linked to the salt mines, the salt houses and the salt mines, the semi-manual processing structures and the industrial brine processing plants, respectively; as well as the crystallization of the rafts in summer seasons that dye the salt mines white and the great mountains of white salt that are located on the edges of the pits and rafts. In addition to the landscape and identity significance of these coastal exploitation systems, we envision another series of heritage values that are inherent to these landscapes. First, the ethnological values, by the traditional salt activity maintained throughout history since the extractive system was established by solar evaporation, which is linked to a whole series of religious rituals (such as the blessing just before beginning of the season of salt extraction) and labor (clearing of rafts, preparations, preparation of work tools), a distribution of functions determined by gender and age, etc. Then, the historical values that are obvious because of the importance of salt for governments and their populations and that have maintained this system for more than two millennia and for which popular wars and brawls have been staged. On the other hand, architectural values linked to the construction of salt systems characteristic of maritime and quasi-identical operations in the Gulf area; and to the characteristic saltworks constructions for other functions such as administrative, organizational or distributive of the salt houses. No less important, the values linked to the Industrial Heritage, with the new systems of salt exploitation and, of course, the natural ones, where poultry species also take center stage. All of them are collected and transmitted in the significant and different landscapes of the salt that still remains (obsolete or active) on the coast of Huelva. Finally, the relevance of some of these landscapes in Huelva has been recognized by UNESCO and by the Andalusian administration. For the first case, the salt pans of Huelva city and Almonte, are part of two cataloging spaces of the Biosphere Reserve and World Heritage, respectively. In the second of these, the Salina Industrial Aragonesa (Huelva) and the Salinas del Alemán (Isla Cristina), are listed in the General Catalog of the Andalusian Historical Heritage and are part of two catalogs of Landscape of Cultural Interest processed by the same administration. In this sense, we believe that it is extremely necessary, given the progressive disappearance of many of these landscapes, for the institutions to promote their protection and use for the historical, cultural and heritage education of the coastal populations of Huelva dedicated to this and other economies linked to the coast, which marked the course of its economic and social development, using the heritage resources that still gives us its landscape and which are the direct evidence that currently persists throughout the fishing-canning history of the province.

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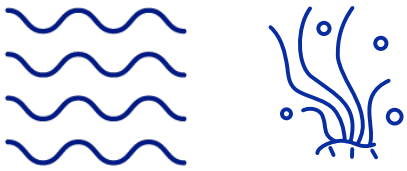
Session 10

The Sea Knowledge

Ecology



Natural seawater HCO_3^- enrichment impairs nitrate homeostasis in *Zostera marina* L. leaves

Jordi Diaz-Garcia*[1] , Rubén Contreras[1] , Jose A. Mercado[1] , Jose A. Fernández[1] , Lourdes Rubio[1]

*Corresponding author: jordidg@uma.es
[1] Department of Botanic and Plant Physiology, University of Málaga, Málaga, Spain

Keywords: Zostera, NRT2, Bicarbonate, Nitrate

Introduction

Increasing atmospheric CO_2 promotes high C assimilation in C3 species; nevertheless, reduces the overall mineral concentrations in the biomass. Among all, N is the main affected, which content diminished by 16% in 689 ppm CO_2 (Loladze, 2014). This impact could be greater in seagrasses, since the marine environment is a natural CO_2 sink, in which increasing the dissolved inorganic carbon (DIC) rises HCO_3^- concentration, and most of these marine plants have non C-saturated photosynthesis and can use HCO_3^- for that. This is the case of *Posidonia oceanica*, which uptakes HCO_3^- using a H^+/HCO_3^- symport mechanism in leaf cells (Rubio et al., 2017). This HCO_3^- is internally dehydrated by Carbonic Anhydrases, arising OH^- and CO_2, which is used in the Calvin cycle. The input of HCO_3^- and the cytosolic pH increase would trigger the activation of S-type anion channels, allowing the passive output of Cl^- and NO_3^- (Xue et al.). The lost of cytosolic NO_3^- could contribute to the impoverishment of N in the tissues by affecting NO_3^- uptake and/or impairing Nitrate Reductase activity. Seagrasses are the only group of angiosperms that colonize marine environments, evolving mechanisms to uptake essential nutrient, as NO_3^- or Pi, from very low concentrations (<10 μM) in a liquid, highly saline (0.5 M Na^+) and alkaline medium (pH 7.9-8.2). In contrast to terrestrial plants, which use H^+ symporters, seagrasses such as *P. oceanica* or *Zostera marina* use a Na^+-dependent high-affinity nitrate or phosphate transporters. Interestingly, in *Z. marina*, the first fully sequenced seagrass, only one gene (*Zosma70g00300.1*) is annotated as a high-affinity nitrate transporter (NRT2). In addition, sequences for NRT2 accessory protein NAR2 and Nitrate Reductase (NR), *Zosma63g00220.1*, *Zosma84g00350.1*, respectively, are also found in *Z. marina* genome. In order to unveil the role of HCO_3^- increase on nitrogen homeostasis in *Z. marina*, high-affinity NO_3^- uptake, C/N biomass ratio and Nitrate Reductase activity have been analysed in plants incubated in natural seawater enriched with 3 mM HCO_3^- for 7 days. In addition, *NRT2*, *NAR2* and *NR* gene expression have been quantified.

Plants incubated in 3 mM HCO_3^- enrichment seawater show higher high-affinity NO_3^- uptake capacity and a high expression of the high-affinity NO_3^- transporter accessory protein NAR2

To evaluate the hypothesis that the prolonged increase of Ci in the medium would modify the uptake capacity of NO_3^- in *Z. marina*, depletion experiments were conducted (Figure 1). After 5 minutes in the presence of 25 μM NO_3^-, plants pre-incubated in NSW+ 3mM HCO_3^- (NSW+C) or in the absence of N (ASW-N) depleted higher external NO_3^- concentration than in control conditions (NSW and ASW+N, respectively).

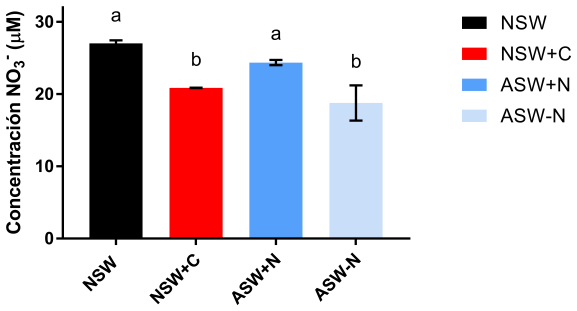


Figure 1: External NO_3^- depletion assays. Before the test, *Z. marina* plants were pre-incubated in NSW; NSW+3 mm HCO3 - (NSW+C); ASW+100 μM NO_3^- (ASW+N) and ASW-N for 7 days at 15°C under photoperiod of 16/8 (light/dark). Assay medium consisted in ASW+25 μM NO_3^-. Data are mean ±SD (n=3) of the external NO_3^- concentration after 5 min of incubation. Different letters denote significant differences (Two-way ANOVA, Tukey test, $p<0.0001$).

As shown in Table 1, no high-affinity NO_3^- uptake was observed in plants incubated in control conditions (NSW and ASW+N); nevertheless, similar NO_3^- uptake rates were observed in plants incubated in high Ci (NSW+3 mM HCO_3^-) or in the absence of N (ASW-N).

Table 1: NO_3^- uptake rates. *Z. marina* plants were pre-incubated as described in Figure 1. NO_3^- uptake rate was calculated after 5 min of incubation in ASW+25 μM NO_3^-. Data are mean ±SD (n=3). Different letters denote significant differences (Two-way ANOVA, Tukey test, $p<0.05$).

Incubation conditions	Incorporation rates ($\mu mol \cdot h^{-1} \cdot gpf^{-1}$)
NSW	-1,39 ± 1,07 [a]
NSW+C	2,57 ± 0,25 [b]
ASW+N	0,92 ± 0,87 [a]
ASW-N	4,02 ± 1,27 [b]

These findings are consistent with the hypothesis that cytosolic NO_3^- could decrease in plants grown in high Ci. Nitrate not only acts as a nutrient, but also as a signal that regulates gene expression. Thus, under conditions of low cytosolic NO_3^- availability the expression of high-affinity transporters (NRT2) and accessory protein NAR2 is induced, increasing the capacity to uptake NO_3^- at micromolar range. In order to investigate the effect of the seawater HCO_3^- increase on the expression of the *NRT2*, *NAR2* and *NR Z. marina* genes, a qPCR analysis was performed using leaves of plants incubated for 7 days in NSW or in NSW enriched with 3 mM HCO_3^- (NSW+C). As shown Figure 2, transcript levels of *NRT2* do not increase in plants incubated in high HCO_3^-. On the contrary, the accessory protein *NAR2* gene does significantly more than 90 times. This result is similar to the strong induction of NAR2 expression observed in NO_3^- deficient terrestrial plants, suggesting that NAR2 is essential for NRT2 stabilization at the plasma membrane to function.

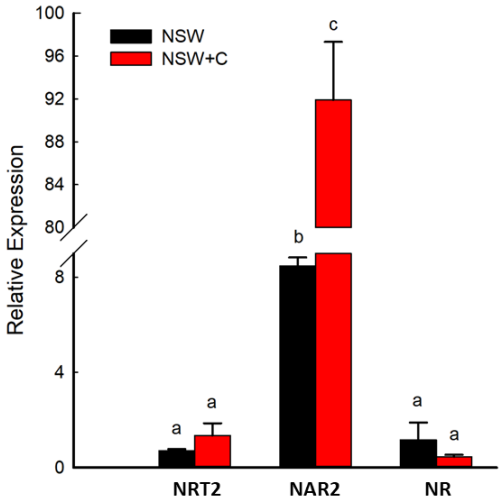


Figure 2: Effect of HCO3 - on NRT2, NAR2 and NR gene expression. Before the test, *Z. marina* plants were incubated for 7 days in NSW and NSW+3 mM HCO3 - (NSW+C) as described in Figure 1. Relative gene expressions were quantified by qPCR. For each gene, relative expression was calculated using TF2D expression level as control. Values are mean ±SD (n=3). Different letters denote significant differences (Two-way ANOVA, Tukey test $p<0.05$).

Interestingly, it has been described that NRT2 transporters in mono and dicots exhibit a strong increase in transcripts under conditions of NO_3^- starvation, except for the case of NRT2.5 versions, which barely modify their expression. In fact, in silico analysis of the only *NRT2* gene identified in the genome of *Z. marina* indicates that is homologous to the NRT2.5 of terrestrial plants (Rubio et al., 2019).

Seawater HCO_3^- enrichment causes N impoverishment in *Z. marina* leaves

The Nitrate Reductase activity could be considered as an indicator of the availability of cytosolic NO_3^- to be assimilated. With this trial we wanted to confirm the effect of HCO_3^- on the cytosolic nitrate decrease. Figure 3A shows higher NR activity in *Z. marina* leaves from plants pre-incubated in control conditions (NSW, or ASW+N) than in leaves of plants pre-incubated in high HCO_3^- or in the absence of N (NSW+C and ASW-N, respectively). These results suggest that the increase of HCO_3^- in the medium could have a negative impact on the amount of cytosolic NO_3^- available to be assimilated. In fact, in *Z. marina* and *Zostera noltei* it has already been described that previous exposure to a NO_3^- low concentration also reduces the enzymatic activity in the leaves and does not increase even though the amount of nitrate increased (Alexandre et al., 2012). On the other hand, leaves of plants pre-incubated in NSW+ 3 mM HCO_3^- show higher C/N ratio than the plants pre-incubated in NSW (Figure 3). However, similar leaves C content was observed in both conditions (33,69 ±1,11 μmol C/mgDW; 34,04 ±0,78 μmol N/mgDW, respectively). Nevertheless, lower N content was detected in leaves of plants incubated in NSW+ 3mM HCO_3^- (2,18 ±0,21 μmol N/mgDW) compared to control conditions (2,39 ±0,08 μmol N/mgDW). This means that the increase in the C/N ratio in plants incubated in high HCO_3^- is due to the diminution of the N content in *Z. marina* leaves.

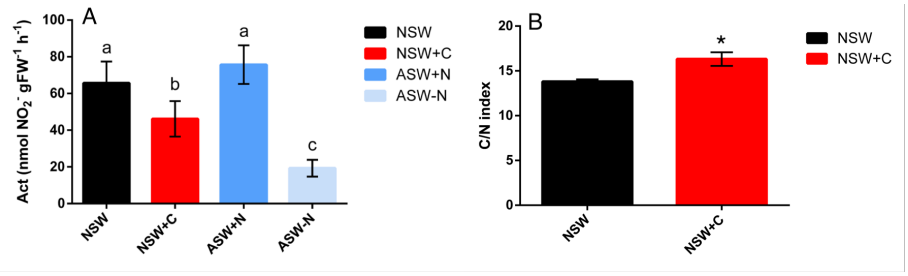


Figure 3: Nitrate Reductase acitivity (A) and C/N ratio (B) in Z. marina leaves. Before the test, plants were preincubated as described in Figure 1. The enzymatic activity was quantified with a modification of Roth and Pregnall (1988) protocol. The content of C and N was determined in lyophilized leaves by atomic absorption spectrometry. Values are mean ±SD (n=3). Different letters in A or asterisk in B denotes significant differences (Two-way ANOVA, Tukey test, $p<0.05$ and Student t-test $p< 0.001$; respectively).

Overall, these results fit the hypothesis that natural seawater HCO_3^- enrichment causes the nitrogen loose from *Z. marina* leaf. Thus, the chronic diminution of the cytosolic NO_3^- concentration could impair nitrogen assimilation and would contribute to the N biomass dilution expected under elevated inorganic carbon.

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Absence of thermal tolerance variability and niche underfilling of two key macroalgae species

Sandra Hernández*[1] , Ana García García[1] , Brezo Martínez[1]

*Corresponding author: sandra.hernandez@urjc.es
[1] Universidad Rey Juan Carlos

Keywords: Ascophyllum nodosum, Chondrus crispus, Physiological thresholds, Distributional range shift, Ecotypes

Abstract

Climate change is affecting the survival of organisms directly. In the marine environment, intertidal and subtidal macroalgae species are drastically reducing their canopy due to their low physiological resilience (Wernberg et al., 2011). Habitat-forming species are depleting their populations and replacement by other species of this type is not occurring (Martínez et al., 2018). This translates into a cascade of simplification of coastal ecosystems, especially in temperate zones where these organisms are dominant. Since physiological tolerance thresholds are usually fixed and species-specific, rapid adaptation to stressful situations such as ocean warming, does not occur. This produces that the populations of low latitudes, where the thermal stress is greater, are not better adapted, and little by little disappear because their Upper Survival Temperatures (USTs) do not increase and their safety tolerance margins decrease (Bennett et al., 2015). We investigated experimentally the ecotypic variation of USTs to sea temperature between European populations along the latitudinal range of two macroalgae species, *Ascophyllum nodosum* (Linaeus) Le Jolis and *Chondrus crispus* Stackhouse. The first one, is a cold-temperate brown algae genetically and morphologically invariable throughout its distribution, and the second one, is a red seaweed of large morphological plasticity and genetic variation, so it will be expected to have more variation in population USTs. No better tolerance to high ocean temperatures at marginal populations was observed, and states of thermal niche underfilling were identified for both species comparing the realized niche with the fundamental thermal determined by thermal thresholds shown in the experiments. These species could occupy latitudes that are thermally favorable on the Portuguese and even African coast, in absence of extreme events or other physical stressors. Nevertheless, the heat waves could be the cause of that unfilled situation because the safety margins are exceeding and prevent the survival of these species. Marine ectotherms were shown to fill their thermal potential niche (Sunday et al., 2012), however, macroalgae and coastal sessile species have not been investigated so far and niche unfilled situations seem to be common. Furthermore, the biogeographic models that related the distribution of macroalgae to ocean isotherms assumed niche-fill states (Lüning, 1990). Our results suggest restricted selective pressure of ocean warming and that additive atmospheric warming and other low tide stressors explain the southern distribution limits.

Acknowledgments

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Dispersion and effect on native fish communities by the invasive alga *Halimeda incrassata*

Toni Vivó Pons*[1] , Josep Alós Crespí[2] , Fiona Tomas Nash[2]

*Corresponding author: tovivo73@gmail.com
[1] Universitat de les Illes Balears, Cra. de Valldemossa, Km. 7.5, 07122 Palma de Mallorca, Spain
[2] Instituto Mediterráneo de Estudios Avanzados, IMEDEA (CSIC-UIB). C/ Miquel Marqués 21, 07190, Esporles, Illes Balears, Spain

Keywords: Alien species, Ecosystem engineers, Mediterranean Sea, Fish community

Preliminary abstract

Invasive species are one of the main factors that impact or alter the functioning of marine ecosystems. Despite the negative effects that they can have on local communities, depending on the trophic level where native organisms are, invasive species could have beneficial effects on certain organisms (Thomsen et al., 2014). The invasive alga *Halimeda incrassata* discovered in 2011 in Mallorca (Alós et al., 2016) being an ecosystem engineer, could induce some important changes on ecosystem functioning, by stabilizing the substrate, competing with native macrophytes or providing a new source of habitat and food to native fauna (Guy-Haim et al., 2018). Here, we study the factors that have influenced *H. incrassata* dispersion through the years and the effect that it has on native fish communities of stabilized sandy bottoms, continuing with the work done by Alós et al. (2018), but in this study we analyse changes on the whole fish community. We have used data from 8 years of sampling in the same area (the MPA of "Badia de Palma", in Mallorca) with underwater cameras, analysing presence/absence of *H. incrassata* and the fish community composition and abundance. Our preliminary results show that native fish communities have changed due to the presence of this invasive algae. Species like *Xyrichtys novacula*, *Pagellus erythrinus* or *Dactylopterus volitans* are more abundant whilst other species like *Bothus podas* have declined due to the presence of *H. incrassata*. Besides these results, factors that contributed the most to the spreeding of *H. incrassata* have to be analysed too. More research has to be done to observe the dynamics and ecology of this invasive alga, and the effects that it has on other habitat-forming or same trophic level species.

Acknowledgments

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An Artificial Neural Network to infer the vertical phytoplankton biomass profile from surface data

Michela Sammartino*[1] , Salvatore Marullo[2] , Rosalia Santoleri[1] , Michele Scardi[3]

*Corresponding author: michela.sammartino@artov.ismar.cnr.it
[1] Institute of Marine Sciences - Italian National Research Council (ISMAR-CNR), 00133 Rome, Italy
[2] Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), 00044 Frascati, Italy
[3] Department of Biology, University of Rome -"Tor Vergata", 00133 Rome, Italy

Keywords: Artificial Neural Network, Vertical chlorophyll-a profile, Mediterranean Sea, Remote sensing

Introduction

Changes in phytoplankton composition and biomass are good indicators of the trophic state of the marine environment, especially within a climate change context. One of the most commonly used proxies for algal biomass in the ocean is the chlorophyll concentration (Chl). Estimates of Chl are provided throughout different techniques, instruments and platforms. Nowadays, at surface, the quantification of Chl concentration has been improved by the use of satellite sensors that provide Chl data at higher spatial and temporal resolution, being more and more employed for the detection of its variability at global and regional scales. Conversely, Chl data along the water column are less available. Vertical profiles of algal biomass are obtained by *in situ* measurements that are usually expensive and time consuming, providing fragmented dataset in space and time. In this context, the development of methods to reconstruct the vertical Chl structure from surface information becomes crucial for the modeling of biological processes occurring in the Mediterranean Sea. To this aim, the high resolution of satellite data and the accuracy of field data were combined within an Artificial Neural Network (ANN, Scardi (1996)) to infer the Chl vertical profile from surface measurements only (Sammartino et al. (2018) and references therein). One of the main advantage of such machine learning approaches relies in their ability at finding the non-linear relationship among different variables without *a priori* assumption. Given the high level of synopticity of satellite data, this method represents a valid alternative approach to classical models to overcome the discontinuity of *in situ* sampling.

Data and methodology

An Artificial Neural Network requires an initial set of data used as co-predictors for the first training and the later test phase. In this work, we employed an ANN (Figure 1) structured with seven inputs and one output and trained with an error back-propagation algorithm (Scardi, 1996). The latter is one of the most known training algorithm where the errors are iteratively traced backward throughout the network in order to minimize the residuals between the observed output and the predicted one (Lek et al., 2000). In this work, a large Mediterranean dataset of temperature and Chl profiles was used for the training and test of the network. The ANN performance has been evaluated either using *in situ* surface data or satellite matchups as inputs, and, separately, validated against *in situ* Chl profiles (Sammartino et al., 2018). All *in situ* data passed a quality control following a set of requirements that provide a total number of 1213 profiles available for the model implementation. The inputs were processed and normalized in the

[0,1] interval. Vertical Chl profiles were at first transformed logarithmically and then scaled as for the other input parameters (see Figure 1). The 70% and 30% of the global *in situ* dataset have been used for the training and test of the network, respectively. A further 30% of profiles were extracted as a subset from the training as an internal validation dataset, used to guide the time length of the learning stage. After several tests, the best performing ANN was chosen according to the lowest Mean Square Error and determination coefficient r^2 obtained in the validation. Nevertheless, it has to be stressed that in the use of ANN approach, there is not a univocal configuration or solution, and the prediction capability is strictly depending on training dataset features.

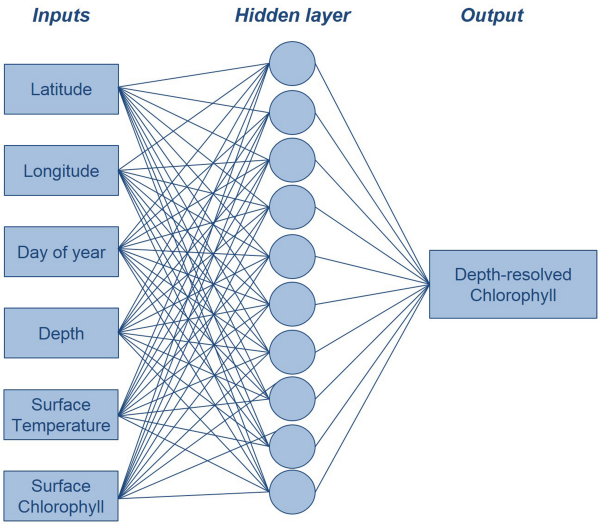

Figure 1: Artificial Neural Network structure used in this study (adapted from Sammartino et al. (2018)).

Results and conclusions

Results of the network validation against *in situ* data showed very promising results. To discern errors introduced by using surface remote sensing estimates as input, from errors due to the ANN performance, the model was validated using both *in situ* surface data and satellite data. The statistics obtained from the validation of the ANN performance on the *in situ* test set and the matchup satellite one were comparable with each other, with an r^2 of 0.69 and 0.63, respectively and the same bias of -0.04 mg m^{-3} (for more details see Sammartino et al. (2018)). An example of the method performance is given in Figure 2 where the mean observed profile of surface Chl concentration ranging from 0.001 to 0.08 mg m^{-3} match very well with the predicted one, also reflecting a similar variability, as showed by the overlapping of their standard deviations (shaded area in Figure 2). The comparison of the statistical results of the ANN validation on *in situ* training and test sets shows the good generalization capability of the network. Monthly satellite Chl and SST estimates were used to infer the vertical Chl field on seasonal scales (not shown). This analysis highlighted the usefulness of the ANN to model the vertical dynamic of Chl in specific areas of the Mediterranean Sea that are deeply impacted by seasonal cycle. Even knowing that a univocal configuration of an ANN cannot exist, its integration with the high resolution of satellite data represents an innovation, which can improve the biological modeling approaches and allow having an estimate of Chl vertical profile where *in situ* data are missing.

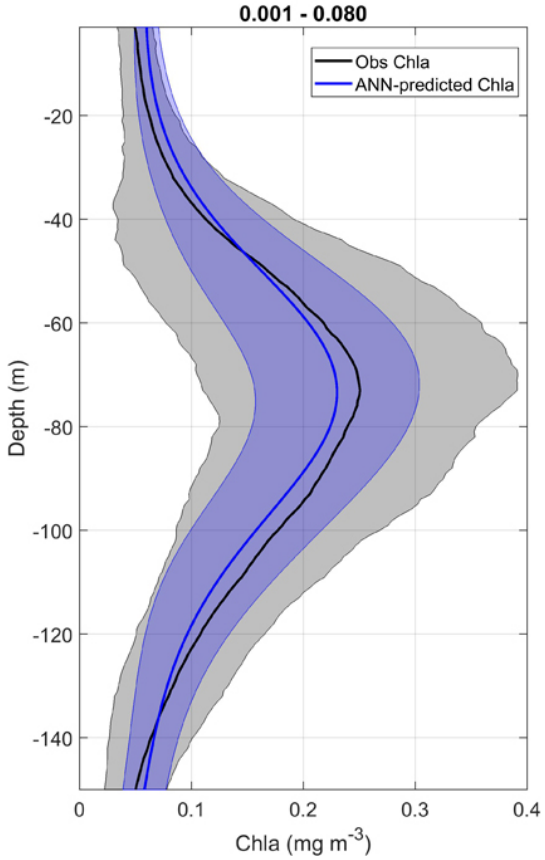

Figure 2: An example of the ANN performance. All the observed Chl profiles falling in the range of surface Chl (0.001-0.08 mg m^{-3}) were averaged as a mean profile (black line) and compared to the correspondent predicted one (blue line). The shaded areas represents their standard deviation (adapted from Sammartino et al. (2018)).

Acknowledgments

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How do seagrasses face herbivory? A case of study in Cadiz bay

Isabel Casal*[1] , Fernando G. Brun[1] , Eva Zubía[2]

*Corresponding author: isabel.casalporras@alum.uca.es
[1] Department of Biology (Ecology Area), University of Cádiz, Puerto Real (Cádiz) Spain
[2] Department of Organic Chemistry, University of Cádiz, Puerto Real (Cádiz), Spain

Keywords: Herbivory, Marine Angiosperms, Natural Products

Introduction

The marine angiosperms or seagrasses are vascular plants with leaves, rhizomes, roots, flowers and seeds that inhabit marine environment. Seagrasses are characterized by developing extensive crowded meadows in shallow coastal areas, forming one of the most important coastal habitats of the biosphere, bearing a great ecological value because of the services and functions they provide, both to humans and marine ecosystems. Seagrasses are distributed along all the coasts of the world, except in Antarctica (Robertson and Mann, 1984) while only four species are found in the Mediterranean Sea: *Zostera noltei* Hornemann (1832), *Zostera marina* Linnaeus (1753), *Cymodocea nodosa* Ascherson (1870) and *Posidonia oceanica* (Linnaeus) Delile (1813). With the exception of the *P. oceanica*, the other three species coexist in the Bay of Cadiz (Figure 1, modified from Brun et al. (2015)).

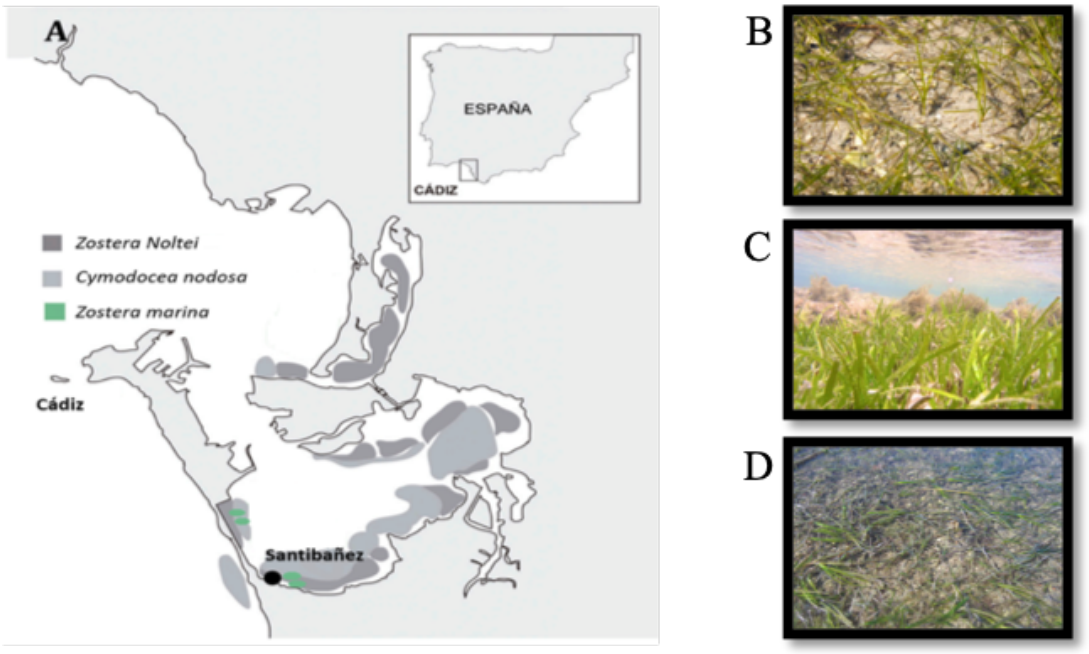

Figure 1: (A) Seagrass habitats in the Bay of Cadiz (B) *Zostera noltei* (C) *Cymodocea nodosa* (D) *Zostera marina*.

Marine angiosperms possess a secondary metabolism that synthetizes specific organic compounds known as natural products. These compounds play important roles in the survival of the plants,

including their involvement in chemical defence mechanisms. One of the ecological functions played by these natural products could be their deterrent capacity against herbivores. The consumption preference of a herbivore over one plant species, is determined by the palatability of their tissues. Plants can modify this palatability through different strategies that are based on chemical, nutritionals, and mechanical adaptations (Fritz and Simms, 1992). Strategies based on chemical adaptations, that is, on the synthesis of natural products, have an important function in restricting the taste quality of marine plants, but also can make these tissues toxic for consumers (Braekman et al., 1998). In spite of that, little is known about the deterrent capacity of natural products in seagrasses and whether species-specific differences within the community may prone some species more vulnerable to herbivore attack. Therefore, the main objective of this work was to study the deterrent activity against herbivores of the extracts from *Z. noltei* and *C. nodosa* by developing a manipulative experiment using the sea urchin *Paracentrotus lividus*.

Material and Methods

Paracentrotus lividus was used (Figure 2), since it is a generalist herbivore inhabiting the Bay of Cadiz and is known to feed in seagrass beds (Vergés et al., 2011).

Figure 2: *Paracentrotus lividus* collected in La Caleta, Cadiz.

Sea urchins were collected by hand in a nearby rocky shore, La Caleta, in Cadiz (SW Spain, 36° 31' 39" N, 6° 18' 46" W). Once in the laboratory, sea urchins were distributed in 2 tanks (30 l; 36 ind./ tank) with aeration, and were fed with *Ulva* sp. for 5 days before starting the assays, to acclimate sea urchins to laboratory conditions. Were prepared butanolic extracts of *C. nodosa* and *Z. noltei* plants. In addition, a butanolic extract of *Z. noltei* was separated by using a sephadex column to obtain the phenolic natural products (rosmarinic acid and flavones). In order to demonstrate that temperate seagrasses (*Zostera noltei* and *Cymodocea nodosa*) contain natural products with deterrent capacity, we conducted a set of trials where various diets included in blocks of agar (Figure 3), composed either of: 1) seagrass lyophilized tissues, 2) extracts of the plants or 3) phenolic natural products isolated from *Z. noltei* (rosmarinic acid and flavonoids) were used to feed *P. lividus*.

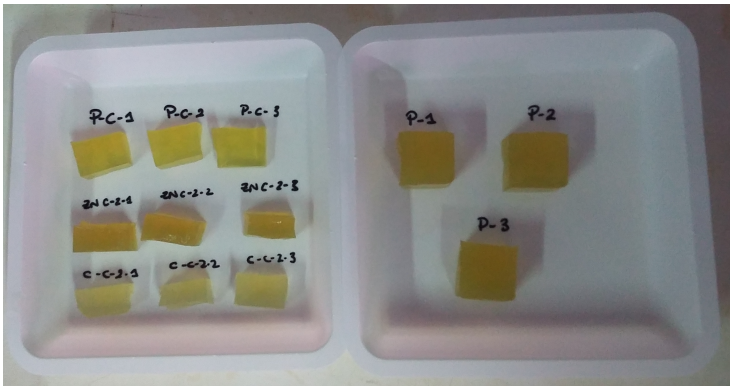

Figure 3: Agar blocks with pure product diets, *Z. noltei* and *C. nodosa*.

At the beginning of each test, each block of agar was weighed, placed for one hour in the aquarium and then removed. The surface was dried with paper and weighed again. The consumption rate was calculated by the difference between the final and initial fresh weight measured in the assay, divided by the time elapsed (1h).

Results and Discussion

The preliminary results showed that the diets formed by lyophilized leaves of *C. nodosa* and *Z. noltei* had higher consumption rates than diets composed by the extracts of these plants. The diets based on lyophilized leaves contained all the nutritional, structural, reserve, and chemical properties, while the diets formed by extracts only contained reserve and chemical properties. The diet formed by phenolic natural products showed the lowest consumption rate. This low consumption rate could indicate that the natural products have a deterrent activity against the consumption by *P. lividus*. Therefore, the diets containing few sugars and a high percentage of natural products showed low consumption rates which could indicate that both the presence of these natural products and the absence of sugars greatly influence the palatability of the tissues and the feeding behaviour of the herbivore.

Acknowledgments

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A shifting balance: responses of mixotrophic marine algae to cooling and warming under UVR

Marco J. Cabrerizo*[1] , Juan Manuel González-Olalla[2] , Presentación Carrillo[3]

*Corresponding author: marcojc@uvigo.es
[1] Department of Ecology and Animal Biology, Faculty of Marine Sciences, University of Vigo, Campus Lagoas Marcosende, 36310 Vigo (Spain)
[2] Department of Ecology, Faculty of Sciences, University of Granada, Campus Fuentenueva s/n, 18071 Granada (Spain)
[3] Universitary Institute of Water Research, University of Granada, C/ Ramón y Cajal, 4, 18071 Granada (Spain)

Keywords: Interactive effects, Light-dark cycles, Mixotrophic phytoplankton, Temperature, Ultraviolet radiation

Abstract

Mixotrophy is a widespread metabolic strategy in ecosystems worldwide (Caron, 2016). Shifts in temperature (T) and light (e.g. the ultraviolet portion of spectrum (UVR)) are key abiotic factors that modulate the conditions under which an organism is able to live. However, whether the interaction between both drivers alters mixotrophy in a global-change context remains unassessed. To determine the interactive T and UVR effects on relative electron transport rates, nonphotochemical quenching, bacterivory, and bacterial production, we conducted an experiment with populations of the haptophyte *Isochrysis galbana* grown mixotrophically, which were exposed to 5°C of cooling (i.e. 14°C) and warming (i.e. 24°C) with respect to the control (19°C) with (or without) UVR over light–dark cycles and different timescales. At the beginning of the experiment, cooling inhibited the relative electron transport and bacterivory rates, whereas warming depressed only bacterivory regardless of the radiation treatment. By the end of the experiment, warming and UVR conditions stimulated bacterivory. These reduced relative electron transport rates (ca. 50% (warming) and higher 70% (cooling)) were offset by increased (35%) cumulative bacterivory rates under warming and UVR conditions. We propose that mixotrophy constitutes an energy-saving and a compensatory mechanism to gain carbon (C) when photosynthesis is impaired, and highlight the need to consider the natural environmental changes affecting the populations when we test the impacts of interacting global-change drivers.

Acknowledgments

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First occurrence of the alien large benthic foraminifera *Parasorites orbitoloides* (Hofker, 1930) and *Euthymonacha polita* (Chapman, 1900) in the Western Mediterranean: their effects on the large-benthic foraminifera community.

Beatriz Alvira Romero*[1] , Albert Fuster-Prohens[1] , Anna Khokhlova[1] , Guillem Mateu-Vicens[1]

*Corresponding author: alvira.beatriz@gmail.com
[1] Càtedra "Guillem Colom Casasnovas", Universitat de les Illes Balears (Palma de Mallorca, España)

Keywords: Alien species, *Euthymonacha polita*, Large benthic foraminifera, *Parasorites orbitoloides*, Western Mediterranean

Introduction

In recent years, the number of marine alien species in the Mediterranean Sea is increasing. Due to the opening of the Suez Canal (1869), the number of species from the Indo-Pacific has been increasing in this basin, including symbiont-bearing foraminifera (Mouanga and Langer, 2014). Foraminifera are protozoa which, mostly of them, form calcium carbonate shells and, therefore, contribute to the formation and composition of marine sediments. These sediments, such as epiphytic foraminifera tests, can partially feed beaches. The entry and settlement of species of foreign foraminifera can lead to a strong modification of the composition of this sediment as well as to the displacement of native species (Mouanga and Langer, 2014). Therefore, detecting and determining the entry paths for new species of tropical origin in the Mediterranean basin is important for two reasons. Firstly, in order to determine how these species could affect other organisms with whom they could establish competitive relationships and, in the second place, to determine if they can have the ability to alter the environment. *Parasorites orbitoloides* and *Euthymonacha polita* are two species of large-benthic foraminifera from the Indo-Pacific region (Figure 1). *P. orbitoloides* presents chlorophyte symbionts. This symbionts bring them the greenish coloration characteristic of the subfamily Archaiasinae. Its presence in the Mediterranean Sea had not been documented yet. *Euthymonacha polita* belongs to the Peneroplidae family, a group that presents red algae symbionts. So far, in the Mediterranean, this species has only been cited in the Aegean Sea, Turkey (Meriç et al., 2010). The mechanisms of arrival to the Western Mediterranean Sea are unknown. One of the main objectives of this work has been to determine if these exotic species could compete or displace the autochthonous symbiont-bearing foraminifera species, as well as, if they could modify the composition of the foraminifera community.

Materials and Methods

The geographical area sampled corresponds to the Balearic archipelago. From each of the three main islands, samples of sediment of the littoral have been collected in different beaches: 15 of Mallorca, 8 of Ibiza and 4 of Menorca. From each beach three subsamples have been collected. Of each subsample, 60 specimens belonging to the seven different species of symbiont-bearing foraminifera of the Western Mediterranean have been counted. The abundance of each specie in each beach have been calculated with the mean of the tree subsamples. Moreover, to see

the effects on the whole community of foraminifera have been used the morphotypes described by Langer (1993) and modified by Mateu-Vicens et al. (2014). Due to each shell morphotype corresponds to a specific autoecological condition, the proportion of each morphotype in the community are indicative of the community conditions. Thus, from each sample, the first 60 foraminifera found were counted and classified under the corresponding morphotype: A, SB, B, C or D. Morphotype A is formed by incrusting sessile forms with long term life span (1 year), SB forms correspond to the symbiont-bearing foraminifera, temporary motile foraminifera with a short-term life span (2-5 months) belong to the morphotype B, morphotype C is formed by motile foraminifera which feed on particle in suspension and have a short-term life span (3-4 months) and, finally, morphotype D is composed by little opportunistic forms with a very short life span (1-2 months). In order to analyse the effects into the symbiont-bearing community as well as in all autochthonous foraminifera community have been built abundance graphics. Furthermore, in order to see if there is a correlation between the different kinds of symbiont of each specie with the morphotypes, a Principal Component Analysis has been made.

Results and Discussion

In the Balearic coast there is no relationship between the presence of these two exotic species and the presence and abundance of the autochthonous symbiont-bearing foraminifera species (Figure 1). *P. orbitoloides* is present in all the islands but with low abundances as well as *Laevipeneroplis laevigatus* (Cushman, 1930), the other species with green algae. Due to the kind of symbiont, *L. laevigatus* is the only species which could be affected by the entrance and settlement of *P. orbitoloides*. That is not observed. Indeed, both have very low abundances so it is unlikely that they compete for resources. Moreover, it is very likely that these two species present different strategies due to their different morphology (Hohenegger, 2009) They present higher abundances in those environments where morphotypes C and B are abundant. So these organisms are mobile and linked to high organic matter environments. Thus, the symbiosis in these cases probably is not so linked to the obtaining of sugars but rather to other advantages such as the enhancement of the calcification. *Euthymonacha polita* is the rarest. Their abundances are generallyvery low, below 5% of the symbiont-bearing foraminifera. Because it has red algae symbionts, their settlement could affect the species of the genus Peneroplis. However, there is no decrease in the abundances of these species in response to the presence of *E. polita*. This lack of response could be due to two factors. First, *E. polita* has very low abundances so it could hardly affect the rest of the species. Second, similar shells are usually a sign of very similar niches or strategies (Hohenegger, 2009) and *E. polita* presents a very different shell compared to the rest of the symbiont-bearing foraminifera. So the niche it occupies has to be different and it does not compete with peneroplids. Analysing the abundances and not the presence of these species with the Principal Components Analysis (PCA), very interesting groupings have been shown (Figure 1). The different species are grouped coinciding with the type of symbiont they harbour, the morphology of their shell and with the abundance of some of the morphotypes. Although all these species belong to the SB morphotype, not all of them show a positive correlation with it. Only those with red algae, specifically the genus Peneroplis, are positively correlated. *Peneroplis planatus* (Fichtel and Moll, 1798) is the most correlated due to its morphology adapted to the epiphytic behaviour (Hohenegger, 2009) while *Peneroplis pertusus* (Forsskal in Niebuhr, 1775) and *Peneroplis arietinus* (Batsch, 1791) would be better adapted to be part of the epifaunal community of the sediment among this vegetation. *E. polita* shows a positive correlation with high abundances of the morphotype D, so it is a more opportunistic species. Both its morphology and its small size are consistent with this strategy (Langer, 1993; Mateu-Vicens et al., 2014). Finally, the abundance of *Sorites orbiculus* (Forsskal in Niebuhr, 1775), the only one with dinoflagellates, is positively correlated to the abundance of morphotype A. *S. orbiculus* is a sessile epiphyte of hard substrates as sea-grass leaves. Due to it does not move in search of food, it depends on photosynthesis. This sessile

strategy is similar to that of morphotype A specimens. To conclude, these two exotic species are present throughout the Balearic archipelago. Their abundances are very low. Being the principal reason why they will hardly imply problems for the autochthonous benthic foraminifera community or for the ecosystem structure. Finally, this work shows that not all foraminifera with symbionts show the same trophic strategies. This group is heterogeneous, the ecology and trophic strategy of each of these species will depend on the kind of algae they harbour.

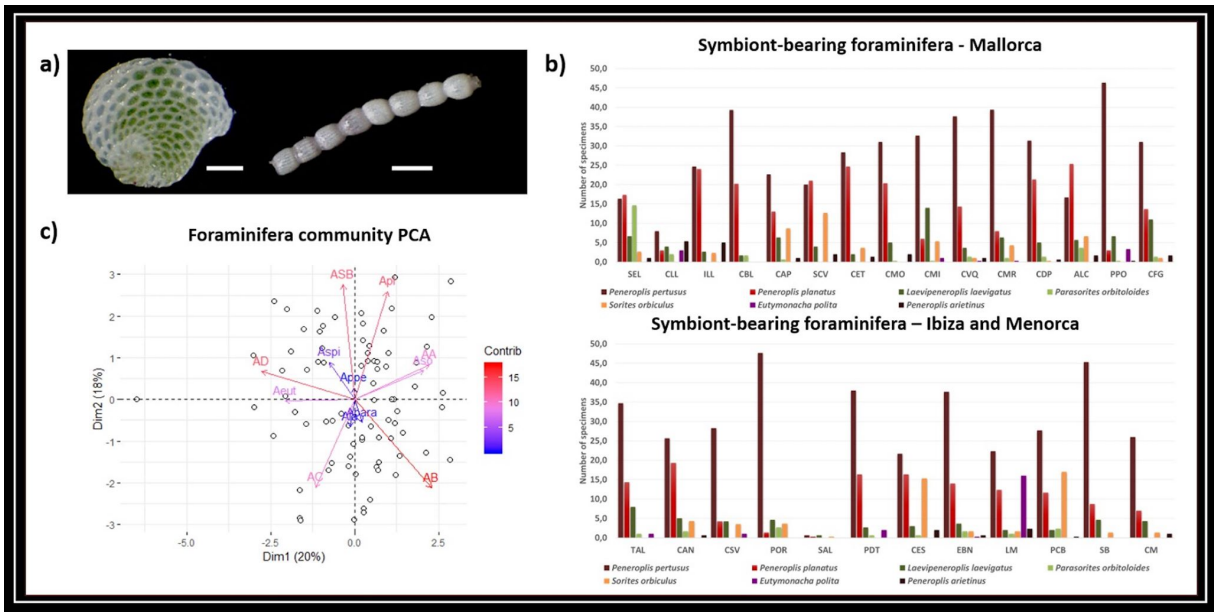

Figure 1: a) Alive *Parasorites orbitoloides* (left) and alive *Euthymonacha polita* (right). The white bar means 300/*mu*m. (b) Abundances of the different species of symbiont-bearing foraminifera of the Western Mediterranean in each sampled beach of the Balearic archipelago. (c) PCA of the abundances of each symbiont-bearing species (*Parasorites orbitoloides* = Apara; *Laevipeneroplis laevigatus* = Ala; *Euthymonacha polita* = Aeut; *Peneroplis pertusus* = Appe; *P. planatus* = Apl; *P. arietinus* = Aspi; *Sorites orbiculus* = Aso) and each morphotypes (morphotype A = AA; morphotype SB= ASB; morphotype B= AB; morphotype C= AC and morphotype D = AD).

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Understanding the biogeographical patterns of marine invertebrates in the remote Azores Archipelago and surrounding NE Atlantic Ocean

Lara Baptista*[1] , António M. Santos[2] , Manuel Curto[3] , Björn Berning[4] , Sérgio P. Ávila[1]

*Corresponding author: laracbaptista@hotmail.com
[1] CIBIO-InBIO, Azores Pole, University of the Azores, Portugal
[2] CIBIO-InBIO, University of Porto, Campus Vairão, Porto, Portugal
[3] Institute for Integrative Nature Conservation Research, BÖKU, Vienna, Austria
[4] Oberösterreichisches Landesmuseum,Geowissenschaftliche Sammlungen, Austria

Keywords: Azores Archipelago, Biogeography, Population genetics, Bryozoa, Gastropoda

Introduction

The colonization of volcanic oceanic islands by marine invertebrates is an interesting topic for island biogeographers. Despite the interest in the unique biogeographical patterns arising often on remote archipelagos, the processes operating in marine-life are far from well-understood. The Azores Archipelago is no exception, being considered a natural laboratory to study evolutionary and biogeographical processes. Several studies revealed strong affinities of Azorean marine taxa with European/Mediterranean taxa, which mismatch the predominantly eastwards ocean-surface circulation (Sala et al., 2013) in the NE Atlantic (Figure 1). This phenomenon, designated as Azorean Biogeographical Paradox (Ávila, 2013), poses marine biogeographical questions regarding the connectivity between marine insular and conspecific populations in the NE Atlantic.

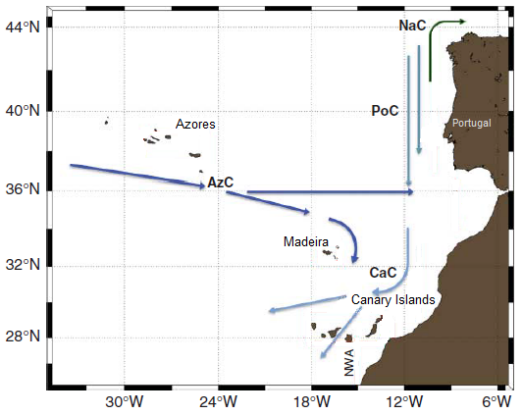

Figure 1: Prevailing western sea-surface circulation in the Northeast Atlantic Ocean, depicting four major surface currents: Azores Current (AzC), Portugal Current (PoC), Canary Current (CaC) and Navidad Current (NaC). Adapted from Sala et al. (2013).

To reach newly formed islands, colonizers must disperse from a source location. In marine invertebrates, dispersal is assured by pelagic larvae, phoresy or rafting of egg-masses, juveniles or adults (Ávila, 2013) and this ability is linked to larval development (Scheltema, 1989): planktotrophic (p) organisms disperse easily, whereas non-planktotrophic (np) species usually have restricted ranges. Thus, population dynamics in the NE Atlantic Ocean is likely different for

p- and np-developers and comparable between taxa with the same larvae type. This study seeks to elucidate the long-distance dispersal of marine invertebrates under complex oceanographic settings, the role of seamounts, ocean-currents and periodic climatic changes in the colonization of the Azores. With the major goal of clarifying the Azorean Biogeographical Paradox, several questions will be addressed: 1 - Are there differences in the genetic structure and diversity of populations of p-/np-developers at the local, regional and broader scales; 2 - What is the role of the ocean-surface circulation and geographic distance in the population structure in the NE Atlantic; 3 - What were the roles of seamounts and periodic climatic changes in the marine invasion of the Azores.

Materials and Methods

A population genetic and phylogeographic study of two shallow-water gastropods and one bryozoan species (Table 1), naturally occurring in the NE Atlantic with contrasting types of larvae (p/np), and therefore different dispersal capabilities, is hereby proposed.

Table 1: Target species, type of larval development and geographical distribution. p – planktotrophic, np – non-planktotrophic. POR–Portugal; MED–Mediterranean; AZO–Azores; MAD–Madeira; CAN–Canaries; CAB–Cabo Verde; NWA–Nortwestern African coast; VIR–Virginia, USA; CRL–North and South Carolina, USA; GME–Gulf of Mexico, CAR–Caribbean Sea, SCA–Scandinavia, BRI–British Isles, NEA–Northeast Atlantic.

Species	Larvae	Geographical distribution
Stramonita haemastoma (Gastropoda)	p	POR, MED, AZO, MAD, CAN, CAB, NWA, VIR, CRL, GME, CAR
Cingula trifasciata (Gastropoda)	np	SCA, BRI, POR, MED, AZO
Reteporella atlantica (Bryozoa)	np	NEA

The target species were sampled by hand-pick collection, integral scrapping of the algal substrate or scuba-diving. The collection scheme (Figure 2) provides representativeness of populations to analyse connectivity at the local, regional and NE Atlantic scales. Individuals from additional, non-sampled locations were obtained through access to collections or provided by collaborators.

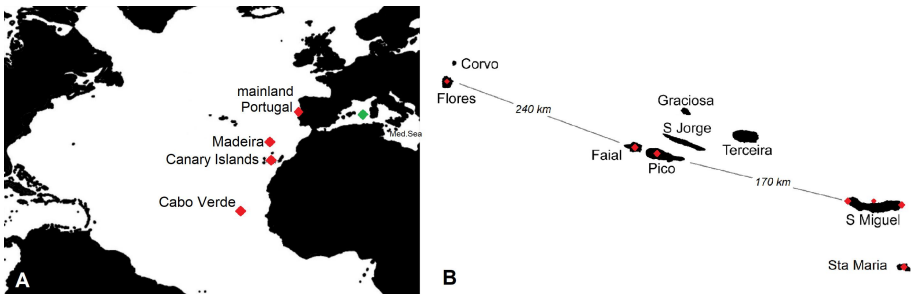

Figure 2: Sample collection scheme in (A) regions out of the Azores area (Madeira, Cabo Verde/Canary Islands, mainland Portugal/Mediterranean Sea) and (B) within the Azores Archipelago (Flores, Pico/Faial, three localities in São Miguel, Santa Maria).

DNA was extracted from the tissue with column-based commercial kits, with a final elution volume of 40-50 uL. Whole-genome low-coverage sequencing with Illumina MiSeqwill be performed for one individual by population of each species. The development of a de novo set of microsatellite markers, preparation of SSR-GBS amplicon library and allele definition will follow the protocol by Curto et al. (2019). Population genetic analyses with appropriate software (GenAlEx, Cervus, STRUCTURE and BayesAss) will be performed in the microsatellite dataset to account for marker variability, polymorphisms and genetic structure patterns among populations. Reconstruction

of demographic patterns across temporal and spatial scales will rely on IMA and BEAST. The inferred patterns will be extensively interpreted to better understand the factors influencing the colonization of the Azores, including the geological evolution of the archipelago, ecological features of the target species, palaeontological information available and a special reference to (palaeo)oceanographic data of the NE Atlantic Ocean. This final task will allow to enlighten the role of long-distance dispersal assisted by the prevailing sea-surface ocean circulation in the population structure, by comparing the patterns between species and larval types at local, regional and NE Atlantic scales.

Expected outcomes

The larval development (p/np) of a species influences its dispersal potentials, which is expected to be reflected in their genetic structure. Accordingly, the np gastropod and bryozoan species, with lower dispersal ability, should exhibit greater genetic differentiation between populations than the widely dispersing p-developers. On the other hand, the patterns obtained for the np species (gastropod *C. trifasciata* vs bryozoan *Reteporella*) are expected to be similar due the dispersal restrictions of their larvae. As the Gulf Stream passes the Azores in a NW-SE direction, at least the two species with np larvae (or adults thereof rafting on floating substrata) should not be able to maintain gene flow in a westward direction under the present oceanographic conditions. If distance, predominant oceanic circulation and geological evolution influence the preferential direction of larval dispersal, we might be able to retrieve a genetic signal. The demographic patterns determined will be interpreted at a broad context, expecting to provide valuable clues to understand present and past biogeographic processes in the Azores Archipelago and NE Atlantic Ocean in general. With this approach, we hope to clarify the origins of Azorean coastal invertebrate fauna and how gastropods and bryozoans colonized newly formed ecological niches, considering the potential role of the Southern Azores Seamount Chain in the process.

Acknowledgments

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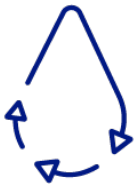
Session 11

The Integrated Sea Management



Maritime Spatial Planning and Marine Environment Protection in Spain

Constanza Sánchez García*[1]

*Corresponding author: constanzasanchezgarcia@gmail.com
[1] Masters Degree student at the University of Málaga

Keywords: Marine environmental policy, Maritime spatial planning, Sustainable management

Introduction and background in the field of the marine environment protection and the management of maritime space

Marine environment is essential for human life. Despite the great importance that it has for human beings, it has taken a long time to them to plan and protect this natural environment. One of the steps on the right path is the maritime spatial planning. It is inevitable to say that a good planning of the maritime space influences in a highly positive way the state of the adjacent littoral. We could define this concept as the "public procedure of analyzing and distributing spatially and temporally human activities in marine areas in order to achieve ecological, economic and social objectives". The main purpose of maritime spatial planning is to promote sustainable development and to identify and manage the use of maritime space for different human activities. It is an initiative with a clear environmental purpose, but also economic and even social, since a sustainable marine environment is a sustainable natural resource that human beings can continue to use for much longer. In recent years, the evolution of maritime spatial planning has been exponential. In this area, the unity between the Intergovernmental Oceanographic Commission of UNESCO and the European Commission needs to be highlighted. Both adopted in March 2017 a Joint Roadmap to accelerate Maritime/Marine Spatial Planning (MSP) processes worldwide. As a result of this collaboration, in 2018, the MSP Forum and the MSPglobal Initiative were created. Both projects are a great contribution to the Decade of Ocean Science for Sustainable Development (2021-2030), as well as to the 2030 Agenda for Sustainable Development. The last step of the European Union in the field of maritime spatial planning is the well-known Directive 2014/89/EU of the European Parliament and of the Council, of 23 July 2014, establishing a framework for maritime spatial planning. The objectives of the EU regulation are "to promote the sustainable growth of maritime economies, the sustainable development of marine areas and the sustainable use of marine resources". In order to achieve them, the coastal Member States are proposed a framework for the management of their maritime space that will lead to the establishment of specific plans in each territory. Of course, the Directive poses these planning obligations from an integrated approach in all senses: ecosystem, geographical and social. Already in our country, we find that the first regulation referring to the protection of the marine environment as such is Law 41/2010, of December 29, on the protection of the marine environment, approved as a transposition of Directive 2008/56/EC of the European Parliament and of the Council, of 17 June 2008, establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). The objective of the state regulation, as indicated in its Article 1, is to maintain the good environmental status of the marine environment and ensure the sustainable use of the resources it offers. To achieve this, the requirements that must be met by the planning carried out are established in the law, indicating as essential instruments for it the Marine Strategies: planning instruments whose scope is a marine demarcation and which indicates the general framework that must be met by

all those administrative activities and sectoral regulations with effects on the specific marine environment. There is a clear link between the Marine Strategies and the maritime spatial plans. The former are the framework for the regulation of a specific demarcation, while the latter are the effective instrument of distribution and coordination of the uses that are permitted within it (Fuentes Gassó, 2017).

Regulation of maritime space in Spain: Royal Decree 363/2017

In compliance with the deadline established for the transposition of Directive 2014/89/EU, on April 11, 2017, the Royal Decree 363/2017, of April 8, was published in the BOE, establishing a framework for maritime spatial planning. The objectives of the Royal Decree, like those established in the Community regulation from which it derives, are fundamentally focused on promoting the sustainable development of the Spanish marine environment and on the sustainable management of all those uses that may converge in the maritime space. As for the scope of application of the Royal Decree it is "all marine waters, including the bed, subsoil and natural resources, in which the Kingdom of Spain exercises sovereignty, sovereign rights or jurisdiction. It will also be applicable to the Spanish continental platform". The standard proposes the development of a plan for each of the five maritime demarcations of our country, namely: North Atlantic, South Atlantic, Strait of Gibraltar and Alborán, Levantine-Balearic and Canary Islands. About the content that the plans must have, we find in article 10 a list of the uses, activities and interests that can come together in the maritime space and which must be distributed spatially and temporarily in the plans for the best management and protection of marine waters. Among them we find the following: fishing areas, facilities and infrastructures for prospecting, exploitation and extraction of oil, gas and other energy resources, minerals and mineral aggregates, and the production of energy from renewable sources, shipping routes and maritime traffic, protected areas, places and habitats that deserve special attention because of their high environmental value and protected species, etc. The calendar established in the First Additional Provision of the Royal Decree gives until March 31, 2021, for the approval by the Council of Ministers of the proposals for management plans. For the time being, Spain has not drafted any of the management plans, whose preparation is entrusted to the Interministerial Commission of Marine Strategies - CIEM and the Maritime Space Management Working Group - GT-OEM.

Conclusions

The dangers that threaten our coasts and marine waters are increasingly greater and more harmful, so our action must be in accordance with this risk, developing appropriate action plans, management and protection of the marine environment. These plans can not lengthen their elaboration process excessively over time, nor can they be fully valid for long periods of time, since the risks and solutions are updated at an amazing speed (Zamora Roselló, 2019). Furthermore, any measure, norm, plan or action must be carried out from an integrated approach, in accordance with what was previously stated in the text. It is an essential attribute of the protection of the environment, which is not threatened by a single type of danger nor is it used for a single type of activity. An important part of this integrated approach is the indissolubility of the marine environment and the coast: any environmental strategy to be developed in one or another area must be connected with all the others that develop in the opposite (Zamora Roselló, 2018). Currently there are different levels of implementation in the different procedures for the management of maritime space that are being carried out in several countries of the world. Maritime spatial planning development and application are clearly growing: in August 2018, approximately 70 countries had developed, or were developing, maritime spatial planning plans in their territories. However, there are many other States that have not yet implemented any measures in this area. In some of them it is necessary the cooperation of other States or agents

for the elaboration of the plans, while in other cases it is the lack of means to implement the strategy which makes external assistance necessary. It is therefore necessary to conclude that there is still much work to be done in the field of maritime spatial planning, not only from the point of view of the planning implementation in the littoral States, but also from the integration of the different sectors that take part in the use we make of our seas.

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A legal approach on marine spatial planning: reflections from Spain

María Remedios Zamora Roselló*[1]

*Corresponding author: remedios@uma.es
[1] University of Málaga, Public Law Department

Keywords: Marine spatial planning, Ocean governance, Territorial planning

Introduction

Marine spatial planning (MSP) is defined by UNESCO as "a public process of analyzing and allocating the spatial and temporal distribution of human activities in marine areas to achieve ecological, economic, and social objectives that are usually specified through a political process" (Ehler and Douvere, 2009). Meanwhile the Directive 2014/89/EU of the European Parliament and of the Council of 23 July 2014, establishing a framework for maritime spatial planning, considers MSP as "a process by which the relevant Member State's authorities analyse and organise human activities in marine areas to achieve ecological, economic and social objectives". And through their maritime spatial plans, Member States shall support sustainable development and growth in the maritime sector, applying an ecosystem-based approach, and to promote the coexistence of relevant activities and uses.

The initial question is the purpose of MSP and, in this sense, the doctrine has identified two approaches, one based on uses and the other on ecosystems. On the basis of the first perspective, MSP would be an instrument to manage the conflicting uses that may occur in the maritime environment and achieve sustainable development. While for the second of the options, the priority would be to guarantee the biological integrity of the marine ecosystem, incorporating a holistic vision. UNESCO and European Union (EU) definitions tend towards the first of the approaches, placing the emphasis on the management of the uses and leaving the biological integrity of the marine ecosystem in a secondary position. And in the EU case we have to highlight its commitment to MSP as a tool for blue growth.

Once we have identified the concept and objectives, we will address two essential elements of MSP in Spain: its relationship with territorial planning and its use for the improvement of ocean governance.

Discussion

First of all, we have to study if MSP is a prolongation of the spatial planning in the maritime space, or if it is timely to establish a new management model, adapted to the maritime singularities. There are different examples: on the one hand, the model of the Netherlands and Germany that have opted for the extension to the sea of their system of territorial organization; and on the other, the case of the United Kingdom that has developed a new and specific system.

The United Kingdom system is based on a regulatory framework which develops a model of governance for maritime environments. This government is under the supervision of a specific organization (Marine Management Organization), a public body with executive but not departmental character. However, many of the basic objectives for marine management follow the territorial space principles, and the elaboration of the maritime plans has adopted the terrestrial planning model.

In Spain, MSP initiatives are the result of the EU Directive; for this reason, it is important to analyze its scope (Sanz Larruga, 2018). The Directive shall apply to marine waters and it shall not apply to coastal waters or parts thereof falling under a Member State's town and country planning, provided that this is communicated in its maritime spatial plans. The Real Decreto 363/2017, the Spanish regulation for MSP, has introduced the same scope; so we consider that this choice limits the joint vision of the coastal space and it moves the MSP away from a unified regulatory model.

Throughout the EU Directive and the Spanish Real Decreto, there are constant references to the need to address the interactions between land and sea, as well as environmental, economic and social aspects. There is evidence that the projection of the activities and uses that are carried out in the marine waters condition a model of coastal ordination and territorial planning. Therefore, developing another new partial policy, means maintaining the traditional distinctions that have not supported the development of a coordinated model of action.

MPS is a joint policy, but there is a predominant tendency where the planning instruments are conditioned by the need to offer a framework of action to a specific activity; in short, provide a legal support to a certain use of maritime space. Hence, there is a trend that clearly advocates a rethinking of MSP initiatives, where other types of principles beyond purely economic ones can be accommodated, and with an overall vision of maritime space.

Because of that, some sectors observe the MSP with skepticism, given the possibility that some visions or concrete models on the management of seas and oceans will end up prevailing, thanks to the interests at stake; and, all this, to the detriment of alternative proposals (Flannery and Ellis, 2016).

In the MSP initiatives it is relevant to note the participation of the different administrative levels involved, the scientists, and the representatives of the economic and environmental sectors. In this respect, the participation of NGOs stands out, because they can offer a great support in the development of concrete initiatives, adding their specific knowledge to the public sector, and overcoming some of their limitations. Likewise, the knowledge and previous studies that these organizations have carried out can also be a guide in this planning process.

These instruments of MSP are a service to society, it should be a priority to communicate and integrate citizens in this area. The development of ocean governance involves the implication in decision making of all social, political and economic actors; the citizen is not a mere passive receiver, but an active subject.

Conclusions

The development of MSP policy is an opportunity to implement planning instruments that really pursue the integration of terrestrial and maritime space. However, the current framework does not reflect the importance of this correlation between terrestrial and maritime planning, or this need for coordination inherent to uses and activities. The maritime-terrestrial interaction seems to remain in a desideratum without a clear or specific content.

We have to wait for the MSP elaboration and implementation, to know how the plans are integrated in the communities and what the reactions are considering all the subjects involved. In this sense, we cannot ignore that the initiatives on MSP, far from being neutral proposals, respond to a policy with a clear ideological component; as happens in the field of territorial planning. Therefore, the States will draw the policy they want to develop on the maritime space, prioritizing some uses over others; and, in this sense, the differences between the chosen models can be remarkable.

This deficit is incardinated with the difficulties of ocean governance, the question is: which is the chosen model? At this point, there are several subjects to be analyzed: citizen involvement, coordination and inter-administrative cooperation, and creation of an administrative organization.

The development of participatory processes requires prior training, awareness, and a commitment to facilitate and integrate the citizen in the planning procedure. The inefficiency in governance results in the non-application of the measures adopted. We find examples of plans with an arduous process of elaboration that finally do not get to be approved, or initiatives are never implemented. In this sense, it is important to overcome the resistance to change at the institutional level, but also of the other actors involved in MSP.

Acknowledgments

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Assessing the Present Health of the Marine Social-Ecological System in Moorea, French Polynesia

María Dolores Castro-Cadenas*[1] , Charles Loiseau[1] , Joachim Claudet[2]

*Corresponding author: marcascad2895@gmail.com
[1] National Center for Scientific Research, CRIOBE, USR 3278 CNRS-EPHE-UPVD, Perpignan, France
[2] National Center for Scientific Research, PSL Université Paris, CRIOBE, USR 3278 CNRS-EPHE-UPVD Paris, France. Laboratoire d'Excellence CORAIL, Moorea, French Polynesia

Keywords: Ecosystem services, Ocean Health Index, Sustainable, Well-being

Introduction

The wellbeing of coastal communities relies strongly on ocean ecosystem services (Halpern et al., 2012; Sandifer and Sutton-Grier, 2014). This important connection is endangered by current unsustainable use of ocean resources and the impacts of climate change (Halpern et al., 2008). There is an important need for social-ecological assessments, in particular on islands where these links are even tighter (Friedlander, 2018), to estimate the health of the ocean. This study aims to produce the first integrated and quantitative assessment of the ocean health in Moorea using Ocean Health Index framework.

Material and Methods

The Ocean Health Index (OHI) is a multidimensional tool that allows to estimate ocean health through time and space in a standardized and integrated manner (Halpern et al., 2012). OHI is based on 10 public goals that represent ocean benefits to humans (Table 1). For Moorea Ocean Health Index assessment, the approach to calculating goal scores was adapted to better suit the social-ecological context of the island.

Results and Discussion

The index score revealed room for improvement of marine and social health. The goals with lower scores were those related to biodiversity conservation (e.g. protected places) and food provision, while goals with higher scores were related to tourism and recreation and economies (Figure 1). At present, the delivery of benefits to local communities is not maximized. Management efforts should be prioritized towards biodiversity conservation and monitoring programmes, particularly monitoring of fish catch to inform fisheries management. Future work could explore the sensitivity of OHI to different spatial management scenarios, such as the establishment of Marine Protected Areas (MPAs), to improve biodiversity conservation and sustainable resource use in Moorea, ensuring the delivery of important ecosystem services to local communities.

Table 1: Description of the benefits measured for each goal and sub-goal (Halpern et al., 2012).

Goal	Sub-goal	Benefit measured
Food Provision	**Artisanal fisheries**	A sustainable supply of seafood by artisanal scale fisheries
Artisanal Fisheries Opportunities		Possibility of artisanal fishing for subsistence and or/ recreation.
Carbon storage		Carbon storage and sequestration in coastal habitats
Coastal protection		Amount of protection provided by marine and coastal habitats against erosion and flood of coastal areas
Tourism and recreation		The ability of the ocean to provide tourism and recreation services to people
Coastal livelihoods and economies	**Coastal livelihoods**	The ability of oceans and coastal systems to sustain people's livelihoods and maintain its quantity and quality for people living on the coast
	Coastal economies	Revenue generated by marine-related industries
Sense of place	**Iconic species**	Conservation of species that are important to local cultural identity
	Protected places	Conservation of geographic areas that are important to cultural identity
Clean waters		The degree to which coastal waters are unpolluted by natural and human pollution sources
Biodiversity	**Habitats**	The conservation status of habitats existing in a location
	Species	The conservation status of native marine species

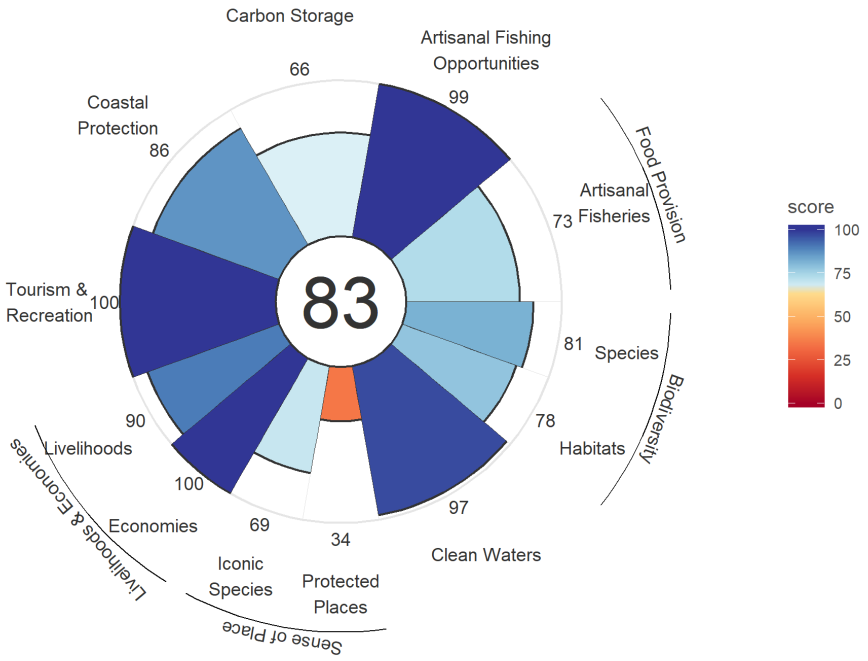

Figure 1: Moorea Ocean Health Index scores for individual goals or sub-goals (coloured petals) and overall (central number) for 2018. Each petal represents the score (radius) and weight (width) for the goal or sub-goal. Scores range from 0 to 100, with colour ranging from red for low scores and blue for high scores.

Acknowledgments

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Understanding cross-border interactions in the European Macaronesia for transboundary marine spatial planning

Víctor Cordero Penín*[1] , Cristina Pallero Flores[1] , Javier García Onetti[1] , Javier García Sanabria[1] , María De Andrés[1]

*Corresponding author: victor.cordero@uca.es
[1] University of Cádiz

Keywords: Marine spatial planning, Ecosystem based approach, Drivers-Pressures-State-Impacts-Responses (DPSIR), Cross-border cooperation, Land-sea interactions

Abstract

Although Marine Spatial Planning (MSP) is acknowledged to be able to both achieve environmental and socio-economic goals simultaneously, it is often challenging to successfully incorporate the ecosystem-based approach into the planning process. In this study, an integrated analysis of the European Macaronesia sea-basin has been undertaken using the Drivers-Pressures-State-Impacts-Responses (DPSIR) framework extended to Drivers-Activities-Pressures-State-Impacts (on human well-being)-Responses (of management) (DAPSI(W)R(M)) for the marine environment (Elliot et al., 2017). This problem-structuring framework has thrown light on the complex system formed by the interactions between the ecological and socio-economic structures of the sea-basin in support of an ecosystem-based MSP (Figure 1).

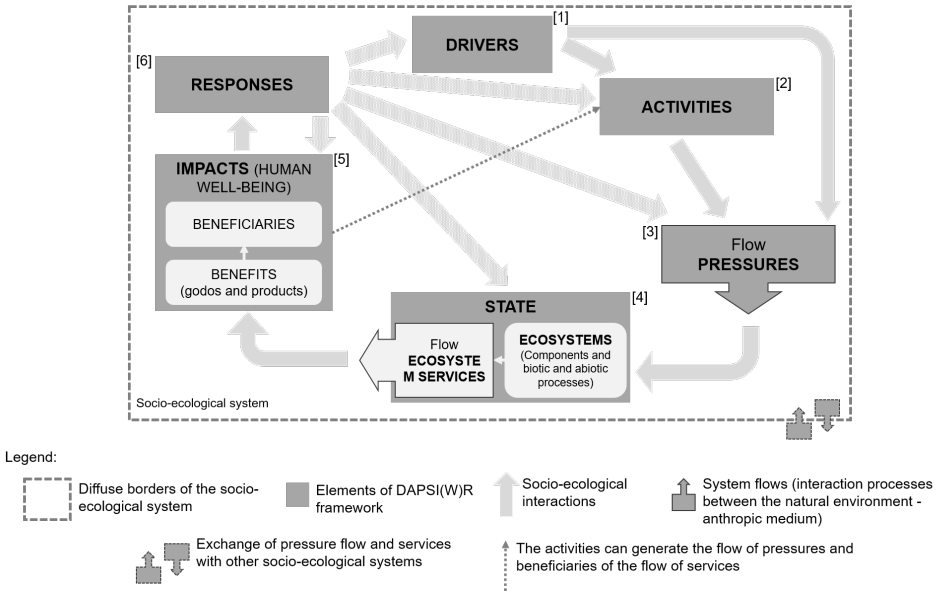

Figure 1: Conceptual scheme of the DAPSI(W)R framework applied to the European Macaronesia.

In this sense, despite the existing significant differences between the archipelagos of the Azores, Madeira and the Canary Islands, this analysis showed that the most developed maritime activity is by far coastal tourism, followed by ports and maritime traffic, fisheries and aquaculture

(European Commission, 2017). At the same time, pressures exerted by these maritime activities are the mechanisms of State change on the natural system, such as invasive species introduction by maritime traffic and aquaculture. This pressures then leads to Impacts on the ecosystem services (Table 1) that are ultimately related to Human Welfare.

Table 1: Ecosystem services of the marine environment of the European Macaronesia.

TYPE OF SERVICE		BENEFIT	BENEFICIARY AND / OR MAIN TRANSFORMER*	IMPORTANCE AND EVOLUTION OF THE SERVICE
Provisioning	Biomass	Traditional foods and by-products	Fishing	↓
		Technified food	Aquaculture	↑
	Abiotic products	Materials	Energy and industry (oil and mining)	↑
		Energy	Energy and industy (non renewable)	→
			Energy and industry (Renewable)	↑
	Genetic material	Biological products	Biotechnology	↑
	Spatial and phisical support	Location of uses and activities	Ports and transportation	↑
		Connectivity (communication and approach of entities, people and products)	Ports and transportation	↑
Regulation and maintenance	Mitigation of pollutants	Reception and purification of substances, toxins, pollutants, garbage, etc..	Fishing and aquaculture	↓
	Climate regulation	Stable climatic conditions (temporary recurrence) Local physical-chemical parameters: temperature, currents	Tourism	↓
	Biological regulation	Stable trophic chain, control of invasive species, disease control, etc.	Fishing and Biotechnology	↓
Cultural	Physical contact and experiences with nature	Recreational activities-tourism	Tourism	↑
		Landscape-aesthetic enjoyment		↓
	Spiritual, ethical, aesthetic and emblematic interactions	Cultural identity, belonging	Local community and Tourism	↓
	Intellectual interactions and cognitive development	Scientific knowledge	Biotechnology, Energy and Industry	↑
Low importance		Medium importance	High importance	Very high importance
Worse service: ↓		The service is maintained: →		Improve the service: ↑

*Main activities and transforming uses of the environmental state and that intervene in the use of the ecosystem service and give rise to the benefits for the population (GVA and jobs). The maritime sectors are considered as the beneficiaries although it is interpreted that in all cases the ultimate beneficiary of ecosystem services is the **general popullation** (in addition to other sectors involved to a lesser or indirect extent).

In general, all regulation and cultural ecosystem services show a deterioration trend except the cultural ecosystem services associated to the increase of scientific research and the increasing opportunities that eco-tourism development offers to get in contact with nature. Hence, provisioning ecosystem services are overall improving or remain stable over time in the case of the biomass of biological marine resources connected to fisheries and non-renewable energy. However, in relation to the benefits, maritime activities do not depend on the good environmental state in the same manner, presenting different levels of resilience that should be considered into the MSP processes (Figure 2).

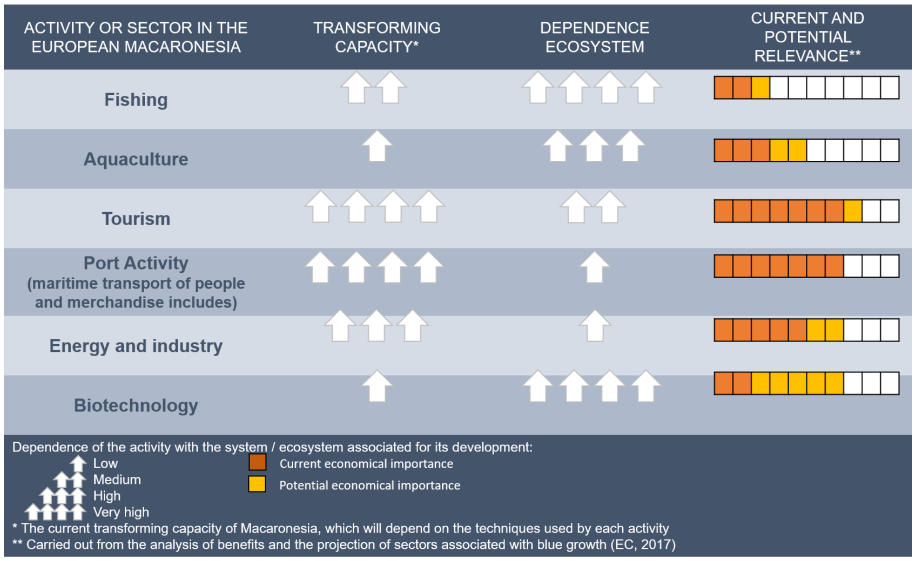

Figure 2: Relationship between the main maritime activities and their well-being contribution to the European Macaronesia based on their capability to transform the ecosystems (through pressures) and their dependence on the good environmental state of ecosystem services.

Additionally, the three archipelagos share common resources and challenges, such as migratory species of tuna and cetaceans or the lack of monitoring and scientific information relating to marine ecosystems. The understanding and visualization of these transboundary dynamics have derived in the identification of common issues for cross-border cooperation (Figure 3) while the segmentation of the Macaronesian sea-basin by some international marine management instruments, such as the fishing areas of the Food and Agriculture Organization of the United Nations (FAO) or the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR), reveals that the ecosystem-based approach will still remain a challenge to be incorporated into marine spatial planning unless special cross-border cooperation mechanisms are adopted.

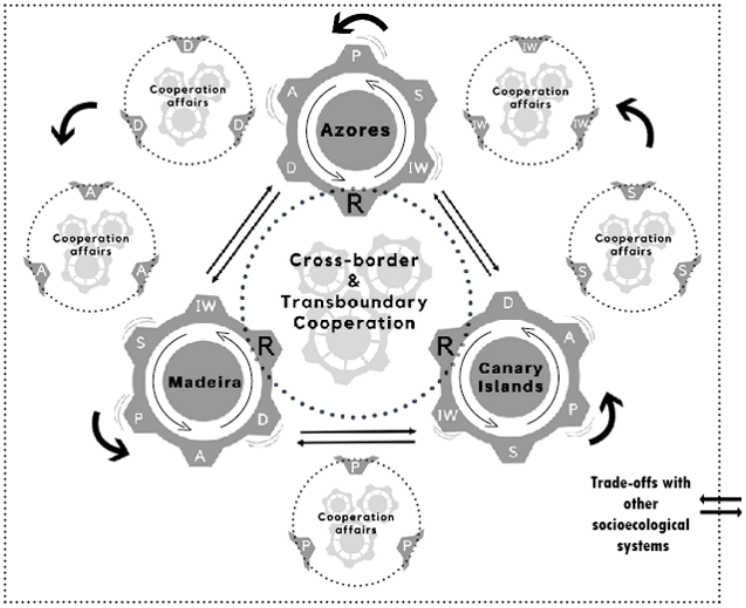

Figure 3: Conceptual framework for cross-border cooperation in the European Macaronesia.

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Neptunus project: providing opportunities for the transition to the circular economy of the seafood sector in the Atlantic Area

Israel Ruiz Salmón*[1] , María Margallo[1] , Jara Laso[1] , Ángel Irabien[1] , Rubén Aldaco[1]

*Corresponding author: israel.ruizsalmon@unican.es
[1] Departamento de Ingenierías Química y Biomolecular, Universidad de Cantabria. Avda. de Los Castros, s.n., 39005, Santander

Keywords: Life cycle assessment, Circular economy, Seafood sector, Atlantic area, Eco-innnovation

Framework

NEPTUNUS project aims to pursue a new transnational clustering concept approach to review, examine and harness key eco-labelling and key enabling eco-innovations that add-value and cross-cut sea food-water-energy domains in order to address barriers and to strengthen these sectors regionally and across jurisdictions in the Atlantic region. A standardised clustering framework to define, evaluate and modify future strategies is necessary, in order to assess interacting governing forces and balance the nutritional, economic and energetic value of the seafood sector so as to shape and inform industry growth aligned and informed by consumer engagement. This overall goal can be achieved by means of two specific objectives: Introducing green economy strategies in the seafood sector to minimize environmental impacts, whereas incorporating competitive products into green markets promoting eco-labelling under a NEXUS approach. Developing strategies and policies based on circular economy and cradle-to-cradle principles that deliver new products and working systems to the seafood sector.

The challenge

European Atlantic countries share commercial alliances and common interests in the production and consumption of food, particularly in the case of seafood. The Atlantic area has abundant natural resources, which places it among the main exporters of seafood resources serving the European market. NEPTUNUS represents a good opportunity to contribute to the economic development of this area, but also implies a high responsibility that needs to be articulated through tangible midland long-term actions. It jointly addresses a global concern and interest in terms of policies and strategies aimed at climate change mitigation, and energy, water and food security (NEXUS approach). To address the challenges posed by these objectives, sustainable and multilateral research cooperation is needed to define integrated methodologies and strategies. The added methodological challenge is to integrate environmental, nutritional and economic variables that meet regional needs through transnational strategies. The establishment of synergies in knowledge and experiences and challenges at the local level will help overcome challenges at a global level.

Acknowledgments

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Coastal planning in Spain

Esther Rando Burgos*[1]

*Corresponding author: erando@uma.es
[1] University of Málaga, Public Law Department

Keywords: Spatial planning, Coast, Maritime spatial planning, Legislation, Spain

Introduction

Coastal areas constitute one of the main assets of the coastal Autonomous Communities, generally strongly linked to tourism and with a considerable weight in their economy. But together with this, they are spaces with important resources and particularly vulnerable environmental values. The relevance of the coastal area as well as its planning and management has been a recurrent theme in the European Union. With the background to the EU Demonstration Programme on Integrated Coastal Zone Management (1999), entitled "Towards a European Strategy for Integrated Coastal Zone Management", the common problems of coastal zones (unplanned urbanisation; decline of traditional environmentally friendly sectors; coastal erosion) were pointed out; o, lack of adequate communication and transport networks) and established the seven general principles for good coastal zone management (a broad perspective; knowledge of the specific conditions of the area; working in harmony with natural processes; decisions that do not jeopardise the future; participatory planning to build consensus; support and involvement of all relevant administrative bodies; combination of instruments). Finally, it would promulgate Recommendation 2002/413/EC of 30 May 2002 concerning the implementation of Integrated Coastal Zone Management in Europe, in which it points out that integrated coastal zone management involves many factors, particularly spatial planning and land use, as well as the need for a broad and comprehensive perspective, taking into account the interdependence of natural systems and human activities that have an impact on coastal zones, but at the same time considering various issues such as the growing threat (both to the environmental and social balance of coastal zones) posed by the increase in population and economic activities. Within this framework, the Protocol for the Integrated Management of Mediterranean Coastal Areas was drawn up in 2008 and ratified by Spain in 2010. At the state level, in its area of competence, the State has also carried out various legislative reforms of interest in recent years. From the approval of Law 2/2013, of 29 May, on the Protection and Sustainable Use of the Coast and the modification of Law 22/1998, of 28 July, on Coasts, to Royal Decree 876/2014, of 10 October, approving the General Regulations on Coasts. This issue needs to be properly matched, precisely because of its position between the maritime and territorial policies that are being developed. On the one hand, the reference to the integrated maritime policy that the European Union has been carrying out materialized in Directive 2014/89/EU, of 23 July 2014, establishing a framework for maritime spatial planning and leading Spain to adopt Royal Decree 363/2017, of 8 April, establishing a framework for maritime spatial planning, is unavoidable. On the other hand, the different coastal Autonomous Communities, making use of territorial planning instruments, have developed different instruments with which they aspire to preserve and increase the protection of their coastal area as well as to regulate the uses and activities compatible with a particularly sensitive area and already strongly transformed.

Coastal planning in the Autonomous Communities

Although coastal spatial planning does not constitute a competential title of its own, it has been expressly incorporated by all the Autonomous Communities in their Statutes of Autonomy, within their competence for territorial planning. This is the frame of reference that leads a good part of the Autonomous Communities to enact territorial planning instruments focused on coastal areas. However, the importance of the issue lies in the need for a joint vision capable of coordinating and harmonising the different plans (coastal, coastal, maritime) which, some pre-existing and others in the process of being drawn up, make possible an appropriate and necessary integration between maritime and terrestrial. In this respect, the coastal space has much to say. As Zamora Roselló (2018) points out, "The very connection between maritime spatial planning and the achievement of the objectives of blue growth affect the joint vision of the terrestrial and maritime environments". If MSP is to be an instrument to help achieve smart, sustainable and inclusive growth, the development of a coordinated scheme for land and sea interaction is key. We fully agree on the need pointed out by the author of a unitary vision of the coastline and its management, as well as the key role that it should have the inter-administrative coordination by the concurrence of different competence titles. As well as in the relevance that the projection of the decisions of planning of the territory and urbanism acquire in this new scenario (Zamora Roselló, 2019). There is no doubt that spatial planning is a public function with an important responsibility in the planning and preservation of coastal areas. However, like any other autonomous competence, in order to know the situation and the development of this matter in the coastal areas of the whole of Spain, and with it a general and global perspective, it is necessary to analyses and know the different instruments developed by them. The particularity of these spheres within territorial planning, the significance they represent, the similarities and particularities with which each coastal Autonomous Community has developed it, or is doing so, and above all, the need for a comprehensive vision of territorial planning in the Spanish coastal areas, encourage to homogenize and deepen in the matter. In this context, attention must be paid to the existence of a large number of instruments which, from the point of view of spatial planning, have focused specifically on coastal areas. So, of the ten Autonomous Communities that have coastal areas, eight peninsular (Galicia, Asturias, Cantabria, País Vasco, Cataluña, Comunidad Valenciana, Murcia and Andalusia) and the two archipelagos (Canary Islands and Balearic Islands), almost all have paid, from the perspective of territorial planning, special attention to their coastal areas. An obligatory reference in this matter is the Autonomous Community of Asturias, the first to approve in 1993, a territorial planning instrument centres on its coastal space, the Directrices Subregionales de Ordenación del Territorio para la Franja Costera. At the other extreme, the Comunidad Valenciana which recently, in May 2018, approved the Plan de Acción Territorial de la Infraestructura Verde del Litoral de la Comunidad Valenciana y el Catálogo de Playas de la Comunidad Valenciana (PATIVEL). More than two decades have passed between the approval of both instruments, in which, gradually, the different Autonomous Communities through specific instruments of territorial planning have focused their efforts on one of the most precise spaces of this figure. Shortly before, in 2015, Andalusia approved the Plan de Protección del Corredor Litoral de Andalucía (PPCLA), after carrying out a reform in its legal framework for the inclusion of this instrument in 2012 and that two years later would be declared null and void by judicial decision. Galicia also has since 2011 with the Plan de Ordenación del Litoral de Galicia (POLG) or Cantabria, Autonomous Community that in 2004 approved the Plan de Ordenación del Litoral de Cantabria (POLC). Previous in time are the Autonomous Communities of the País Vasco, in 2007, with the Territorial Sectoral Plan of Protection and Planning of the Plan Territorial Sectorial de Protección y Ordenación del Litoral de la Comunidad Autónoma de País Vasco, Cataluña that since 2005 has the Plan Director Urbanístico del Sistema Costero or Murcia, which in 2004 approved the Directrices y Plan de Ordenación Territorial del Litoral de la Región de Murcia. Paradoxically, it is the archipelagos that for the time being do not have a spatial planning

instrument specifically designed for their coastal areas. In the case of the Canary Islands, in 2004 it agreed to initiate the procedure for drawing up the Directrices de Ordenación del Litoral, but so far they have not seen the light of day. The Balearic Islands, for its part, opts to include the criteria and guidelines for the planning of their coastal space in the general regulations of the sub-regional territorial plans of each of the islands.

Conclusions

If the joint vision of the territory, beyond the administrative limits drawn on it, are a reality (and a necessity) for which we have been advocating (Rando Burgos, n.d.), the question seems increasingly complex due to the singularities that it presents in particularly vulnerable areas and subject to strong pressures, such as the coasts. And we say it is complicated, insofar as it is no longer just the planning of these coastal spaces, from the territorial perspective, but this must be properly combined with the management of coastal spaces and with the relatively recent standard of reference that from the state framework comes to establish the bases for maritime spatial planning. All this broadens the scope of the much-needed inter-administrative coordination between the different territorial Administrations involved in defining not just a territorial model but one that will become a reference territorial-maritime model in the coming years. Advocating from now on for coordination, consensus and agreement between the present and the future of our territories and our seas, betting on a common goal, is becoming more precise today than ever and the different public policies as well as the Administrations involved must focus their efforts on this.

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The "Cachucho", Marine Protected Area and Special Conservation Area.

Noelia Pérez Nogueira*[1] , María Ángeles Bárcena Pernía[1]

*Corresponding author: noeliapnogueira@usal.es
[1] University of Salamanca

Keywords: The Cachucho, Le Danois Bank, Marine Protected Area, Reefs 1170

Background

This paper is the product of the recompilation and synthesis of the legislation referring to the protection of the marine environment, both at the national and international levels. This information has been acquired through the Ministry for the Ecological Transition, catalogues, species lists, the Interpretative Guide of the Spanish's Maritime Habitats Inventory, documents of the OSPAR commission, etc. Furthermore, the information was also compiled from oceanographic research projects such as LIFE+ INDEMARES or ECOMARG, as well as from the draft published on December 2018 by the Ministry for the Ecological Transition for the renewal of the management plan for the Marine Protected Area in order to move towards the public participation procedure.

Marine Protected Area

When referring to a Marine Protected Area (MPA) we are alluding to a natural space designated for the protection of the different ecosystems, communities or biological/geological elements belonging to the marine environment - including the intertidial and subtidal areas - that, given their rarity, fragility, importance or singularity, deserve a special protection (as stated in the Law 42/2007 of the Nature and Biodiversity Heritage, approved on the 13th December) where the MPA figure is designated. For the correct conservation of the MPA, agendas or management instruments will be approved, and limitations regarding the exploitation of natural resources will be established. The previous investigation surrounding the issue of the "Cachucho" as well as its declaration as MPA in 2011 (Royal Decree 1629/2011, from the 14th November), was carried out by a multidisciplinary group, being among its members representatives from the IEO, from the Autonomous Organization for National Parks, from the Directorate General (DG) of Coasts, from the DG for the Biodiversity, from the DG for the Merchant Marine, from the Ministry of Foreign Affairs, from the DG of Fishing Resources of MAPA, from the Ministry of Fishing and Environment of the Principality of Asturias, from the National and Province Fraternitie League of Asturian Fishers, the National Organism of Fishing Associations, the Regional Center for the Coordination of the Southern Waters, WWF/Adena and Oceana (INTEMARES, 2018). The "Cachucho" is located on the continental shelf of the Cantabrian Sea - facing the Asturian locality of Ribadesella - about 65 km away from the coastline (Figure 1).

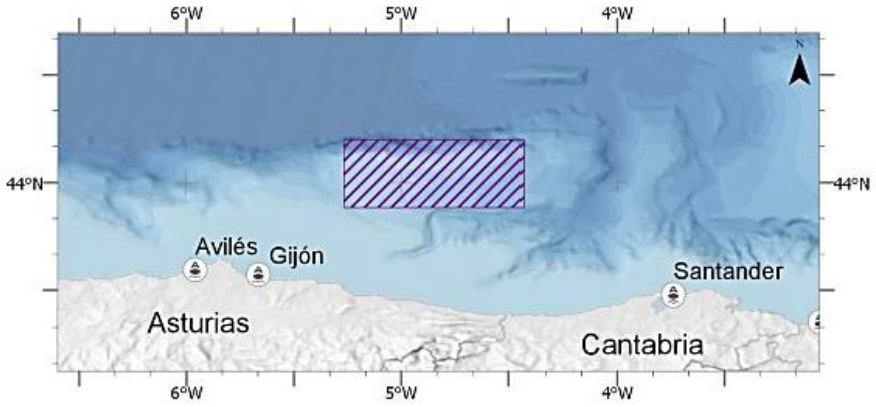

Figure 1: Location and size of Marine Protected Area.

It comprises the outskirts of the submarine mountain known as Le Danois Bank, and it is presumed to be a singular environment because of its morphological characteristics as well as its location (Heredia et al., 2008). The bank counts with a horst type structure, with a total slope that overpassess the 4000 meters, it is parallel to a sedimentary internal basin with an average profundity of 850 meters (The Asturian Basin), as well as to the abyssal plain (Figure 2).

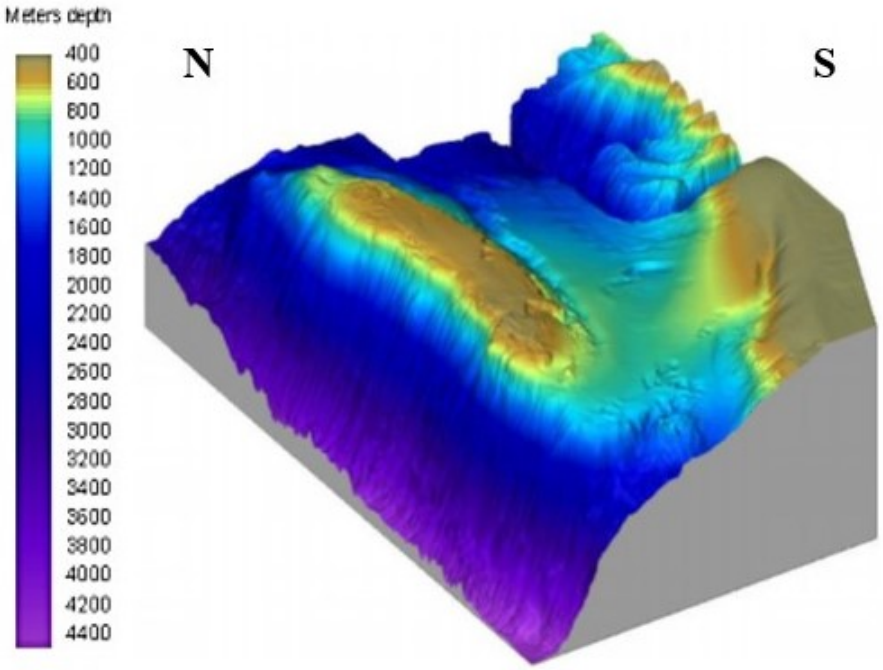

Figure 2: 3D image of the continental slope, Le Danois Bank, Asturian Basin, Lastres Canyon and continental shelf (from North to South).

Furthermore, in the surroundings of the bank there are several submarine canyons. Its characteristics and location allow for the development of several habitats, including the type of habitat of communitarian interest 1170/Reefs, formed by cold-water corals and sponges fixed to hard rock, that at the same time provide refuge to many other species. Its presence, along with that of the community interest's taxa known as loggerhead turtle (*Caretta caretta*) and that of the bottlenose dolphin (*Tursiops truncatus*), justifies its declaration as Special Conservation Area (ES9ATL01) by the Red Natura 2000 in 2011, in accordance with the 92/43/CEE Counsel

Directive, of the 21st May 1992, relating the conservation of natural habitats along with the wildlife species and flora, under which the annexes I and II respectively compile the reefs and these taxa (INTEMARES, 2018). Biogenically there are 6 different types of reefs:
- Bathyal Rock *Callogorgia verticillata*, white gorgonia with preference for irregular substrates.
- Bathyal Rock with *Paramuricea* cf. *placomus* (species in revision, could be of genus *Placogorgia*, had never been found in that low latitudes), yellow gorgonian with preference for areas with a strong slope.
- Bathyal Rock with *Dendrophyllia cornigera*, yellow coral that is developed at smaller profundity than the rest of species, reason for its wide development in the bank's roof.
- Bathyal Rock with black corals *(Leiopathes, Antipathes)* found at the greatest profoundities.
- Bathyal Rock with great Hexactinellid sponges (*Asconema*).
- Bathyal Rock with great demosponges *(Geodiidae, Axinellidae* and *Pachastrellidae)* (Sánchez et al., 2015) Traditionally, the "Cachucho" has been a fishery area of great importance, where the mechanical damage resulting from the employment of bottom trawling fishing techniques is believed to be a plausible reason for the severe degradation of the reefs (THIC 1170) undergone on the upper parts of the bank. Nevertheless, given the disappearance of this impact due to the denomination of the area as a protected zone, its future perspectives and state of conservation are estimated to be favorable. The gaps in the previous research and information regarding the area make it impossible to evaluate the evolution of the loggerhead turtle and bottlenose dolphin (INTEMARES, 2018). Another 8 cetaceans appear in the "Cachucho" and the leatherback turtle (Dermochelys coriacea) compiled in the Habitats Directive, and 13 species of seabirds compiled in the 2009/147/CE Directive of the European Parliament and Council, from the 30th November 2009, aimed at the conservation of wild fowls. On the other hand, the list of endangered or declining habitats and taxa published on the Agreement for the protection of the marine environment of the Northeast Atlantic (OSPAR Agreement, Paris 1992) gathers 14 taxa comprising fish, reptiles and birds, as well as 4 different habitats: Deep-Sea Sponge Aggregations, *Lophelia pertusa* reefs, Seamounts and Sea-pen and burrowing megafauna communities (OSPAR, 2010).

Conclusions

Thus, the "Cachucho" constitutes a greatly important space for the marine protection at the national level for it is a hotspot of biodiversity thanks to the local upwellings provoked by the altering of the marine currents as a consequence of the irregularity in the topography of the seafloor created by the submarine mountain, the existence of habitats of different characteristics (slope, profundity, substratum...) and its unusual proximity to the coast, that favors the presence of a great variety of species that inhabit the "Cachucho" either permanently or in a frequent manner when migrating. The "Cachucho" also presents a high rate of endemicity, a trait common of submarine mountains, probably because the area has a limited space for reproduction. More than 740 species have been mentioned, being at least 6 of them catalogued as new (Heredia et al., 2008). Moreover, the "Cachucho" is a favorable area for the reproduction of multiple species, several of them being of commercial interest, so it has important repercussions on the sustainability of the area's fisheries.

Acknowledgments

We would like to thank the Faculty of Agrarian and Environmental Sciences, especially to the dean team for the trust in Noelia supporting part of the assistance to this congress. We also would like to thank Professor José A. Flores Villarejo for his enthusiasm in teaching us Oceanography at an inland University.

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Ecosystem services and urban development in the Bay of Cadiz

Maria de Andres*[1] , Juan Manuel Barragan[1] , Javier García Sanabria[1]

*Corresponding author: maria.deandres@uca.es
[1] Universidad de Cádiz

Keywords: GIZC, Cities, Ecosystem Based Management, Socio-Ecological Systems

Introduction

In Spain, the coastal zone has been configured as one of the most important spaces for the population and the economy. A process where people, activities, large infrastructures and facilities are concentrated in what is basically a wide strip, is known as "littoralisation" (de Andrés et al., 2017). The deterioration of coastal areas, in a prominent manner during the last five decades, is well studied and documented.
The Bay of Cadiz is a sub-region of Andalusia (Spain), located in the southwest of the Iberian Peninsula, it is considered an urban area in its entirety. The boundaries of the Bay of Cadiz are not specifically stipulated using administrative delimitations that usually coincide with the boundaries of the coastal municipalities, nor with the Bahía de Cádiz Natural Park (de Andrés et al.).
This communication focuses on the study of the Bay of Cadiz as a Social-Ecological System. Impacts on ecosystem services will, therefore, be studied as a consequence of the changes in land use that have occurred in recent decades due to urban sprawl. This will allow for the proposal of integrated coastal management processes.

Metodology

The model used for the delimitation is based on the conceptual framework developed by Barragán and de Andrés (2016) which establishes the basis for the delimitation of the coastal areas of Spain from a social-ecological perspective. This model interprets the coastal zone as the geographic space (marine, intertidal and terrestrial), according to geographic-ecological, social-economic and political-administrative criteria. The Social-Ecological System (SES) designed for integrated management, is composed of three distinct geographical units: a) coast, b) coastal zone and c) coastal-influence zone. The study of the issues of the Bay of Cadiz from a social-ecological approach is obtained from a DPSIWR (Driver - Pressure - State - Impact - Well Being - Response) analysis of the ecosystem services of the Bay of Cadiz (Figure 1).

Results

The results show that coastal ecosystems, mainly saltlakes, beaches and dunes and tidal saltmarshes, are the ones that offer the most services to the population. Regulatory services such as erosion control or climate regulation stand out; But also cultural services, mainly recreational and tourism. Food supply is provided from coastal ecosystems, thanks to the fishing stock, among others. The support service is mainly associated with the creation of habitat, which in the case of coastal ecosystems is characterized by the harbouring of endemic and vulnerable species (Table 1).

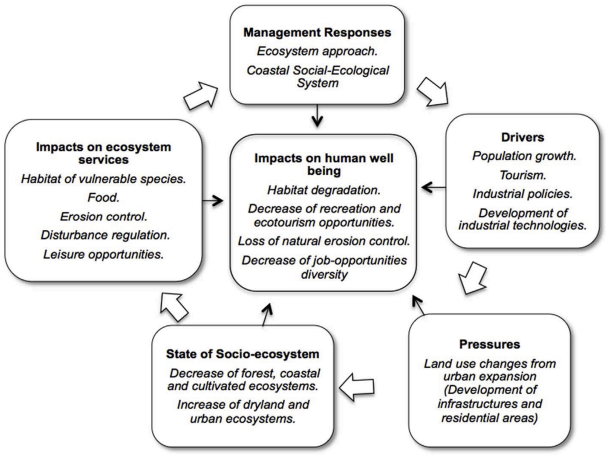

Figure 1: DPSIWR framework in the Bay of Cadiz.

Table 1: Ecosystem services in the Bay of Cadiz.

	Services	Uses y benefits	Forest	Scrubland	Grassland	Tidal saltmarsh	Non-tidal saltmarsh	Seagrasses	Seaweeds	Rocky coast	Salt lakes	Beach and dunes	Marine environment	Wetland	River	Green urban areas	Agroforest	Irrigated cultivated area	Rainfall cultivated area
Support	Habitat	Biodiversity maintenance	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		Vulnerable species	■	■		■	■				■	■	■						
		Fisheries stock				■		■		■	■		■		■				
		Nursery and reproduction				■		■		■	■	■	■						
		Military defence			■					■		■							
	Transport	Nautical transport				■					■	■	■		■				
	Sewage assimilation	Sewage assimilation				■				■		■	■		■				
Provision	Food	Fisheries				■				■	■	■	■		■				
		Plants and seaweeds	■	■		■			■		■								
		Fruits and vegetables															■	■	■
		Salt									■								
	Fresh water	Fresh water												■	■				
	Building resources	Wood	■																
		Rock								■									
		Sand										■	■		■				
	Energy	Biofuel	■						■								■		
	Biochemical	Cosmetics				■			■		■								
Regulation	Climate regulation	Carbon absorption	■	■	■	■	■	■	■		■	■	■	■		■	■	■	■
		Local temperature				■					■	■	■		■				
	Water regulation	Water purification	■	■	■	■	■	■	■		■	■	■	■	■				
	Erosion control	Soil retention	■	■		■	■	■		■	■	■		■			■		
	Disturbance regulation	Protection from flooding	■	■		■	■				■	■		■	■		■		
		Protection from extreme events	■	■		■	■	■		■	■	■	■	■	■		■		
	Productive soil	Fertile soil															■	■	■
	Pollination	Pollination	■	■	■	■	■				■	■		■		■	■	■	■
	Grazing land	Grazing			■														
	Noise reduction	Buffer from noise	■	■									■	■		■	■		
	Air purification	Oxygen liberation	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■
Cultural	Education	Environmental education	■	■	■	■	■	■	■		■	■			■	■			
	Research	Research	■	■	■	■	■	■	■	■	■	■	■	■	■		■		
	Recreational and ecotourism	Leisure opportunities	■	■	■	■				■	■	■	■			■			
		Paths	■	■	■	■	■				■								
		Nautical activities				■					■	■	■		■				
		Birdwatching				■					■	■	■						
		Sport fishing								■		■	■		■				
		Sun and beach										■							
	Aesthetic	Landscape	■	■		■					■	■	■	■	■				
		Saltlake infrastructures									■								
	Sense of place	Sense of place									■	■	■						
	Iconic species	Coastal bird life				■					■	■	■						

The population growth in the Bay of Cadiz in the last 60 years has been associated with unprecedented urban development, accompanied by an increase in economic uses and activities and, consequently, an increase in the area occupied by infrastructures and equipment destined to

satisfy services that guarantee human well-being. In this sense, it is worth noting that while the population has grown by twice as much as it was in 1956, the area given to urban centres and residential developments has multiplied by 8 in the same period (Figure 2).

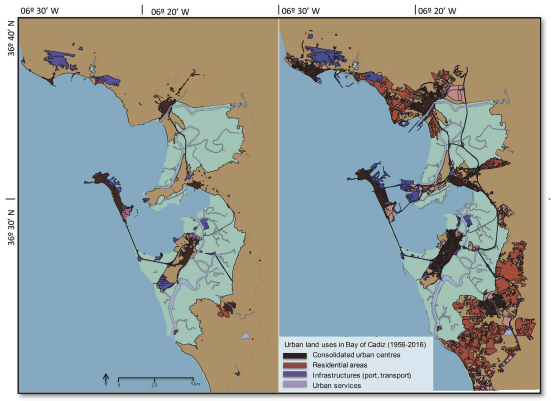

Figure 2: Urban land uses changes in the Bay of Cadiz (1956-2016).

Urban expansion and the development of economic activities have led to the regression and fragmentation of certain ecosystems. To this effect it is essential to know the impact that this regression causes on the services that ecosystems offer the population in order to address it's management with an ecosystem approach. The results of this analysis show the trend and abundance of ecosystem services in the Bay of Cadiz (Table 2).

Table 2: Ecosystem services evolution and trends in the Bay of Cadiz.

Type	Services	Uses and benefits	Trend and existence
Support	Habitat	Biodiversity maintenance	↗
		Vulnerable species maintenance	↓
		Fisheries stock	↘
		Nursery and reproduction	↘
		Military defence	↑
	Transport infrastructures	Nautical transport	↘
	Sewage assimilation	Sewage assimilation	↘
Provision	Food	Fisheries	↘
		Plants and seaweeds	↓
		Fruits and vegetables	↘
		Salt	↘
	Fresh water	Fresh water	↗
	Building resources	Wood	↘
		Rock	→
		Sand	↗
	Energy	Biofuel	↓
	Biochemical	Cosmetics	↘
Regulation	Climate regulation	Carbon absorption	↘
		Local temperature	↘
	Water regulation	Water purification	↘
	Erosion control	Soil retention	↓
	Disturbance regulation	Protection from flooding	↓
		Protection from extreme events	↓
	Productive soil	Fertile soil	↘
	Pollination	Pollination	↘
	Grazing land	Grazing	↑
	Noise reduction	Buffer from noise	↘
	Air purification	Oxygen liberation	↘
Cultural	Education	Environmental education	↑
	Research	Research	↗
	Recreational and ecotourism	Leisure opportunities	↑
		Paths	↗
		Nautical activities	↗
		Birdwatching	↗
		Sport fishing	↗
		Sun and beach	↗
	Aesthetic	Landscape	↓
		Saltlake infrastructures	↘
	Sense of place	Sense of place	↘
	Iconic species	Coastal bird life	↘

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MarSP: Cross-border cooperation in the Macaronesian region

Claudia Millán Caravaca*[1] , Javier García Sanabria[2] , Javier García Onetti[3] , Cristina Pallero Flores[2] , Víctor Cordero Penin[2] , María de Andrés García[2] , Eleonora Verón[4]

*Corresponding author: cmillancaravaca@gmail.com
[1] University of Cork, Postgraduate student
[2] University of Cádiz, Research Group on Integrated Coastal Zone Management
[3] University of Cádiz, Geographical Regional Analysis Research Group
[4] National University of Mar del Plata; Geography Department

Keywords: Marine spatial planning, Macaronesian maritime spatial planning, Ecosystem-based management, Cross-border cooperation, Stakeholder engagement

Introduction

The present dissertation is based on the Macaronesian Maritime Spatial Planning (MarSP) project, specifically in analysing cross-border cooperation processes between archipelagos. Within the EU, the Macaronesian region consists of three archipelagos: the Azores, Madeira (both belonging to Portugal), and the Canaries (Spain). These three Atlantic archipelagos, located off the coast of Europe and Africa, share a common volcanic origin and gentle climate which have created the perfect conditions for a rich array of both marine and terrestrial species and habitats. The MarSP project aims to coordinate the different marine sectors' activity in a shared space. Not only is MSP focused on reducing pressures generated by anthropogenic actions, but it may also lead towards sustainable development. Such a desirable goal would not be possible without considering the stakeholders. Johansson (2016) highlights the significance of stakeholder participation throughout the whole decision-making process. Stakeholders involved from early stages, who are well-informed and whose opinions are taken into account, are more likely to support the outcomes of this process. Previous studies have highlighted the lack of coherence of the decision-making process, which leads to a failure in governance. At the same time, there is a growing need to undertake cross-border MSP initiatives, engaging countries that share a common marine region. Scientific research has proven the advantages of creating a sense of collective purpose and trust among authorities in the MSP planning process. Transboundary MSP often results in a coherent set of national marine spatial plans applied to the study area. These plans present the following benefits: a joint assessment of current conditions covering the whole marine region, a forward-looking strategy for that region, and a set of guidelines and principles mutually agreed by all participating countries (GEF LME:LEARN, 2018; Carneiro et al., 2018).

Literature review

For this dissertation (i.e. MarSP project), the application of DAPSI(W)R has resulted in a schematic simulation of both socioecological systems and their main interconnected processes. Besides, this framework promotes cross-sectoral integration by establishing the role of different stakeholders and economic sectors (Pallero Flores et al., 2018). The general diagnosis made using DAPSI(W)R is completed with an atlas of the Macaronesian Region. The objective of this collection of maps is to establish those geopolitical dimensions of maritime spatial planning within the MarSP project (Suárez de Vivero and Palacios Guerrero, 2019). Equally important is the

analysis of good practices from past MSP processes regarding cross-border cooperation. Different case studies have been presented such as the CTI-CFF; Lesser Sunda Ecoregion; Grenada Bank, St. Vincent and the Grenadines; and Rhode Island Ocean SAMP amongst others. Besides, valuable lessons derived from good practices may be applied to other MSP processes. Both the good practices selected and lessons derived from them have characteristics in common with the Macaronesian Region. The chosen criteria have been the existing cross-border cooperation mechanisms across jurisdictions (e.g., international, national, regional, and local); land-sea interactions (e.g., land activities cause impacts in marine areas); remote location (e.g., outermost marine regions); key sectors affected by international jurisdiction (e.g., fisheries, shipping), and the formation of high sea pockets (e.g., ABNJ located in between marine areas under Portuguese or Spanish jurisdiction) (Pallero Flores et al., 2019).

Methodology

Recommendations on cross-border cooperation in Macaronesian marine areas are underpinned by the DAPSI(W)R framework, regional atlas, and analysis of good practices. These guidelines act as a basis for the design of pilot projects which address critical marine management issues such as different legislation, institutions and priorities (depending on the Member State); lack of resources, control of illegal activities and communication between countries (Pallero Flores et al., 2019). Based on these findings, a series of workshops have taken place in the Azores, Madeira, and the Canaries. An initial survey to determine proposals for pilot projects has been filled out by stakeholders from different backgrounds. Given the results of this first questionnaire, seven proposals have been designed focused on a variety of topics (e.g. marine protected areas; marine tourism; maritime security; fisheries; ports and shipping; regional coordination; research and training). Once the pilot projects have been designed, a second series of workshops takes place. Then, the proposals are presented to the stakeholders for their perusal. After discussion, two pilot projects will be selected to be further developed. As the last step of this participative process, two representatives from each archipelago will value the final proposals.

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Blue energy and marine spatial planning. What is going on in southern Europe.

Pablo Quero García*[1] , Juan Adolfo Chica Ruiz[2] , Javier García Sanabria[2]

*Corresponding author: pablo.querogarcia@gmail.com
[1] Research Group of Integrated Coastal Zone Management (GIAL), University of Cadiz, Spain.
[2] INDESS (Research Universitary Institute for Sustainable Social Development), University of Cadiz, Spain.

Keywords: Marine spatial planning, Renewable energy, European Union, Mediterranean, Blue energy

Abstract

The 2050 target of the European Union to decarbonize the economy by 80-90 % will undoubtedly see marine renewable energies playing a key role. The deployment of offshore facilities within the framework of Blue Growth creates tension as competition for other uses of the sea intensifies as economic exploitation of marine resources increases. The European Union has chosen marine spatial planning (MSP) as an instrument to plan the harmonious development of activities in marine areas of Member States, and the sustainable use of offshore resources, including blue energy. Directive 2014/89/EU requires Member States to approve maritime spatial plans that determine the spatial and temporal distribution of activities and uses in their marine waters before 2021. Some Member States, mainly in the North Sea basin, have already applied MSP as a way of easing tensions while promoting blue energy (Ehler, 2011). Not surprisingly, the United Kingdom, Germany, Denmark, the Netherlands and Belgium are home to 98% of all offshore wind capacity connected to the grid in Europe in 2017 with 4,016 turbines and 14,451 MW installed. The southernmost marine areas of the continent also have considerable energy potential that, as yet, has not been exploited due to the limitations imposed by a narrow and steep continental shelf, not to mention legal and administrative barriers (Soukissian et al., 2017). This increases the costs of installation and maintenance, decreasing profitability and forcing the selection of locations near the coastline, which, in turn, creates tension due to competition for use of space, not to mention the public's rejection of the visual impact. These factors have dampened the commitment of public and private developers who have given priority to other more profitable and less problematic energy sources (e.g. solar photovoltaic and onshore wind). Marine spatial planning processes that are currently taking place in these Member States are therefore an opportunity to resolve these conflicts and promote sustainable use of blue energy sources in southern Europe. This article evaluates the way in which the processes of marine spatial planning that are currently being developed in southern European countries are integrating clean energy generation policies for the oceans. For this purpose, MSP initiatives of the five Southern Member States with marine renewable energy objectives defined in their national energy planning have been studied: Spain, Greece, Italy, Malta and Portugal. In each case, a set of six items were analyzed (maritime and energy policy; institutions; strategic instruments; operational instruments; information and knowledge; and public participation) with the aim of defining the elements of MSP that impact on the development of renewable energy use in the oceans. Results shown some general characteristics and factors for the MSP initiatives discussed that will affect the development of the offshore renewable energy sector in the marine areas of southern Europe (Table 1).

Table 1: General features of marine spatial planning of Southern European Member States with impact on blue energy. Source: authors' own.

General features of the MSP in the south of the EU that impact on blue energy
Maritime and energy policies are changing, lack definition or are nonexistent. States lack a general vision for the maritime area that determines national priorities for the development of the marine space. Sectoral policies shape the distribution of uses at sea.
Model of fragmented marine governance with responsibilities distributed over different departments. Inter-administrative committees are imposed as a formula to coordinate government action on the marine space.
Use of ocean zoning as a tool to organize uses and activities in the marine environment. The main spatial conflicts take place within the coastal and marine tourism sector.
Dated and non-specific approval processes that are slowed down by requiring the participation of a relatively large number of agencies.
Unstructured and inefficient data management, with incomplete and incompatible data series.
No mechanisms are anticipated that ensure the involvement of the stakeholders during the MSP process in an adequate time-frame or manner. Participation is limited to formal consultations within the procedures for preparing plans and environmental prevention.

One of the main difficulties identified is a lack of defined policies for blue energy, and a general lack of vision for the maritime area. This, in turn, determines the national priorities for development of marine space. The process of maritime spatial planning currently being implemented in these regions is confirmation of a valid instrument to form a solid and safe strategic framework that sets the course for the maritime sectors in the medium-long term. MSP could also stimulate cross-border coordination and the adoption of transnational solutions that contribute to overcome specific barriers of blue energy in southern Europe (Afrokomi-Afroula and Nikitas, 2015). The shortcomings identified in marine governance of the countries studied are also an obstacle for the marine renewable energy industry. There are no key bodies that bring together competences related to maritime sectors. This means that management responsibilities for marine space and its uses are divided into different departments, creating complex institutional frameworks that hinder decision-making processes and finally translate into confusing and inefficient authorization procedures. The formula adopted in some countries of inter-administrative committees can partially solve the problem of institutional fragmentation by improving the horizontal coordination of sectoral policies (including that related to marine renewable energy) (Guerra, 2018). Coastal tourism has to be considered as another key element for future development of renewable offshore in southern regions of Europe as it is of great economic and social importance. The implementation of MSP in these States must keep this factor in mind and design specific tools to reduce conflicts between offshore energy use and tourism (Bush and Hoagland, 2016). It can be concluded that the Portuguese maritime spatial planning is the most suitable to facilitate the launch of offshore renewable energy use: there are defined policies with clear objectives for blue energy and drivers for management of the Portuguese marine space; a hierarchy of responsibilities with an authority specifically responsible for coordinating marine affairs; the authorization processes has been simplified for the developers through the creation of a single window; and areas have been defined for the location of renewable electric generation projects in the sea. It is also a flexible system that includes procedures for modification of management plans post approval. Although it is true that the process of planning maritime space in Portugal is in its second planning cycle, and this means a greater degree of maturity. With the exception of isolated cases of a sectoral nature, for most of the Member States of southern

Europe, this is the first instance of maritime spatial planning. It will be interesting to see which models are finally implanted in each region and if they finally employ the necessary elements to promote the use of blue energy in their waters.

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PART II

POSTERS

Microalgae biomass cultivated in agro-industrial wastewater as potential ingredient for feeding Siberian sturgeon (*Acipenser baerii*): Effect on digestive enzymes

Antonio Jesús Vizcaíno Torres*[1] , María Isabel Sáez Casado[1] , Alba Galafat Díaz[1] , Miguel Salazar Martínez[1] , Tomás Francisco Martínez Moya[1] , Katia Parati[2] , Andrea Di Biase[3] , Bongiorno Tiziana[2] , Montedoro Marina[2] , Francisco Gabriel Acién Fernández[4] , Francisco Javier Alarcón López[1]

*Corresponding author: avt552@ual.es

[1] Departamento de Biología y Geología, Univ. Almería. Sacramento s/n La Cañada S. Urbano 04120, Almería, Spain

[2] Istituto Sperimentale Italiano L. Spallanzani - Loc. La Quercia, 26027 Rivolta d'Adda (CR), Italy

[3] AIA-Agricola Italiana Alimentare, Spa, Brand Veronesi Mangimi, Verona, Italy

[4] Department of Chemical Engineering, University of Almeria, 04120, Almeria, Spain

Keywords: Microalgae, Siberian sturgeon, Digestive functionality, Agro-industrial wastewater, Enzymatic hydrolysis

Introduction

The use of microalgae in aquafeed formulation has been successfully studied in different species. However, the excessive cost of production is one of the major bottlenecks for their generalized use in commercial feed formulation. For this reason, the growth of microalgae in agro-industrial waste is of particular interest in the context of a circular economy, not only for recycling valuable nutrients but also for reducing the cost of microalgae production (Pittman et al., 2011). To our knowledge, few studies have evaluated the possibility of using microalgae produced in that manner as protein source for aquafeeds (Tomás-Almenar et al., 2018). On the other hand, the existence of a cellulosic-rich cell wall in microalgae could limit the bioavailability of nutrients for carnivorous fish species. For this reason, previous enzymatic hydrolysis of microalgal biomass before inclusion to aquafeeds could improve its performance as dietary ingredient. Given the above considerations, the aim of this study was to assess whether the biomasses of *Nannochloropsis gaditana* and *Scenedesmus almeriensis* produced in a sustainable bio-refinery using diluted in pig manure are suitable for feeding Siberian sturgeon. The effect of i) the microalgae species used for supplementation of experimental diets, ii) the nutrient source used for producing the microalgal biomasses, and iii) the inclusion or not of a previous enzymatic hydrolysis for the disruption of the cell wall, on the digestive enzymatic activities of fish was evaluated.

Materials and methods

Microalgae biomasses produced on synthetic medium (SM) and on 1:10 (v/v) diluted pig manure (PM) were provided by Estación Experimental "Las Palmerillas" (Fundación Cajamar, Almería, Spain). Enzymatic hydrolysis of microalgal biomasses was carried out using an *in vitro* model. For the feeding trial, nine diets were formulated to be iso-proteic and iso-lipidic; a microalgae-free diet (Control; CT) and 8 test diets suplemented with 10% microalgae biomass (Table 1). Feeding trial was carried out at the Istituto Sperimentale Italiano L. Spallanzani (Italy). After

a 15-day acclimation period, fish were randomly distributed in 27 polypropylene circular tanks. Experiments were conducted in triplicate (9 feeds x 3 tanks each feed), and fish were fed with the different diets twice per day (9:00 and 17:00 h), 7 days per week at a rate of 3% of their body weight for 6 weeks. At the end of the trial, fish were individually weighted and then, euthanized by clove oil overdose (200 ppm) followed by spine severing. For enzymatic activity analysis, intestines from three fish per tank was differentiated in anterior and posterior intestine. Samples were homogenized to obtain enzymatic extracts in order to determine trypsin, chymotrypsin, leucine aminopeptidase, alkaline phosphatase and total alkaline protease according to Vizcaíno et al. (2014). In addition, digestive proteases were separated and visualized in substrate-SDS-PAGE electrophoresis gels.

Table 1: Experimental diets used in the feeding trial.

Diet	
Control diet (CT)	Microalgae-free diet
Diet 1	10% *N. gaditana* crude (grown on synthetic medium)
Diet 2	10% *N. gaditana* crude (grown on diluted pig manure)
Diet 3	10% *N. gaditana* hydrolysed (grown on synthetic medium)
Diet 4	10% *N. gaditana* hydrolysed (grown on diluted pig manure)
Diet 5	10% *S. almeriensis* crude (grown on synthetic medium)
Diet 6	10% *S. almeriensis* crude (grown on diluted pig manure)
Diet 7	10% *S. almeriensis* hydrolysed (grown on synthetic medium)
Diet 8	10% *S. almeriensis* hydrolysed (grown on diluted pig manure)

Results and discussion

At the end of the feeding trial sturgeon fed Diet 4 (*N. gaditana* hydrolysed, and grown on PM) showed higher final body weight than fish fed on CT diet ($p<0.05$). Fish fed any type of *S. almeriensis* showed the lowest body weight, compared to CT fed fish. In order to determine the possible effect of dietary treatments on digestive enzyme activities, three factors were considered; i) algae (*N. gaditana* vs S. *almeriensis*), ii) fertilizer (SM vs PM), and 3) treatment (crude microalgal biomass vs hydrolysed microalgal biomass; Table 2). Proteolytic activity was significantly affected by the microalgae species. Trypsin activity level in proximal intestine was higher in fish fed on *Nannochloropsis*-supplemented diets (Table 2) whereas total alkaline protease was higher in fish fed *Scenedesmus*-supplemented diets. However, in these last fish it was evidenced the higher total proteolytic activity the lower growth performance, which might reflect a compensation mechanism against dietary changes (Santigosa et al., 2008). A significant effect of the type of fertilizer used for growing microalgae was observed. Leucine aminopeptidase and alkaline phosphatase activity levels in proximal intestine were significantly lower in fish fed on diets supplemented with microalgae grown on PM ($p<0.05$). Finally, it was also found an effect of using microalgal biomass enzymatically treated before dietary inclusion on proteolytic activity level. Hydrolysed microalgae-supplemented diets induced a reduction in the level of total alkaline protease activity in distal intestine. This fact could be due to the increase in the bioaccesibility and bioavailability of nutrients when pre-hydrolysed microalgae biomasses were used for supplementation of experimental diets.

Table 2: Effect of the different factor (algae, fertilizer and treatment) on digestive enzyme activities measured in intestinal extracts of sturgeon at the end of the feeding trial.

Factor	Algae		Fertilizer		Pre-treatment	
	PI	DI	PI	DI	PI	DI
	p value		*p value*		*p value*	
L-aminopeptidase	0.327	0.758	0.024	0.219	0.403	0.620
Alkaine phosphatase	0.405	0.006	0.021	0.819	0.212	0.101
Trypsin	0.039	0.404	0.321	0.114	0.346	0.885
Chymotrypsin	0.375	0.123	0.639	0.479	0.094	0.506
Total alkaline protease	<0.001	0.049	0.471	0.730	0.525	0.012

Codes. PI: posterior intestine; DI: distal intestine. To determine possible differences in digestive enzyme activities between each two Algae, Fertilizer and Treatment, a comparison of means was used (Student's t -test).

PI: posterior intestine; DI: distal intestine. Differences in digestive enzyme activities for each factor, i) Algae (*N. gaditana* vs *S. almeriensis*), ii) Fertilizer (synthetic medium vs diluted pig manure) and iii) Treatment (crude biomass *vs.* hydrolysed biomass), were stablished by a comparison of means (Student's *t*-test). In addition, zymography confirmed that fish fed on the experimental diets showed a similar pattern of alkaline protease that was observed in control specimens (Figure 1).

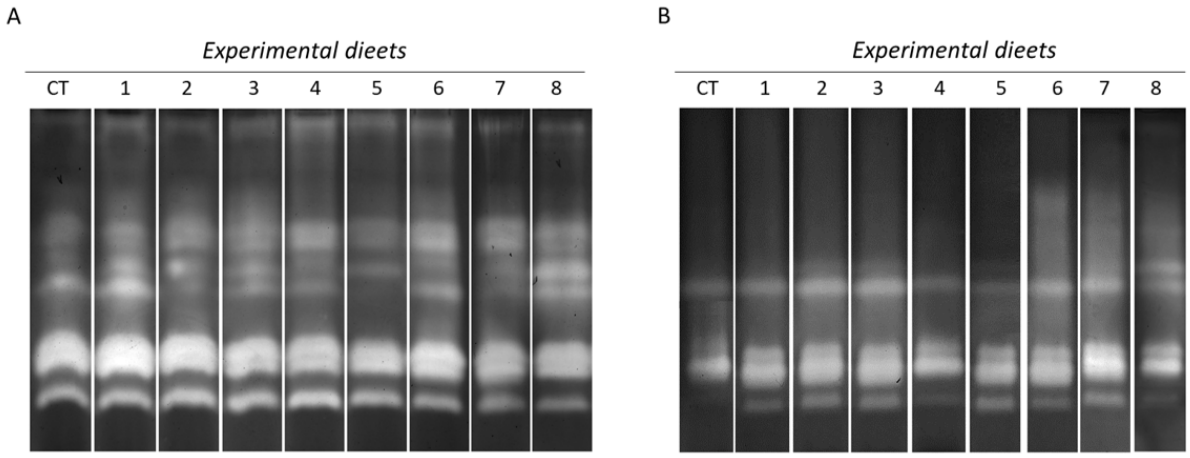

Figure 1: Zymograms of alkaline proteolytic activity in the anterior (A) and posterior (B) intestine of juvenile sturgeon fed on the experimental diets. The number in each lane represents the experimental diet.

In conclusion, the data observed in this study confirmed the potential for using both microalgae for feeding Siberian sturgeon juveniles. Overall, the experimental diets tested, based on microalgae grown on SM and on PM, ensure a balanced and complete level of the nutrients, suitable for the growth of sturgeon juveniles, particularly in the case of *N. gaditana*.

Acknowledgments

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Physiological recovery in two rajidae species: a new tool to manage bottom trawling discards

Cristina Barragán-Méndez*[1] , Miriam Fernández-Castro[1] , Ignacio Sobrino[2] , Juan Miguel Mancera[1] , Ignacio Ruiz-Jarabo[1]

*Corresponding author: cristina.barragan@uca.es
[1] University of Cádiz
[2] Spanish Institute of Oceanography (IEO)

Keywords: Discards, Survival, Rajidae, Physiology, Recovery

Introduction

European Union Common Fisheries Policy (CFP, EU 2013) proposes an obligation to land discards following a schedule based in different areas, fisheries and species. Once the regulation comes into force all catches of species managed by quotas/catch limits and minimum landing sizes should be landed (article 15 of the CFP) (EU, 2013). However, an exception to this law includes that those species which survival is scientifically proven, can be released into the sea. As fishing processes induce stress responses in captured animals (Frick et al., 2010), survival is conditioned by their physiological recovery. Thus, it is mandatory to study physiological recovery for each captured species. The main goal of the present study is to evaluate survival and recovery capacity of two commercially relevant elasmobranch species in the South of Europe after bottom trawling: *Raja clavata* and *Leucoraja naevus*.

Material and methods

To achieve these goals, two experiments were performed aboard an oceanographic vessel in the Gulf of Cadiz (SW Spain). To evaluate survival rates, animals were randomly selected from each haul. These animals were measured, weighed, sexed and kept in recovery tanks in a flow-throw system. Along a 48 h time course, survival was evaluated. This experiment was carried out in several hauls for each species. A second experiment was performed in order to study the physiological recovery capacity of these animals in its plasma and skin mucus. Thus, plasma and dermal mucus samples were collected at time 0 h (immediately after the fishing process) and after 24 and 48 h recovery in onboard tanks. These samples were immediately frozen. The analysis of collected tissues included stress biomarkers related to energy management such as lactate. Lactate is an anaerobic metabolic product of glycolysis in high energy expenditure situations, increasing its concentration in the blood after episodes of acute stress, and returning to basal levels after physiological recovery of the animal (Skomal, 2007).

Results and discussion

High survival rates were found for the two species. *Raja clavata* showed a survival of 73 ± 16 % and *Leucoraja naevus* of 81 ±17 % (Mean ± SEM).

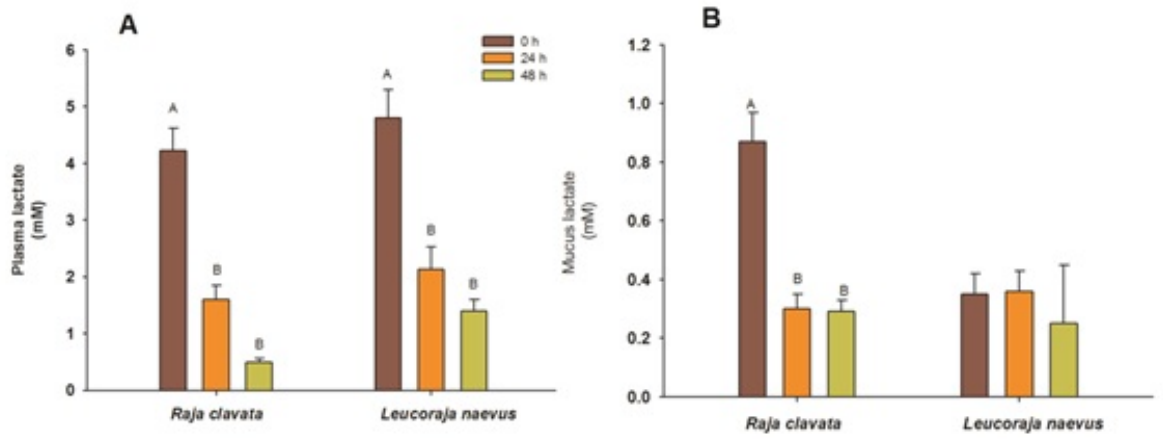

Figure 1: A Plasma lactate in *Raja clavata* and *Leucoraja naevus*. B Mucus lactate in each species. Different letters indicate significant differences along time for each species (one-way ANOVA, Tukey *post-hoc* test, $p < 0.05$).

Results from the recovery experiment (Figure 1) highlighted lactate as the main stress biomarker in these animals under these conditions. Significantly higher plasma lactate concentrations were observed just after bottom trawling in both species. However, these values decreased after 24 and 48 recovery h. In *R. clavata*, mucus lactate concentration (Figure 1) showed a similar trend than in the plasma; *L. naevus* evidenced no major changes in mucus lactate along the experiment. In conclusion, it has been observed that *R. clavata* and *L. naevus*, captured by bottom trawling in the Gulf of Cadiz, show high survival rates. The results obtained herein may pave the way to standardize survival methodologies that will include physiological recovery of captures as a new approach to improve fisheries and ecosystem management.

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In vitro protein hydrolysis of *Ulva compressa* and *Ulva rigida* by the digestive enzymes of turbot (*Psetta maxima*)

Alba Galafat Díaz*[1] , Antonio Jesús Vizcaíno Torres[1] , María Isabel Sáez Casado[1] , Cristina Rodríguez Rodríguez[2] , Alma Hernández de Rojas[3] , Xabier Moreno-Ventas[4] , Nina Larissa Arroyo[5] , Tomás Francisco Martínez Moya[1] , Francisco Javier Alarcón López[1]

*Corresponding author: albagalafat@gmail.com

[1] Departamento de Biología y Geología, Escuela Superior de Ingeniería, Universidad de Almería, La Cañada de San Urbano, s/n 04120, Almería, España

[2] Instituto Español de Oceanografía, C.O. de Santander, Planta de Cultivos Marinos, Bº Carbonera, s/n 39012, Monte, Santander, Cantabria, España

[3] Instituto Español de Oceanografía, C.O. de Gijón, Avda. Príncipe de Asturias 70bis, 33212 Gijón, Asturias, España.

[4] Universidad de Cantabria. Dpto. de Ciencias y Técnicas del Agua y del Medio Ambiente. Avda. Los Castros, 44, 39005 Santander, Cantabria, España

[5] Investalga AHTI SL. Centro de Desarrollo Tecnológico de la Univerisdad de Cantabria. Avda. Los Castros, 44, 39005, Santander, Cantabria, España.

Keywords: Macroalgae, Turbot, In vitro evaluation, Protein hydrolysis, Digestive enzymes

Abstract

During the last decade, macroalgae arise as interesting candidates to be used as additives in aquafeeds owing to their chemical composition and the presence of biologically active compounds like pigments, polysaccharides and vitamins, that can exert beneficial effects on fish. Several works have shown that the use of macroalgae at low inclusion levels produces a positive effect on growth and nutrients utilization, as well as an improvement in carcass quality, intestinal microbiota, disease resistance and stress response (Magnoni et al., 2017; Moutinho et al., 2018). However, beyond chemical composition, nutrient bioavailability is an important factor defining the nutritional quality of novel feed ingredients, although, there is little information related to the way digestive enzymes hydrolyses the algal protein.

Taking into account the above consideration, the main objective of the present study was to assess the chemical characterization of two macroalgae species, *Ulva compressa* and *Ulva rigida*, and the in vitro evaluation of protein hydrolysis of these macroalgae by turbot digestive enzymes (*Psetta maxima*).

Freeze dried biomasses of *U. compressa* and *U. rigida* were provided by Instituto Español de Oceanografía. Characterization of macroalgal biomasses was carried out by means of the crude protein, total lipids and ash analysis, using standardized methods. In addition, bioavailability of protein and carbohydrates from macroalgae was determined using an in vitro methods as described in Alarcón et al. (2001). Throughout the course of the in vitro hydrolysis of macroalgae by turbot digestive enzymes, samples were withdrawn at different time intervals in order to evaluate the degree of hydrolysis expressed as Coefficient of Protein Degradation (CPD), and the total amino acids released (Church et al., 1983). In addition, reducing sugars released was also quantified (Miller, 1959).

The protein content in both *Ulva* species was about 25%, and the total lipids was around 2.5% of dry biomass. Regarding ash content, *U. compressa* was 30%, while *U. rigida* showed around 26% (Table 1).

Table 1: Values are mean ± SD of triplicate determination. Values in the same row with different lowercase letter indicate significant difference ($p < 0.05$)

	U. compressa	*U. rigida*	*p*
Crude protein (%, dm)	21.37 ± 17.54	27.62 ± 8.78	*0.3160*
Total lipid (%, dm)	2.54 ± 0.07	2.62 ± 0.06	*0.3310*
Ash	32.97 ± 0.77^{b}	26.41 ± 0.52^{a}	*0.0097*

Proteinogram of *U. compressa* showed a complex protein profile made up of several fractions with a wide range of molecular weight (Figure 1). In general, proteins of these macroalgae species were hydrolysed by turbot proteases, showing a progressive reduction in optical density after 120 min of reaction. On the other hand, proteinogram of *U. rigida* revealed only two protein bands that barely showed hydrolysis after 120 min of assay (Figure 1). In general, CPD values were higher in *U. compressa* (49%) compared to *U. rigida* (21%) (Figure 1). Overall, turbot proteases release around 20 g amino acids 100g protein-1 after 90 min of in vitro hydrolysis, being values lightly higher for *U. compressa* (Figure 2). Finally, values obtained after quantification of reducing sugars revealed a small release throughout the whole in vitro hydrolysis assay, which seems to reflect a scarce digestion of the macroalgae cell wall. All this results suggest that protein of *U. compressa* and *U. rigida* can be hydrolysed by turbot digestive proteases.

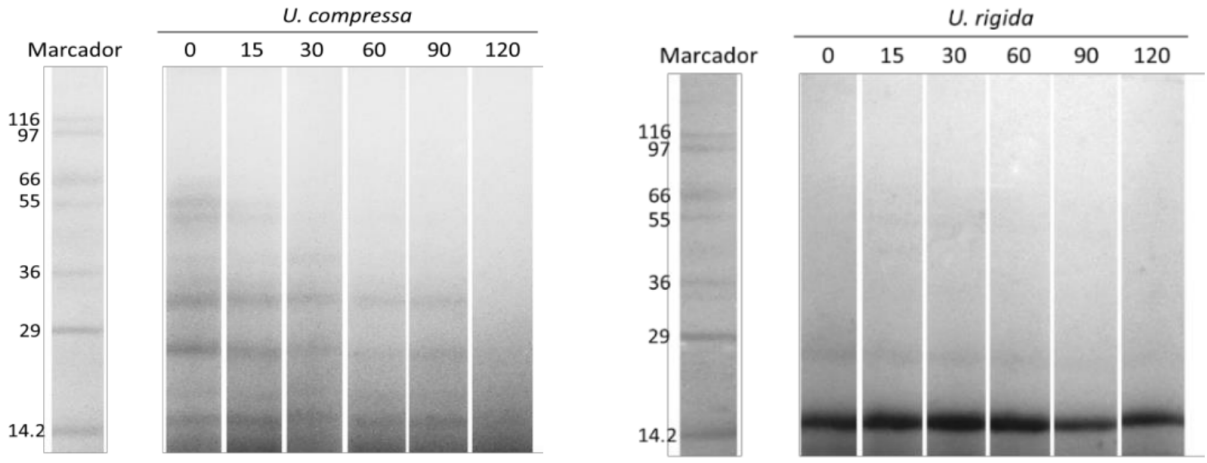

Figure 1: Protein pattern in SDS-PAGE of *U. compressa* and *U. rigida* (units of molecular weight: kDa).

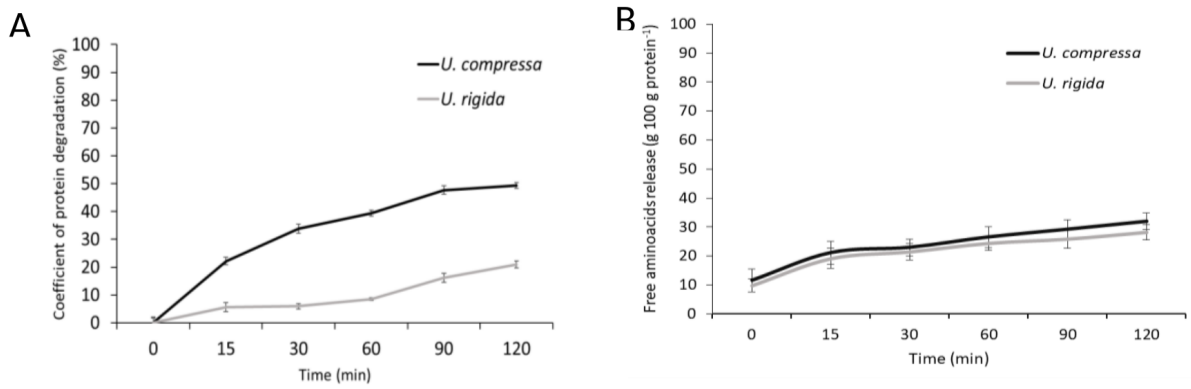

Figure 2: A) Changes in the optical density (OD) (measured as pixels per cm^2) of the main protein fractions throughout the enzymatic in vitro hydrolysis. B) Concentration of free amino acids released (g 100 g $protein^{-1}$) during the in vitro proteolysis.

Acknowledgments

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Morphometric differentiation between juveniles of bluefin tuna and little tunny caught in Western Mediterranean Sea

Jairo Castro-Gutiérrez*[1] , Sámar Saber[2] , David Macías[2] , María José Gómez-Vives[2] , Matxalen Pauly[3] , Josetxu Ortiz de Urbina[2]

*Corresponding author: jairocastrogtz@gmail.com
[1] Universidad de Málaga, Departamento de Biología Animal, 29071 Málaga.
[2] Instituto Español de Oceanografía, Centro Oceanográfico de Málaga, Puerto Pesquero, s/n, 29640 Fuengirola, Málaga.
[3] IPD, Investigación Planificación y Desarrollo, S.A.

Keywords: Morphometric characters, Taxonomy, *Thunnus thynnus*, *Euthynnus alletteratus*, Juveniles

Introduction

Bluefin tuna (*Thunnus thynnus*, L. 1758) and little tunny (*Euthynnus alletteratus*, Rafinesque 1810) are highly migratory species that inhabit Atlantic Ocean and Mediterranean Sea (Collette and Nauen, 1983). The Mediterranean Sea is a spawning area for both species (Alemany et al., 2010; Oray and Karakulak, 2005; Rodríguez-Roda, 1964). Juveniles of tuna species can be found in mixed schools and catches. The very young juveniles of bluefin tuna and little tunny are difficult to differentiate. Differentiation of species is essential for biology, fishery management and conservation studies. Genetics is an accurate method to discriminate species but is expensive, time consuming and results are not immediate. The objective of this study is to identify morphometric characters to discriminate between juvenile of bluefin tuna and little tunny.

Material and methods

Juvenile of bluefin tuna and little tunny were caught between August and September from 2016 to 2018 from four localities throughout Western Mediterranean Sea. A total of 142 bluefin tuna (ranged from 121 to 357 mm) and 101 little tunny (ranged from 85 to 315 mm) were analysed (Table 1). The individuals were caught as bycatch by purse seine targeting small pelagic species (anchovy and sardine) and sport fishery. Juvenile of bluefin tuna (smaller than the minimum landing size) were collected under the provision of the International Commission of the Atlantic Tunas (ICCAT) Atlantic Wide Research Program for Bluefin Tuna (GBYP).

Table 1: Bluefin juvenile and little tunny catches used in this study.

Species	Area	N	Years	FL range (mm)
Bluefin tuna	Málaga	21	2017	147 – 269
	Almería	7	2017	195 – 288
	Valencia	33	2018	224 – 303
	Tarragona	81	2017 – 2018	121 – 357
	Total	**142**		
Little tunny	Tarragona	81	2016 – 2018	85 – 196
	Valencia	20	2018	214 – 315
	Total	**101**		

The specimens kept frozen at -20°C until further analysis in the laboratory, except ten specimens of bluefin tuna that were analysed in fresh. For each individual fork length (FL) was measured with an ichtyometer to the nearest mm or a caliper to the nearest 0.01 mm. The excess of water was eliminated with a piece of paper and then, body weight (BW) was measured to the nearest 0.01 g (± 0.01). Thirteen morphometric measurements were recorded: (1) Fork length, FL; (2) Snout length, SL; (3) Eye diameter, ED; (4) Post-orbital length, PO; (5) Head length, LH; (6) First pre-drosal length, LD1; (7) Maximum body height, H; (8) Pectoral fin length, LP ; (9) Second dorsal height, HD2; (10) Anal fin length, LA; (11) Caudal fin height, CC; (12) Dorsal fins distance, FD; (13) Mouth cleft length, MCL. All measurements were taken on the left side by consensus. A digital caliper was used to measure the morphometric variables (2-13) to the nearest 0.01 millimeter (± 0.01). A non-parametric Kruskal-Wallis Test was performed to determine if there are statistically significant differences between the morphometric variables. To identify the variables that can explain the differences between populations the principal component analysis (PCA) was used. Barlett's Sphericity test and Kaiser-Meyer-Olkin test was performed to check the factorization validity conditions. All statistical analyses was performed with R statistical software with a significance level of α= 0.05.

Results

The variable "caudal fin height" was discarded of the statistical analysis because the objectivity of the measure was conditioned by the fin shape after defrosting. Samples with at least one not-registered variable were discarded. Then a total of 127 bluefin tuna and 82 little tunny were analysed. The variables were scaled to the fork length or to the head length to remove any possible size effect.

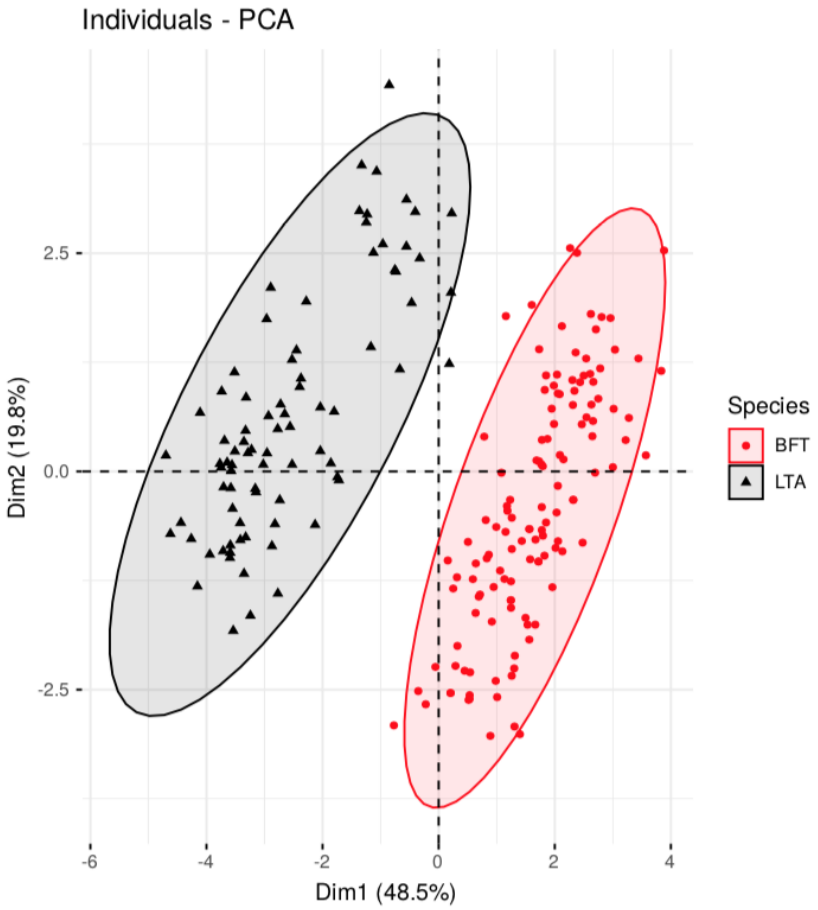

Figure 1: Fig. 1

Kaiser-Meyer-Olkin (KMO) adequacy value (KMO = 0.866) indicate that the sampling is adequate for Factor Analysis (KMO values between 0.8 and 1 indicate the sampling is adequate). Bartlett's sphericity tests the hypothesis that the correlation matrix is an identity matrix, which would indicate that the variables are unrelated and therefore unsuitable for structure detection. Barlett's sphericity test (χ^2 = 1696,96; p < 0.001), provided a correlation value between the variables sufficient to apply the factor analysis. The number of principal components in the PCA is based on: a) Eigenvaluees and/or cumulative variance quantity explained and b) based on a scree plot (Peres-Neto et al., 2005). The PCA showed that the first five principal components account for 88.04% of the total variance of the original thirteen morphological variables (48.5% for PC1, 19.8% for PC2, 9.2% for PC3, 6% for PC4 and 4.5% for PC5). The variables included in the first component were: H, HD2, LD1, LH, LP, MCL, and SL. The variables included in the second component were: ED, PO, FD and LA. The position of individuals in the principal components are represented in Figure 1.

Acknowledgments

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Macroalgae as an alternative source of fish feed: Biochemical composition and antimicrobial activity

Jorge García-Márquez*[1] , Julia Vega[1] , Félix L. Figueroa[1] , Salvador Arijo[1]

*Corresponding author: j.garciamq@gmail.com
[1] University of Málaga

Keywords: Aquaculture, Antimicrobial activity, Macroalgae, Toxicity

Introduction

Intensive aquaculture usually makes the cultivated specimens vulnerable to infectious diseases, some of them produced by *Vibrionaceae* species, causing economic losses in the sector. In addition, the pathogenic diseases are treated by an abusive use of antibiotics, inducing a negative impact on the environment and generating antibiotic-resistant pathogens (Buschmann, 2001). Macroalgae are considered a sustainable alternative to include in fish feed, due to its high concentration of bioactive compounds with antioxidant, immuno-stimulant and/or antimicrobial activities. These beneficial capacities are not attributing to a specific molecule but to a pool of them. On the other hand, macroalgae can also be an option to reduce the use of flours and fish oil in fish feed. Before to include any substance in fish feed, it is necessary to determine any potential toxic effect on fish. The acute toxicity can be measure easily through the Microtox test. This test is based on the sensibility of *Vibrio fischeri* bioluminescence to toxics, measuring changes in the light output following exposure to the samples. If the substance tested has an acute toxic effect, the bioluminescence decreases in few minutes. The aim of this research was the evaluation of several macroalgae as potential additive for fish feed. For that, the biochemical composition (proteins, lipids and carbohydrates), the antimicrobial activity against three fish pathogens and the potential production of acute toxicity of the algae were evaluated.

Materials and methods

Seven different macroalgae (one Ochrophyta, five Rhodophyta and one Chlorophyta) collected from different areas of Malaga and Cadiz coasts were used in this study. For the biochemical composition, total proteins were calculated from the elemental N determination using the N-protein conversion factor (Lourenço et al., 2002), lipids through the sulpho-phospho-vainillin method (Mishra et al., 2014) and carbohydrates according to the phenol-sulfuric procedure (Dubois et al., 1956). The antimicrobial activity of the algae extracted in two different solvents (Distilled water and ethanol 50°) was tested by the agar well diffusion method (Rattanachuay et al., 2010). A suspension of *Photobacterium damselae* subsp. *piscicida* Lg41.01, *Vibrio harveyi* Lg16.00 or *V. alginolyticus* was swabbed over the surface of tryptic soy agar medium, supplemented with 2% NaCl (TSAs). Several wells of 5 mm of diameter were made on the agar surface. The extracts were inoculated into the agar wells and the plates were incubated at 22 °C for 24 h. After the incubation, the inhibition area was recorded. The acute toxicity of the extracts was analyzed by the Microtox test. In this test, a suspension of *Vibrio fischeri* was inoculated to different concentration of the algae extracts. After 15 min incubation, the luminiscence was recorded. The percentage reduction in bioluminescence was measured relative to a control (without extract) to determinate the concentration of algae extract inhibiting 50% of luniniscence (EC50).

Results and discussion

Macroalgae are usually rich in proteins and carbohydrates, but poor in lipid content (Figure 1). The protein content varies between 8 and 16%. The highest content was observed in *P. umbilicalis* and the lowest in *C. cornea*. The concentration of carbohydrates was highest in *U. faciata* and *P. umbilicalis* (12 and 10%, respectively) and the lowest content appeared in *S. vulgare* (only 2.5%). The highest lipid content appeared in *S. vulgare* and *U. fasciata* (2.3 and 1.3%, respectively).

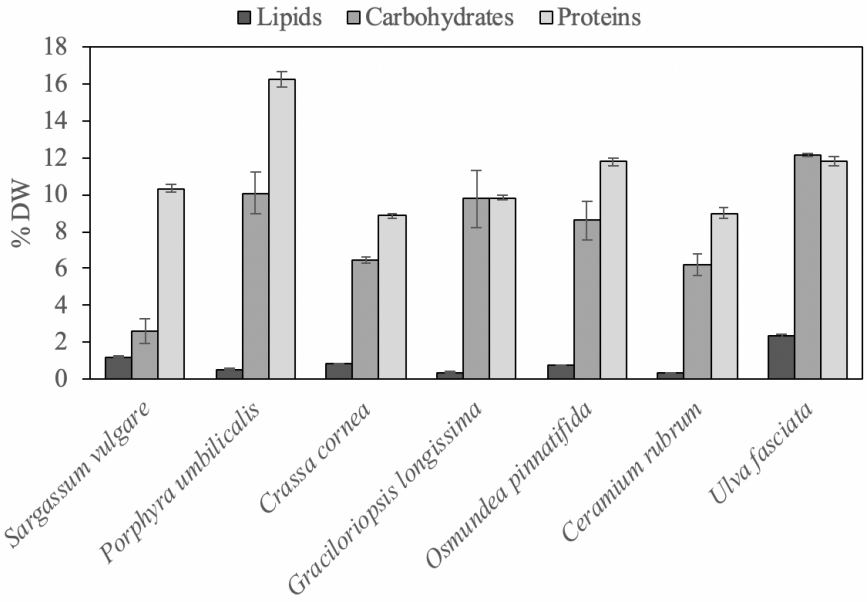

Figure 1: Biochemical composition of the different macroalgae expressed as percentage of dry weight (DW) and showed as average ± standard deviation.

In relation to the antimicrobial activity, only five of the seven species inhibited the growth of more than one bacteria (Table 1). *S. vulgare* and *U. fasciata* extracts were not able to inhibit any of the pathogens tested, and *P. umbilicalis* only inhibited *V. alginolyticus* when extracted in ethanol 50°. *V. harveyi* and *V. alginolyticus* were inhibited with the ethanol 50° extract of *C. cornea*, *G. longissima*, *O. pinnatifida* and *C. rubrum*. As for the distilled water extraction, only *C. rubrum* inhibited *P. damselae* subsp. *piscicida*.

Table 1: Antimicrobial activity against three fish pathogens of the algae extracts as measured by agar well diffusion method. (+): Presence of zone of inhibition. (-): Absence of zone of inhibition.

Macroalgae	Solvent	*V. harveyi*	*V. alginoliticus*	*P. damselae*
Sargasum vulgare	H_2O	-	-	-
	Ethanol 50º	-	-	-
Porphyra umbilicalis	H_2O	-	-	-
	Ethanol 50º	-	+	-
Crassa cornea	H_2O	-	-	-
	Ethanol 50º	+	+	-
Graciloriopsis longissima	H_2O	-	-	-
	Ethanol 50º	+	+	-
Osmundea pinnatifida	H_2O	-	-	-
	Ethanol 50º	+	+	+
Ceramium rubrum	H_2O	-	-	+
	Ethanol 50º	+	+	-
Ulva fasciata	H_2O	-	-	-
	Ethanol 50º	-	-	-

The selection of the macroalgae to test their acute toxicity was based on which of them inhibited more than one pathogen. The potential toxicity of *C. cornea*, *G. longissima*, *O. pinnatifida* and *C. rubrum* in ethanol 50° extract was calculated by the observation of the decrease of the bioluminescence of *Vibrio fischeri*. Only in two species (*C. cornea* and *O. pinnatifida*) the resultant luminescence diminished, being *O. pinnatifida* the most toxic substance, with an effective concentration 50 (EC50) of 0.13 mg mL^{-1}. In *C. cornea*, an EC50 of 11.14 mg mL^{-1} was obtained. *G. longissima* and *C. rubrum* extracts even showed an increase in luminescence compared to the controls. This effect is compatible with a hormesis effect. In conclusion, the high content in proteins and carbohydrates of some macroalgae can allow the substitution of part of fish oil and flours in feed. The results of the in vitro assays demonstrated that some of the algae tested are capable to inhibit the growth of some fish pathogens. However their potential toxic effect has to be considered before adding this extract to the fish feed. In vivo trials will be necessary in order to prove if the antimicrobial potential of the algae is maintained without generating adverse effects on the fish.

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Genome and Phylogenetic Analysis of Genes belonging to the Immune System of the flatfish *Solea senegalensis*.

Aglaya Garcia-Angulo*[1] , Manuel A. Merlo[1] , María E. Rodríguez[1] , Silvia Portela-Bens[1] , Emilio García[1] , Alberto Arias-Pérez[1] , Ismael Cross[1] , Thomas Liehr[2] , Laureana Rebordinos[1]

*Corresponding author: aglaya.garcia@uca.es
[1] Área de Genética, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz
[2] Institute of Human Genetics, Jena University Hospital, Friedrich Schiller University Jena

Keywords: Solea senegalensis, Bacterial artificial chromosome (BAC), Immune system

Introduction

Solea senegalensis is a flatfish species belonging to the Pleuronectiformes order. In the last 10 years, the production of *S. senegalensis* in Spain has increased from 32 to 747.15 Tn, which illustrates the rapid growth of interest in production of the species (FAO) . Several studies have been carried out to improve the production of the Senegalese sole. One of the key points to improve the production of the species farmed in aquaculture is the knowledge of genes involved in the immune system. In this work, a cytogenetic mapping, micro-synteny analysis and comparative mapping of 14 BAC clones containing immune-related genes (*hmgb2*, *trim25*, *irf5*, *b2m*, *tlr3*, *tlr8*, *lysg*, *tnfα*, *tap1*, *il10*, *mx*, *calr*, *trim16*) have been realized between *S. senegalensis* and farmed relevant species (*S. maximus*, *O. niloticus*, *C. semilaevis*) and reference species (*G. aculeatus* and *D. rerio*). Moreover phylogenetic analysis has been realized among different species of mammals, reptiles and fish using five concatenated immune genes.

Results

The 14 BAC clones were localized on six different chromosome pairs using the multiple fluorescence in situ hybridization technique (Liehr, 2009). The comparative mapping analysis showed *C. semilaevis* is the closest species to senegalese sole (Figure 1) and *D. rerio* the species with the greatest number of genomic rearrangements.

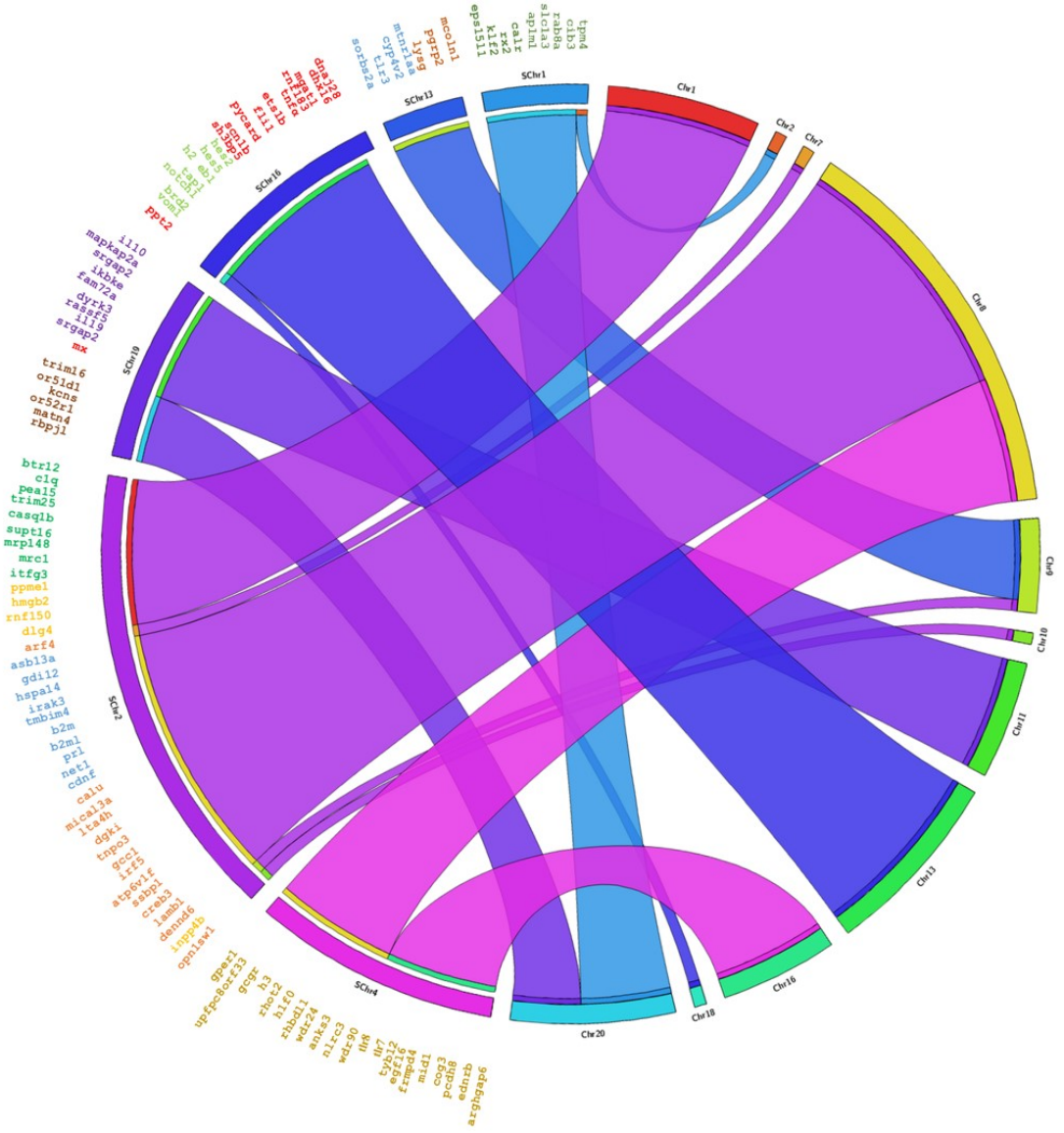

Figure 1: Circos analysis in the species *C. semilaevis*. On the left side the distribution of the BAC clones of Senegalese sole distributed in chromosomes can be observed. The crossing lines show their corresponding localization in the *C. semilaevis* chromosomes.

The analysis of micro-synteny supported these results and showed that many candidate genes have conserved the genomic surrounding, especially the genes *il10*, *tlr3*, *tlr8*, *nlrc3* and *calr*. The phylogeny analysis clearly separated two main clusters: fishes and tetrapods (Figure 2). Within the group of teleosts fishes, *S. senegalensis* appeared included in a subgroup together with the flatfish species *C. semilaevis*, *P. olivaceus* and *S. maximus*.

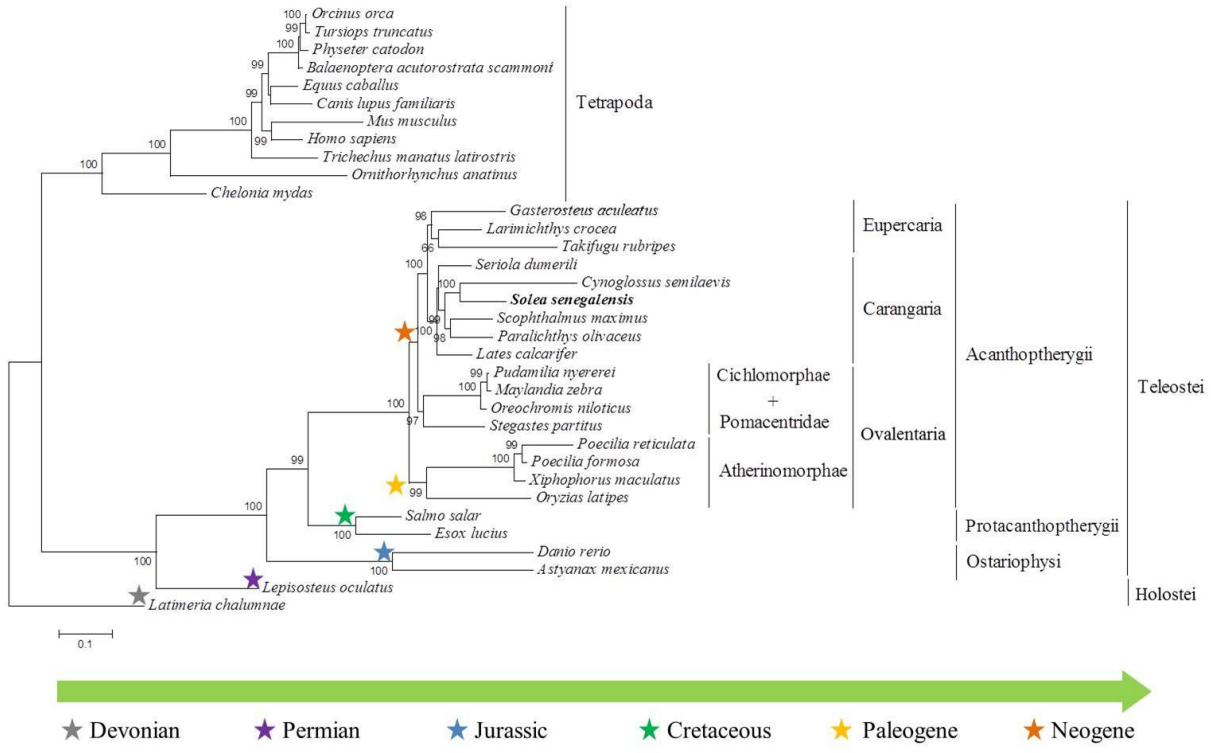

Figure 2: Phylogenetic tree constructed from five immune-system genes (*tlr3*, *tlr8*, *nlrc3*, *calr*, *ikbke*) of different species.

Conclusion

From these results we obtained three main conclusions, the immune system genes studied tend to be grouped together in the genome of *S. senegalensis* which could represent an evolutionary advantage. Large parts of the genomic regions analysed tend to be conserved in the species most closely related to *S. senegalensis*, and the rate of variability of the immune system genes studied is not very high.

Acknowledgments

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Study of the intestinal microbiota of *Solea senegalensis* specimens after the administration of the probiotic *Shewanella putrefaciens* SpPdp11 by Next Generation Sequencing

Marta Domínguez-Maqueda*[1] , Silvana Teresa Tapia-Paniagua[1] , Inés García de la Banda[2] , María del Carmen Balebona Accino[1] , Miguel Ángel Moriñigo Gutiérrez[1]

*Corresponding author: martadm@uma.es
[1] Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga
[2] Instituto Español de Ocenanografía

Keywords: Aquaculture, Probiotic, Microbiota, *Solea senegalensis*, *Shewanella putrefaciens*

Introduction

Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (Araya et al., 2002). The use of probiotics is a key tool to protect farmed fish, in many cases predisposed to stress and/or infection under intensive culture conditions. In this way, *Shewanella putrefaciens* Ppd11 (SpPdp11) is a microorganism applied to farmed fish such as *Solea senegalensis* and *Sparus aurata* that has demonstrated probiotic effect, promoting the growth and a better efficiency of feed utilization, stimulating the immune system of *S. senegalensis* and *S. aurata*, and the stress tolerance of *S. senegalensis* specimens to high stocking densities (Tapia-Paniagua et al., 2014). In addition, its capability to modulate the intestinal microbiota of these farmed fish has also been demonstrated using Denaturing Gradient Gel Electrophoresis (DGGE). At present, the Next Generation Sequencing (NGS) methodology is a better and more sensitive way to evaluate the composition of the microbiota and to analyze the effects on it of different factors, such as the dietary supplementation with a probiotic. In this context, this is the first time that the effect of the probiotic on the intestinal microbiota of *S. senegalensis* is analyzed using the NGS methodology.

Materials and methods

SpPdp11 cells were cultured following the methodology previously described by Tapia-Paniagua et al. (2014). The commercial pellet diet LE Europa GR2 (16% total lipids and 57% crude protein, Skretting, Spain) was used as control (diet C). The same diet was supplemented with SpPdp11 cells following the methodology described by Vidal et al. (2016) (diet AP). Specimens farmed *S. senegalese* juveniles (30 ± 5 mean weight) from the Spanish Institute of Oceanography (Santander, Spain) were acclimated for 2 weeks prior to the experimental period. Then, fish were randomly distributed in two tanks by diet. The weight of the fish was measured at 0, 15, 30, and 45 days of feeding. Fish from each group were fed 8 times a day for 45 days with the corresponding diet. Three fish of each tank were sacrificed and whole intestines were obtained. Fragments of 0.5 cm of the anterior and posterior intestine were collected and stored at -80 C for intestinal microbiota analysis. DNA extraction was carried out following the methodology previously described by Tapia-Paniagua et al. (2010). DNA were sequencing by Chunlab Inc. (Seoul, South Korea). Bioinformatic flow was generated by the open source software package MOTHUR (version 1.3), reads were analysed by Greengenes (version 2013) and statistical analysis were analysed by R

Software and open source software online Microbiome Analyst after all random subsampling was conducted to normalize the data size to 7200 reads.

Results and discussion

In comparison with the fish fed with the control diet, the growth was higher in fish fed with AP diet at 15 and 30 days and significantly higher at 45 days (Figure 1).

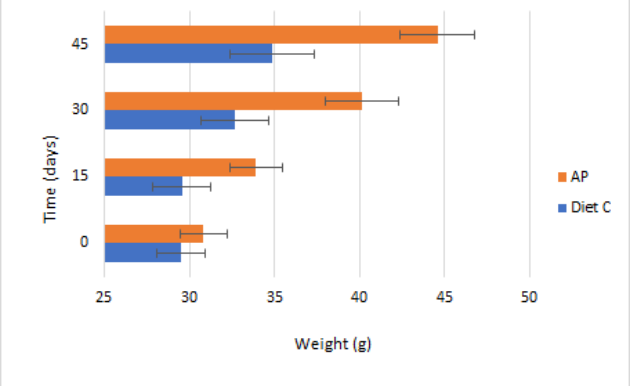

Figure 1: Weight rate (g) of *S. Senegalensis* receiving during 45 days the control diet (Diet C) and the probiotic diet (Diet AP).

In total, 319174 raw reads were obtained for both forward and reverse directions after sequencing. The mean read depth per sample was 26597 - 3223,6 (mean - SD) sequences per red direction. Singletones were removed and a total of 599 OTUs at 97% gene similarity cut off were obtained.

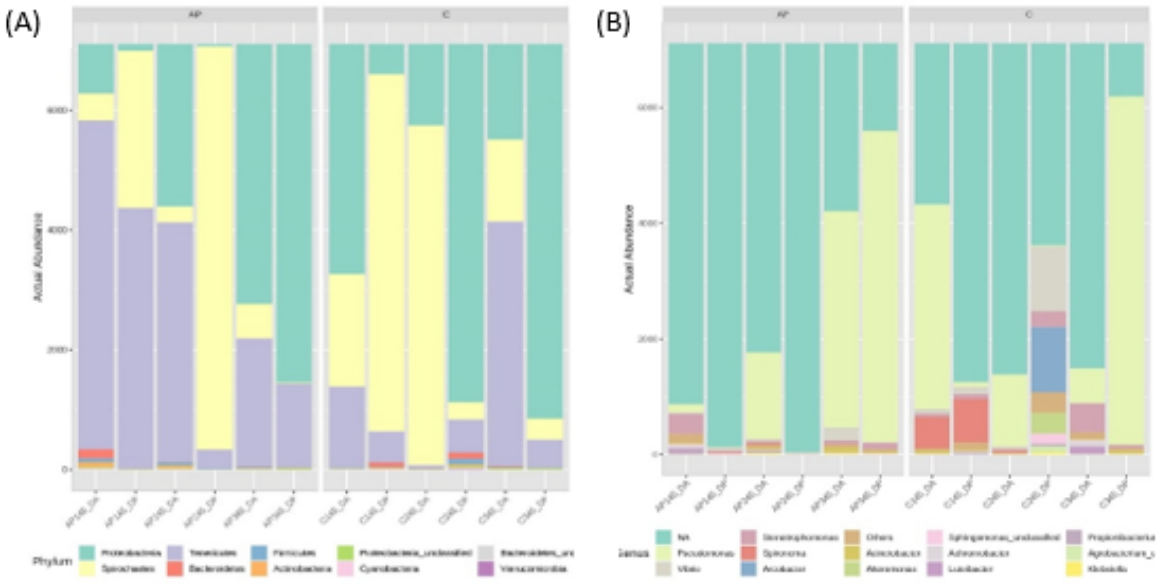

Figure 2: Composition of intestinal microbiota at phylum (A) and genus (B) level in the anterior (DA) and posterior (DP) intestine of specimens of *Solea senegalensis* fed a control diet (diet C) and the probiotic diet (diet AP).

Results from taxonomical analysis showed that C and AP diets had a similar composition of intestinal microbiota. The most representative phyla were Proteobacteria, Tenericutes and Spirochaetes. AP diet increased abundance of Tenericutes in constrast with Spirochaetes (Figure 2). At genus level, results showed a considerable presence of *Pseudomonas* in both diets and fragments of intestine. Others representative genus were *Propionibacterium*, *Spironema*, *Stenotrophomonas*, *Vibrio*, *Arcobacter* and *Achromobacter*. It seems to be a higher presence of *Spironema* in C diet than AP diet (Figure 2). This study present Next generation sequencing (NGS) for studying the microbiota, so we can observe microbial variability between treatments, even non cultivable microorganisms or very poorly represented microorganisms. In general, genera observed, such as *Stenotrophomonas*, *Vibrio* and *Spironema* have been previously reported as intestinal predominant in *S. senegalensis* (Tapia-Paniagua et al., 2014). *Pseudomonas* have been described because of interacting positively with epithelial cells in the intestinal mucosa and exerting an important role like antagonist in salmonids. A positive feature is the presence of *Propionibacterium* which species seems to reduce the antinutritional effects of lectins and exert anti-inflammatory properties in mixtures with species of *Lactobacillus*. In addition, the administration of *S. putrefaciens* like symbiotic with sodium alginate confer a form of synergism, enhancing beneficial effects of the probiotic and a better growth of fish due to an improvement on feed utilization.

Acknowledgments

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Phaeodactylum tricornutum culture: lipid productivity

Cristina Pérez de Mora*[1] , Nathalie Korbee Peinado[1]

*Corresponding author: Cristiprzdm@gmail.com
[1] Departamento de Ecología. Facultad de Ciencias. Universidad de Málaga. Campus Universitario de Teatinos s/n 29071 Málaga - Spain

Keywords: Fatty acids, Fluorescense, Lipids, Phaeodactylum Tricornutum, Protein

Introduction

The continuous population growth that has taken place in the last decade has caused that in addition to uncontrolled increase in the use of food resources, the management of them is being done inappropriately. The combination of population growth and poor management has resulted in different industries having to look for alternatives to supply an ever-increasing population. One of these alternatives has been to look for new sources of effective supply, capable of supplementing a scarce basic diet in some functional groups such as omega 3 and omega 6 PUFAs. This source of supply has been found among others, in marine bacteria, in microalgae and as in the present study, in diatoms, which due to their high adaptive capacity, the speed of growth, their lipid and protein composition, are a source of interest for the production of many commercial products, from food for humans as in agriculture, aquaculture, biotechnology or medicine (Venezuela and Venezuela, 2015) In this sense, the study of diatoms under different light qualities is important, because it will provide basic knowledge on the behavior of different species, to achieve efficient, clean and renewable production. The objective of the present study was to evaluate the photosynthetic activity of photosystem II, the lipid and protein production of *Phaeodactylum tricornutum* subjected to different light qualities

Materials and methods

P.tricornutum, species with which we work is a diatom formed by a cell wall that surrounds the entire cell, usually composed of silica called frustula. This diatom is not very abundant in the environment, are found mainly in unstable environments such as estuaries and intertidal zones. In these environments, salinity and temperature can change rapidly as a result of the effects of tides and solar radiation. The ability to acclimatize to these fluctuations is related to the pleomorphic character of the cells of *P.tricornutum*. The medium supplied throughout the process for the development and maintenance of *P.tricornutum* culture was medium F2. It is a synthetically enriched seawater medium designed primarily as a source of nutrients for coastal algae, especially for diatoms. Biomass for cultivation and preacclimatization was obtained in an incubation chamber with a controlled ambient temperature that remained constant, around 22 C. The cultures were illuminated with LED fluorescent lamps (Phillips TLD 36W54-765), using a 16h photoperiod: 8h darkness. For the experimental design, 9 transparent erlenmeyers were inoculated (to favor a much more homogeneous distribution of radiation) at an initial cell concentration of 5,400,000 cells/mL and carried with F2 medium up to a volume of 800 ml. The cultures were subjected to three different light treatments. Firstly, a control treatment with three replicates was established, with a white light intensity of around 40 micromols m^{-2} s^{-1}. For the second treatment, the same white light was used as the base light and was supplemented with

red LED lights with a luminous intensity of 14 micromol m^{-2} s^{-1}. Finally the third treatment was supplemented with blue LED lights, in addition to the white light used as a base light. All three treatments were carried out in triplicate. To determine the dry weight, fresh weight ratio, a total of 10 different volumes was taken, around 300-350 mL, the total amount of cells in that volume was calculated, lyophilized to obtain a dry weight expressed in mg. This same amount was divided by the amount of total cells resulting in an amount X of Ps (micrograms)/million cells. The photosynthetic yield (II) was evaluated through the photosynthetic capacity of *P.tricornutum* through an indirect measurement system, with a modulated wave amplitude known as Junior PAM. The samples were incubated in darkness for 15 minutes, once adapted to darkness was determined by the Fv/Fm ratio, being Fv a variable fluorescence defined as the difference between the maximum fluorescence level and the minimum fluorescence level F0. The total amount of carbon and nitrogen was determined through dry algal biomass, according to the manual (CNH Perkin-Elmer 2400 (USA)). The values of carbon and nitrogen were expressed in (mg/g (Ps)). For the determination of total lipids the technique described by (Folch et al.(1957) for the determination of total lipids was carried out, applying to 3 different treatments with a total of 3 replicates per treatment. The obtaining of proteins was made by means of the application of the method of (Louren¸co et al.2002), multiplying the Mg/g/(Ps) of nitrogen by the factor F, (F= 6,25). Finally, for the statistics, the average and the standard deviation were determined, in order to know if there are statistically significant differences, through the ANOVA program with Tukey's HSD test. For the elaboration of the figures, the Microsoft Office Excel program was used.

Discussion

The stimulation of microalgae metabolism is the main promoter of the synthesis of bioactive elements, of growth or of any beneficial activity by means of remote interaction between photosynthetic organisms. The effect of the different qualities of lights in *P.tricornutum*, supposed the necessary stimulation to quantify results of interest. This light treatment subjects the cells of *P.tricornutum* to unusual incubation processes, therefore through the measurement of fluorescence (PSII) it has been possible to collect information about the physiological state of the photosynthetic apparatus of *P.tricornutum*, being the response, indicator of damage or alteration in it. Differences in photosynthetic efficiencies were observed according to treatment considering that a decrease in the Fv/Fm ratio indicates a reduction in the photochemical efficiency of the PSII and a disturbance of the photosynthetic apparatus, the lowest results of Fv/Fm were found in the red light, while the highest were found in blue light. These values are associated with an excess of light, caused by the medium-long wavelength emitted by the red LED and that this excess energy acts as a limiting factor of carbon metabolism causing an increase in overexcitation in the photosynthetic apparatus.

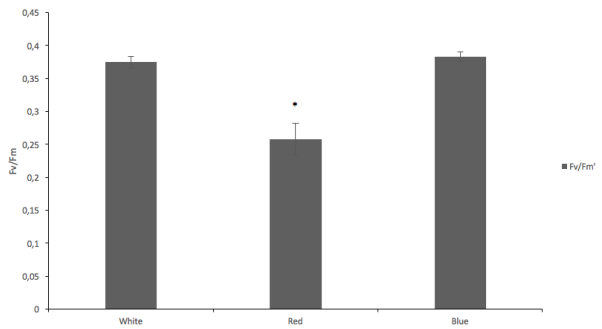

Figure 1: Photosynthetic performance of photosystem II; INITIAL VALUE: 0,402. * is located below the bar that has caused significance in each treatment.

Both carbon and nitrogen are essential elements of biological cycles, so the relationship between the proportionality of C and N in the metabolism of the cell is another variable that shows its physiological state. Some analysis results mention as in the case of *Chlorella sp.* that a ratio of C/N = 5 percent led to a limitation of the growth of the species. These high results may be due among their reasons to a possible period of photo-inhibition of photosynthesis, however the C/N ratio between 1 and 3 percent improved the growth potential of *Chlorella sp* (González-Delgado et al., 2017). This, contrasted with what was observed in the experiment (Figure 2) clarifies that in the case of *P.tricornutum* the percentages of C/N show similarities with the conditions of *Chlorella* since the values are between 1 and 2 percent of their weight.

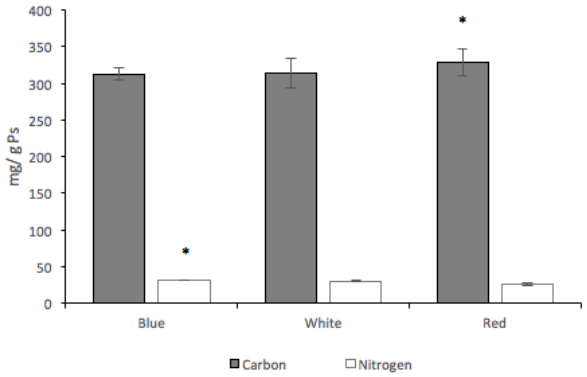

Figure 2: Internal content of mg/g (Ps) of C versus internal content mg/g (Ps) N expressed in mg/g (Ps) in dry weight for the biomass of *P.tricornutum* in the different light treatments; Initial value; C : 222,1 mg/g (Ps) and N: 162,3 mg/g (Ps). * is located under the bar that has given meaning to each treatment content of mg/g (Ps) of C versus internal content mg/g (Ps) N expressed in mg/g (Ps) in dry weight for the biomass of P.tricornutum in the different light treatments; Initial value; C : 222,1mg/g (Ps) and N: 162,3 mg/g (Ps). * is located under the bar that has given meaning to each treatment.

The internal concentration of nitrogen conditions whether or not there is greater protein synthesis. *P.tricornutum* although it showed a low protein productivity, if we refer to what we are experimenting in this study, the treatment of lights did influence protein productivity, being the highest values, determined in the control of white light where it was not subjected to *P.tricornutum* to any type of experiment, this being the most similar to natural light. Between the two remaining treatments, it was observed how the values suffered a notable increase, in the samples irradiated with blue light. This was due to the direct impact of the blue light wavelength on the membrane proteins, and the cellular demand required for the maintenance of the homeostatic processes and the photosynthetic process (Xue et al., 2005). Proteins are of special importance since they have structural and regulating function of enzymes and hormones (Venezuela and Venezuela, 2015). So a good concentration of these have various applications in the market. Primarily act as a dietary supplement, as numerous foods show a deficit at the protein level, this is often supplemented by proteins/amino acids extracted from species such as *P.tricornutum*, this supplementation is also applicable to the diets of many animal species. The internal carbon of *P.tricornutum* was favored by the limitation of N existing in the organism, so the amount of lipids existing in the cultures, was greater in those who were exposed to red light, where the limitation of nitrogen is large and tended to accumulate carbon in the membrane, followed by this the values obtained by the white light control were determined and finally the concentrations of lower total lipids were found in the cultures exposed to blue light. In this case the wavelength of the blue light for *P.tricornutum* did not contain enough energy to stimulate

lipid biosynthesis. Similarly it occurred in the trial with a culture of *Rhodomonas sp.* and *Tetraselmis sp.* where the accumulation of lipids was greater in inoculated treated with red light (Arica, 2015).

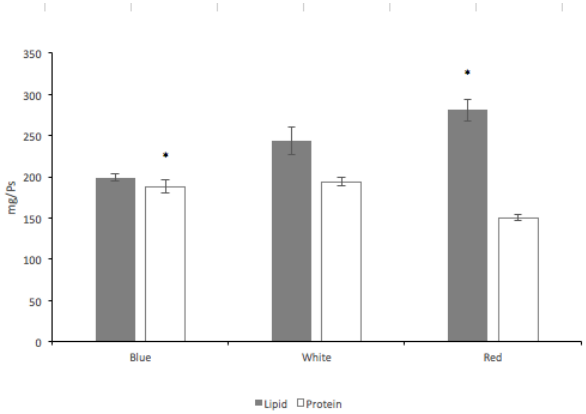

Figure 3: Concentration of Productivity of Lipids versus the concentration of the productivity of Proteins expressed in mg/g Ps for the biomass of *P.tricornutum*; INITIAL VALUE; Lipids: 161.3 mg/g (Ps) and Proteins: 100.4 mg/g (Ps). * is located below the bar that has caused significance in each treatment.

That the amount and proportion of fatty acids present in *Phaeodactylum* was not conditioned by the type of light may have been due to numerous environmental variables, such as an excess of light. However, there is evidence that light quality and intensity affect the polyunsaturation (Martínez Macías, 2017) of the fatty acids present in this diatom. It was observed improportions of the quantity found of monounsaturated acids (MUFAs) against polyunsaturated acids (PUFAs) being the latter much more abundant, contrasting this with what was observed in the study carried out by (Arica, 2015) where in the same way the quantity of polyunsaturated acids doubles that of monounsaturated, on the contrary the results expressed in the analysis. The results expressed in the analysis of *T. pseudonana* where the quantity of MUFAs was much higher than that of PUFAs. Xue et al. (2005) disprove in some way what was observed in this experiment, which leads us to think that it is not only the quality or luminous intensity the variable that has a main role in the polyunsaturation or monosaturation of fatty acids. The biocomposites analyzed do not only have the function of acting as a food supplement. One of the areas that has experienced the most growth of this type of research has been the aquaculture microalgae/diatoms are an important source of food and additives for fish offspring, rotifers, crustaceans... and so on (Martínez Macías, 2017). In addition, not only do they provide a food source for aquaculture, but the rationalised addition of proteins/lipids and so on to cultures improves the quality of larval and bivalve development.

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Effects of probiotic *Shewanella putrefaciens Pdp11* on *Solea senegalensis* infected with *Vibrio harveyi*

Isabel M. Cerezo*[1] , Silvana Tapia-Paniagua[1] , Jorge García-Márquez[1] , Rocio Piñera[2] , M. Angeles Esteban[2] , Miguel Angel Moriñigo[1]

*Corresponding author: b22ceori@uma.es
[1] Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga
[2] Departamento de Biología Celular e Histología, Campus de Espinardo s/n, Universidad de Murcia

Keywords: Solea senegalensis, Vibrio harveyi, *Pdp11*, Probiotic

Introduction

Spain was pioneer in the cultivation of Senegalese sole (*Solea senegalensis*), making this specie an alternative to traditional farming, thanks to acceptance by consumers, price and demand in our markets. Therefore, a study to improve the production of sole could provide business opportunities.
The use of *Shewanella putrefaciens Pdp11* on cultivated specimens of *Solea senegalensis* has been shown to have a marked probiotic effect, being involved in improvements on growth and immune response (Vidal et al., 2016). The gut microbiota has been considered as one of the most important factors influencing host metabolism, glucose and lipid metabolism, fat distribution and growth performance. The ability of probiotics to colonize the intestine changes the intestinal microbiota and therefore the host's metabolism (Falcinelli et al., 2015).
Vibriosis has become the economically most important disease in marine fish culture, affecting a large number of species. It is a disease caused by pathogenic *Vibrio*, which causes internal and external bleeding in the host. In many cases, strains of the genus *Vibrio* are opportunists, infecting when the host organism is immune suppressed or stressed, with the frequency of infection often being attributable to intensive culture and adverse environmental conditions.
Vibrio harveyi Lg 16.00 causes internal and external hemorrhages, with great affectation in the liver and kidney, in specimens of S. senegalensis. Previous work suggests that one of its pathways of entry of the pathogen could be intestinal and that the administration of the probiotic *Pdp11* significantly decreased the mortality of individuals affected by this pathogen (Tapia-Paniagua et al., 2014).
In this research, the probiotic ability of *Pdp11* to interfere in the oral transmission produced by *V. harveyi Lg 16.00* in *S. senegalensis* is studied. To this end, the objectives are: (1) To observe variations in the glucose metabolism in liver and kidney, by fluorodeoxyglucose (18-FDG); (2) histology and histomorphology study of liver; (3) variation of lipids in the intestine and intestinal content.

Materials and methods

Four groups of *S. senegalensis* (1.2-1.5 kg) were tested (1) control, with a commercial feed diet; (2) control + vibrio, fed with a commercial diet and received a single oral dose (1ml/kg) of the suspension of *V. harveyi Lg 16.00* (10^8 cells/ml); (3) *Pdp11*, fed with commercial diet and oral suspension of 1ml/kg of the probiotic *Pdp11* (10^8 cells/ml) (4) *Pdp11* + Vibrio, which was fed with the diet of the *Pdp11* group and with a single oral dose (1ml/kg) of the suspension of *V. harveyi Lg16.00*. All the specimens were fed every 8 hours with the detailed diets during a period

of 6 days; the infection was carried out 18h before sampling.
18–FDG marking: After infection with the pathogen, all specimens received intraperitoneally fluorodeoxyglucose (18-FDG), (150 uCi/kg). The specimens were kept in the tanks for 30 minutes to assure the distribution of the radiopharmaceutical by the organism. After sacrifice them, they were analysed by positron emission tomography (PET) in the facilities of the Molecular Imaging Unit (CIMES-UMA).
Liver histology and histomorphology: Liver samples were fixed in 4% paraformaldehyde and included in Paraplast. The sections were obtained with the help of a microtome and were stained with hematoxylin-eosin following the routine methodology. Complete images of the stained sections were digitized and 3 captures were studied at 1000x of each one. The lipid area of the liver was measured using ImageJ Freeware (version 1.51) as described in Safer (2017). In this way, the total percentage of lipids present in the liver of the specimens studied was calculated.
Intestinal lipids: The lipid content was measured after extraction with chloroform / methanol (2:1 v/v) according to the method described by Folch et al. (1957). The lipids were dissolved in toluene and the fatty acid methyl esters (FAME) were obtained by transesterification with sulfuric acid (1%) in methanol.
Statistical differences were determined using t-test; P-values <0.05 were considered significant.

Results

The results obtained showed a significative increase in the concentration of the radiolabel in the liver and kidney of the control infected with pathogens, in contrast to those who received the probiotic. This result indicates a higher metabolic rate in the organs of individuals affected by the pathogenic strain. This decrease in marked glucose indicates that the probiotic *Pdp11* interferes with the pathogenic process of *Vibrio harveyi Lg 16.00*.
Regarding the histological samples, the lipid area (μm^2) were studied in the hepatocytes of the different treatments. Although the results obtained were not significant, due largely to the heterogeneity of the samples, it was observed that in livers of the Pdp11 + Vibrio group there was a tendency to decrease the lipid area (Figure 1).

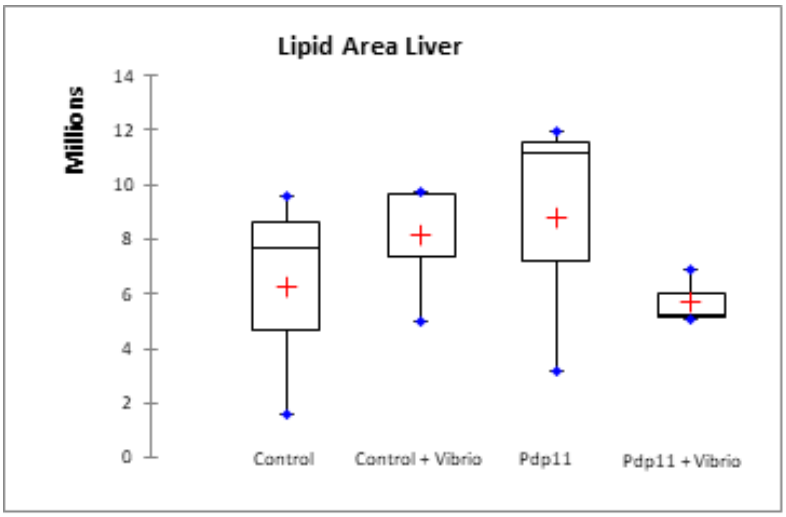

Figure 1: Box plot of lipid area from liver tissue of each treatment.

A decrease in the lipid content in the liver of the Pdp11 + Vibrio group could be related to the energetic need of the organism against infection. The ability of the probiotic to activate the immune system makes the response faster and more effective; this can cause an increase in the

metabolism of the fish that has depleted the glucose reserves, the lipids will be used for energy. Finally, preliminary results on the amount of lipids in the intestine and the intestinal content of different diets, does not show significant differences. However, it is necessary to compare how intestinal lipid change with the presence of *V. harveyi Lg 16.00*.

Acknowledgments

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Functional value of *Nannochloropsis gaditana*. Effect of a treatment to disrupt its cell wall.

Rosario Martínez Martínez*[1] , Cristina Mesas Hernández[2] , Laura Cabeza Montilla[2] , Gloria Perazzoli[2] , Raúl Ortiz Quesada[2] , Octavio Caba Pérez[2] , José Carlos Prados Salazar[2] , Consolación Melguizo Alonso[2] , Pilar Aranda Ramírez[1] , María López-Jurado Romero de la Cruz[1] , Milagros Galisteo Moya[3] , Jesús María Porres Foulquie[1]

*Corresponding author: rosariomz@ugr.es

[1] Departamento de Fisiología, Facultad de Farmacia. Instituto de Nutrición y Tecnología de los Alimentos. Centro de Investigación, Deporte y Salud. Universidad de Granada

[2] Departamento de Anatomía y Embriología, Facultad de Medicina. Instituto de Biopatología y Medicina Regenerativa, Centro de Investigación Biomédica. Universidad de Granada

[3] Departamento de Farmacología, Facultad de Farmacia. Universidad de Granada

Keywords: *Nannochloropsis gaditana*, Functional value, Antioxidant capacity

Introduction

Nannochloropsis gaditana is a marine microalga which was isolated for the first time by Lubián (Lubián, 1982) from the Cádiz Bay. This microalga is mainly used as live food in aquaculture due to its high content in polyunsaturated fatty acids and it is a good source of pigments as well. In recent years, the research about *N. gaditana* has been focused in the development of new fuels and its potential of producing different bioactive compounds. In this regard, is important to ensure the breakdown of its thick and complex cell wall to effectively extract these compounds. Therefore, the aim of this work was to apply a novel treatment to weak the cell walls of *N. gaditana* in order to enhance the extraction of the different bioactive compounds. We compared the nutritional and functional value of the treated and untreated microalga. The applied treatment is under patent and we do not show the specific procedure carried out.

Material and methods

Functional extracts.- Two different functional extract using both types of microalga were assayed. The ethanolic extracts were prepared using an extraction solution of ethanol: water: HCl (50:50:0.2) and protein hydrolysate was carried out following the protocol described by Kapravelou et al. (2013). Briefly, the protein was extracted in basic pH conditions and immediately hydrolysed using two different recombinant proteases. The extraction yield from both methodologies was calculated. Antioxidant capacity.- The antioxidant capacity from the ethanolic extracts was determined through the measurement of the total polyphenols. Moreover, the polyphenols from the ethanolic extract were identified by mass spectrophotometry. All the compounds were identified based on their retention times (RT) and mass, and tentatively identified using MassLynx software. The total polyphenol content from the protein hydrolysate was determined as well.
In vitro digestibility.- The microalga was subjected to an *in vitro* digestibility. Briefly, both types of microalga were digested using a mixture of digestive enzymes in a system of equilibrium dialysis. The final products of digestion (Dialysate: product potentially absorbable and the retentate: product that is not absorbed and could act on the colon) were collected. Dialyzable microalga was expressed as percentage of the total present in each digestion sample. The capacity to inhibit lipid peroxidation and the polyphenolic profile were determined in order to check if the antioxidant capacity is maintained after the digestion process.

Results and discussion

The yield and total polyphenol content of both functional extracts (ethanolic extract and protein hydrolysate) was significantly higher in the treated *vs* untreated microalga (Figure 1). The *in vitro* digestibility of the microalga showed that the percentage of potentially absorbable microalga was two-fold in the treated *vs* untreated microalga (Figure 1). Regarding the capacity to inhibit lipid peroxidation, the dialysates from both types of microalgae showed antioxidant capacity, with significantly higher values in the dialysate of the treated compared to untreated microalga. However, significant differences were not found between the retentates of both microalgae. The identification of the bioactive compounds from the ethanolic extract showed a total of eight compounds from untreated *vs* ten from treated *N. gaditana* (Table 1). After the identification of the bioactive compounds in the dialysate, only some of them had the capacity to cross the dialysis membrane and therefore, being potentially absorbable (data not shown). *Nannochloropsis gaditana* has a complex cell wall that must be disrupted in order to increase its nutricional value (Teuling et al., 2019). Likewise, in this work we show that its functional value is increased when the wall cell is disrupted. The treatment applied to the microalga led to a better extraction of bioactive compound (mainly polyphenols) and a better extraction of proteins, thus resulting in a product with higher concentration of polyphenols as well. Moreover, the higher antioxidant capacity from the treated *vs* untreated microalga is maintained after a digestion process. All these data, show that, to breakdown the cell wall of *N. gaditana*, it is a good strategy to get a higher functional value from this microalga.

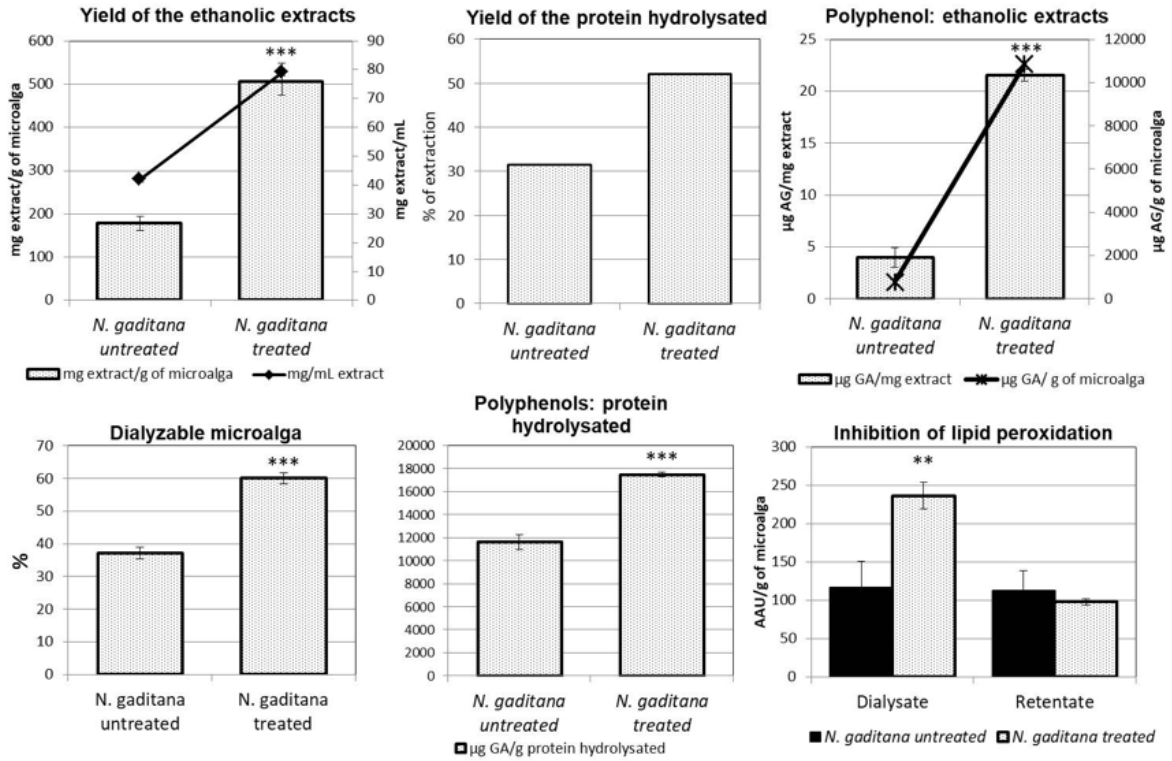

Figure 1: Yield of ethanolic extract and protein hydrolysated from *Nannochloropsis gaditana*, antioxidant capacity and percentage of dialyzable microalga

Table 1: Bioactive compounds of *Nannochloropsis gaditana*

Compound	RT*	[M-H]$^-$	PPM*	Molecular formula	Mass Fragment		
Nannochloropsis gaditana untreated							
1	1.90	325.1088	-14.5	$C_{12}H_{21}O_{10}$	231.0415	217.0779	125.0277
2	2.18	255.0313	7.8	$C_{14}H_7O_5$	128.9957	120.0279	103.0257
3	2.36	341.1286	14.7	$C_{16}H_{21}O_8$	309.1410	231.0499	131.0376
4	3.47	455.0996	4.0	$C_{23}H_{19}O_{10}$	332.0827	176.0189	119.9931
5	4.66	487.3072	2.5	$C_{29}H_{43}O_6$	393.2438	293.1449	173.0310
6	4.80	279.0689	11.5	$C_{17}H_{11}O_4$	248.0401	241.0796	227.0212
7	8.09	387.2019	-9.3	$C_{19}H_{31}O_8$	351.1888	325.1966	265.1518
8	12.70	575.3142	-13.6	$C_{32}H_{47}O_9$	403.2211	301.2129	265.1341
Nannochloropsis gaditana treated							
1	2.01	549.0905	4.6	$C_{24}H_{21}O_{15}$	247.0692	227.0257	119.0652
2	2.50	431.1163	7.4	$C_{18}H_{23}O_{12}$	265.1312	248.0569	164.0708
3	3.06	455.2188	-7.5	$C_{30}H_{31}O_4$	265.1512	125.0348	113.0126
4	4.52	589.3022	1.5	$C_{32}H_{45}O_{10}$	393.2641	265.1356	227.0283
5	5.22	655.4081	3.7	$C_{35}H_{59}O_{11}$	593.3040	590.3131	565.2737
6	6.69	631.3138	3.2	$C_{34}H_{47}O_{11}$	556.3114	393.2368	241.0997
7	7.14	635.3352	-12.4	$C_{34}H_{51}O_{11}$	554.3320	407.1617	241.1268
8	10.89	527.2603	-8.0	$C_{30}H_{39}O_8$	405.1748	369.2156	293.2113
9	11.13	553.2725	13.7	$C_{28}H_{41}O_{11}$	407.1414	369.2225	173.0797
10	13.02	555.2905	18.0	$C_{28}H_{43}O_{11}$	443.2647	265.1794	128.0935
*RT: retention time, PPM: error							

Acknowledgments

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First nutritional characterization of mysid *Mesopodopsis slabberi* at the Guadalquivir estuary

Pablo Cardenas Camacho*[1] , Catalina Fernández Díaz[1] , César Vilas Fernández[1]

*Corresponding author: pablo.cardenas@juntadeandalucia.es
[1] Instituto de Investigación y Formación Agraria y Pesquera IFAPA-Centro IFAPA "El Toruño", Consejería de Agricultura, Pesca y Desarrollo Sostenible - Junta de Andalucía

Keywords: Mysid, *Mesopodopsis slabberi*, Antioxidants, Niche, Carbon

Introduction

Mysid *Mesopodopsis slabberi* (Van Beneden, 1861) is commonly found in estuaries and coasts of the North Atlantic and the Mediterranean Sea. *Mesopodopsis slabberi* is considered omnivorous, feeding on a wide range of preys including detritus, phytoplankton and microzooplankton. It is an important component and resource of the estuaries trophic network, preyed by numerous fish and crustacean decapod species with high commercial interest, channeling energy from low to high trophic levels (Vilas et al., 2008). Our goal is to characterize its nutritional value, seasonal variations and analysis of stable isotopes (^{13}C and ^{15}N) to know better its possible use as a new marine resource and trophic position

Material and methods

Samples were caught in the Guadalquivir River, in a located point called "Esparraguera" (36.894004, -6.230326) at 20 km from the river mouth. Environmental parameters for each sampling are shown in Table 1. Biochemical analyzes were made for each season and two life stages (adults and juveniles) following methodologies by Jiménez-Fernández et al. (2018) and Jiménez-Prada et al. (2018). Stable isotopes ^{13}C and ^{15}N were analyzed by elemental analyzer and ^{13}C modified using lipid correction by McConnaughey and McRoy (1979). Statistical analysis were performed by the statistical software R and SIBER package for stable isotopes analysis. It uses Bayesian statistics for the approximation of trophic niches using ellipses that have 40% of the data density (95% probability). A series of two-way ANOVA was used for the comparison of variances.

Table 1: Environmental parameters for samplings at spring (May), summer (July) and autumn (September).

	T(°C)	SAL(‰)	OD (mg/L)
MAY (SPRING)	19.75±0.07	11.45±1.09	6.61±0.11
JULY (SUMMER)	25.8±0.07	8.76±0.42	5.62±0.05
SEPTEMBER (AUTUMN)	25.85±0.14	10.08±0.26	5.95±0.10

Results and discussion

Elemental composition by season and stage of *M. slabberi* is shown in Table 2. Significant differences were found for proteins between adults and juveniles over the observed months ($p < 0.05$ in all cases), being September whenthe highest protein content was observed. Lipid content

did not vary throughout the studied months ($p > 0.05$) but were significantly higher in juveniles than in adults ($p < 0.05$). The C:N ratio did not show significant differences between adults and juveniles ($p > 0.05$). *Mesopodopsis slabberi* shows a high proportion of both proteins and lipids so could be a good feeding resource.

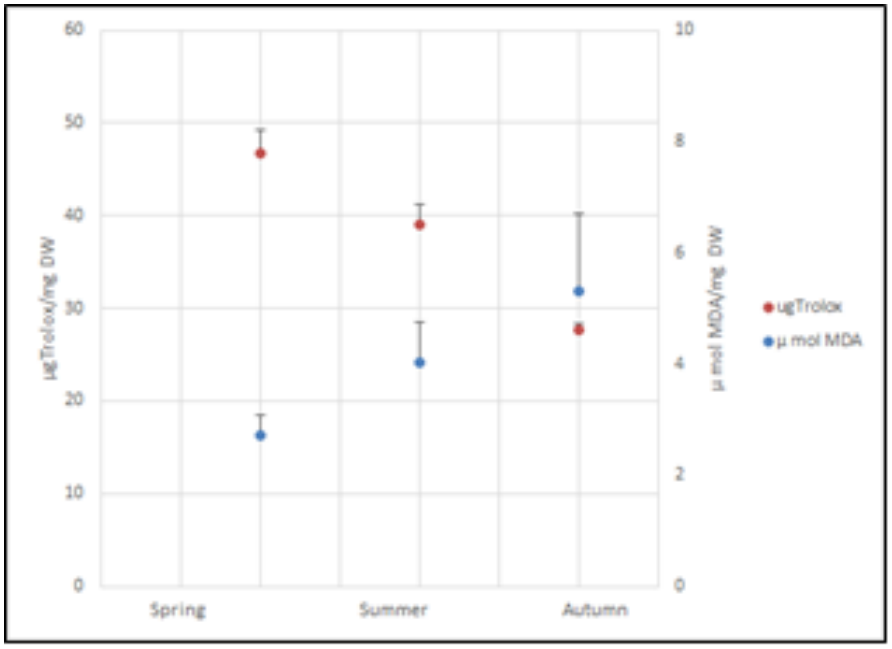

Figure 1: Total Antioxidant Capacity-TAC (μg Trolox equivalent mg^{-1} DW) and lipid peroxidation-LP (μM malondialdehyde mg^{-1} DW) of *Mesopodopsis slabberi* adults at spring, summer and autumn 2018.

We found an interesting seasonal opposite trend for both TAC and LP, where the concentration of antioxidants is highest during the spring and the lowest at autumn, while LP increases from spring to autumn, when the concentration of antioxidants are lower due to the generation of free radicals with oxidative stress (Figure 1). Index TAC/LP clearly decreases throughout the year (17.2 at May, 9.6 at July and 5.2 at September). This pattern could be related with the environmental temperature experimented by adults during its previous 2-3 months of life cycle - highest for autumn and lowest at spring. The high content of antioxidants of *M. slabberi* adds value to its nutritional profile so worthy of being considered an interesting alternative live food or supplement in diets for the culturing of young marine fish with commercial interest.

Table 2: Elemental composition by stage and season of *Mesopodopsis slabberi* throughout 2018. The percentages of each analysis reflect the average and standard deviation of the results obtained. N/A= Not available data.

	Spring		Summer		Autumn	
	Adults	Juveniles	Adults	Juveniles	Adults	Juveniles
mg DW	1.13 ± 0.15	0.046 ± 0.01	0.867 ± 0.07	0.0123 ± 0.008	0.9 ± 0.072	0.023 ± 0.006
% ASH	9.86 ± 0.74	11.3 ± 0.64	8.07 ± 1.02	8.7 ± 1.82	12.92 ± 0.76	13.74 ± 0.41
% PROTEIN	52 ± 10.4	43.5 ± 4.86	49.5 ± 6.73	55.3 ± 3.55	70.7 ± 9.65	58.3 ± 8.18
% CARBO	2 ± 0.83	3.35 ± 0.47	2.5 ± 0.65	2.73 ± 0.85	2.13 ± 0.3	3.3 ± 0.55
% LIPIDS	9.4 ± 1.6	NA	9.25 ± 0.55	15.27 ± 2.53	9.8 ± 0.79	12.99 ± 1.36
C:N	3.62 ± 0.24	2.95 ± 0.63	3.22 ± 0.22	3.3 ± 0.05	3.33 ± 0.24	3.43 ± 0.10

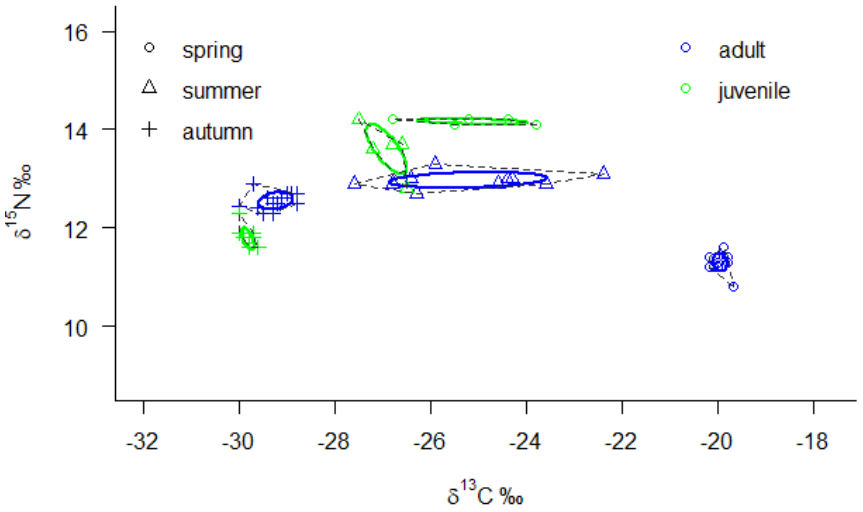

Figure 2: Niche analysis of the *Mesopodopsis slabberi* population sampled at Esparraguera. Ellipsoid containing: (Blue) Adults; (Green) Juveniles for Spring (Circle), Summer (Triangle), Autumn (Cross)

Isotopic relationship between ^{13}C and ^{15}N isotopes in Figure 2 shows how carbon covers a wide range and a clear seasonal pattern. Late winter-early spring adults present the highest body mass (Table 2) and isotopes values (-20 ± 0.1 ‰δ^{13}C and 11.3 ± 0.18 ‰δ^{15}N) corresponding to a marine realm, showing a recent migration from coastal areas, while just released juveniles ^{13}C indicates a more estuarine carbon source and could get higher ^{15}N due to estuary freshwater and antropogenic sources enrichment. Carbon signal at summer (similar for both adult and juveniles - 2.5% Standard Ellipse Area overlap; 99.65% prob.95 overlap) and spring-juveniles (released within the estuary) reflects a more estuarine feeding (-22 to -28 ‰), while autumn data show a higher influence of freshwater realm (-28 to -30 ‰) - heavy rain episodes just before sampling could influence this shift. Carbon values obtained in summer were similar to those obtained by Carrasco and Renzo (2011) for *Mesopodopsis africana* (-27.3 ± 0.13 ‰δ^{13}C) in a south African temperate estuary at the beginning and at the end of the dry season. ^{15}N shows significant differences between adult and juveniles only at May (11.3 ± 0.18 ‰), and juveniles during May (14.19 ± 0.04 ‰) ($p < 0.05$). This seasonal feeding shift could also be related with the observed decrease of adults antioxidant capacity from spring to autumn, when low nutritional value phytoplankton could be predominant after high freshwater influence.

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Evaluation of enzyme additives on the nutritional use of feeds with a high contents of plant ingredients for juveniles of *Mugil cephalus*

Francisca P. Martínez Antequera*[1] , Laura Bermúdez Villaescusa[2] , Mª Jesús Aznar García[1] , Fco. Javier Moyano López[1]

*Corresponding author: paqui_78@hotmail.es
[1] Department of Biology and Geology, University of Almeria, 04120 Almeria
[2] CTAQUA. Commercial Dock S/N. 11500. The Port of Santa María. Cádiz

Keywords: Aquaculture, Plant ingredients, Additives, Mullet

Introduction and justification

Mugilidae are an interesting group of fish for aquaculture because of their omnivorous profile, fast growth and resistance to environmental variations (Abellán and Arnal, 2013). However, the production of commercial feeds for mullets is still scarce. The selection of ingredients in theose species is oriented to a great use of plant by-products. Nevertheless, such use may be limited for their contents in several anti-nutritive factors, such as phytate and non-starch polysaccharides (NSP) (VKM, 2009). Nevertheless, as it occurs with feeds for terrestrial species, it is possible to counteract or decrease these negative effects by using enzyme additives that may increase the bioavailability of pentoses and phosphorus. Considering this, the present work was oriented to assess the effect of an enzymatic pre-treatment on the nutritional value of feeds with high contents in plant ingredients for juveniles of grey mullet (*Mugil cephalus*).

Material and methods

Two different experiments were designed. The experiment 1 evaluated the effect of using an enzymatic mixture of analytical quality for pre-treatment of two feeds with a high content (more 65% in weight) of plant ingredients like rice bran, rapeseed and guar meals. The experiment 2 evaluated in a similar way the use of a commercial enzyme mixture when applied both on a commercial feed for carp, routinely used for feeding mullets, and on an experimental feed containing a great proportion of rice bran and brewers waste. The result of the enzymatic treatment on the bioavailability of key nutrients was assessed by; analysis of the variation in the composition of the feeds after the enzymatic treatment (exp. 1 and 2), conducting in vitro and in vivo digestive evaluation tests (exp 1) and in vivo nutritional evaluation (exp 2). The fish used, supplied by the company PIMSL, had an initial weight of 5 g at the beginning of experiment 1 and 12 g at the beginning of experiment 2. All data obtained were statistically treated by ANOVA followed by LSD using Statgraphics Centurion software.

Results and discussion

Experiment 1: the enzymatic treatment significantly increased the potential bioavailability of both reducing sugars and pentoses from the fraction of carbohydrates present in the untreated feeds and a significant high reduction of the phytate content was observed. The *in vitro* digestive simulation assays confirmed a significantly higher release of pentoses from the enzyme treated

feeds when compared to controls. On the other hand, the *in vivo* digestibility assays evidenced that the enzymatic treatment determined a significant increase in the digestibility of protein for one of the feeds, and a decrease in thatof phosphorus in the other. It is suggested that because of the poor development of the intestine in young fish, the increase in digestive bioavailability of a significant fraction phosphorus previously present in the form of phytate was not accompanied by the corresponding increase in the absorption capacity of such element at intestinal level.
Experiment 2: the enzymatic treatment determined a significant increase in the amount of available pentoses while decreased the content of phytate initially present in both feeds. The growth trial showed a significant improvement in the efficiency of food utilization and growth rates in fish receiving the experimental feed with the enzyme treatment, although these parameters were not as good as those obtained with the commercial feed (Table 1). The improvement of the nutritional use of plant ingredients in aquaculture through the use of enzymes has been highlighted by different authors (Castillo and Gatlin, 2015). These authors suggested among the potential positive effects, the reduction in the viscosity of the digestion, the increase in the bioavailability of some nutrients, the reduction in the elimination of fecal waste, as well as a modification of the microbioal profile that improves the immunological status of fish. The results obtained in the present work seem to confirm some of such positive effects at least in the case of *M. cephalus* and oriented the future development of commercial feeds based on vegetable by-products for this species.

Table 1: Growth and efficiency in food utilization in experimental groups. Values in a row not sharing a common superscript are significantly different with $p<0.05$.

	COMMERCIAL CONTROL	**COMMERCIAL CONTROL + ENZYME**	**PLANT EXPERIMENTAL**	**PLANT EXPERIMENTAL + ENZYME**
INITIAL WEIGHT (g/fish)	12,02 ± 0,33 a	12,35 ± 1,05 a	11,89 ± 0,55 a	12,38 ± 0,16 a
FINAL WEIGHT (g/fish)	16,60 ± 0,60 a	16,70 ± 2,03 a	13,21 ± 0,49 b	15,36 ± 0,04 c
INCREASE (g/fish)	4,58 ± 0,29 a	4,35 ± 1,00 a	1,33 ± 0,28 b	2,98 ± 0,15 c
FEED CONSUMPTION (g/fish)	10,02 ± 0,17 a	9,93 ± 0,96 a	8,65 ± 0,45 b	9,23 ± 0,17 c
FCR (g feed/g fish)	2,19 ± 0,11 a	2,33 ± 0,36 a	**5,89 ± 1,48 b**	**3,11 ± 0,19 c**
SGR (g/100g fish/day)	0,75 ± 0,02 a	0,70 ± 0,1 a	**0,25 ± 0,05 b**	**0,50 ± 0,03 c**

Acknowledgments

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Analysis of the distribution and growth of the clam (*Scrobicularia plana*) and the razor clam (*Solen marginatus*) in the Bay of Cadiz

Anyell Caderno*[1] , Francisco Hortas[1] , Remedios Cabrera-Castro[1,2]

*Corresponding author: anyellc9402@gmail.com
[1] Departamento de Biología, Facultad de Ciencias del Mar y Ambientales. Universidad de Cádiz. Avda. de la Universidad, 11510 Puerto Real, Cádiz, Spain. Campus de Excelencia Internacional del Mar (CEIMAR).
[2] Instituto Universitario de Investigación Marina (INMAR). Campus de ExcelenciaInternacional del Mar (CEIMAR).

Keywords: Bay of Cadiz, Bivalves, Shellfish, Distribution, Minimum sizes

Abstract

Various extractive activities take place in the Bay of Cadiz Natural Park. One of them is shellfishing, which is characterized by catching different species of benthic invertebrates including *Scrobicularia plana* and *Solen marginatus*. Both species are highly appreciated gastronomically, indicating their great demand and commercial interest in the area. Moreover, the high rates of unemployment in the region have meant that for years, shellfishing has been a complimentary activity that helps to subsist. In this area, the number of illegal shellfishing has far exceed the legal number and has spread to various regions of the Natural Park (Figure 1).

Figure 1: Illegal shellfishing in Playa de la Cachucha, Puerto Real, Cadiz.

The starting hypothesis for this work was that the average sizes of capture for these two species has been decreasing over the years since this activity is not well regulated by law which

could be a clear indication of overexploitation. On the other hand, they are filtering species, and since they are collected illegally, they do not go through purification and dispatch centers and are sold directly to the consumer without any regulation. In this work the average sizes of catches for both species were studied in specimens that the shellfishes sell outside the legal circuit and the growth for both species was determined for the usual shellfish areas of the Bay of Cadiz. The samples were obtained monthly between January and July 2019. Biometric analyzes were performed for both species (length: width, height and thickness of the valves and weight: total weight, weight of the valve, meat weight and dry weight, Figure 2).

Figure 2: *Solen marginatus.*

At the same time, the counting of marks or rings of growth was made taking a representative sample of each month for each age class. In addition, the observation and monitoring of shellfish was carried out in the different areas where this activity is carried out. A total of 408 specimens of *S. plana* and 478 of *S. marginatus* were obtained, among which individuals with sizes less than those established by the legislation in force were found. With the counting of rings a growth pattern was established taking into account the number and the increase thereof. The most frequent ring numbers observed for *S. plana* were 15 and 16, which corresponded with an average length of 38.94 mm. In the case of *S. marginatus*, the most frequent ring numbers were 12 and 13, with an average length of 73.93 mm.

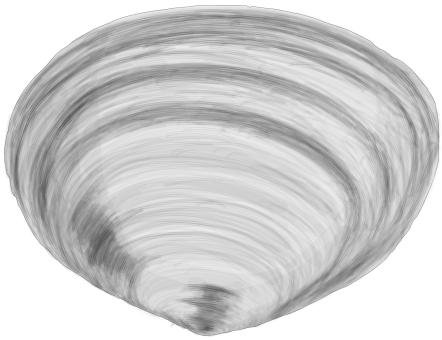

Figure 3: *Scrobicularia plana*. Author: Raúl Marín Aragón.

Currently, a proposal is being developed according to the Von Bertalanffy growth model in order to establish the growth parameters for these molluscs in the Bay of Cadiz. This study aims to provide a better understanding of both species that will serve to manage their populations in the medium and long term adequately.

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Spawning season for Atlantic bonito *Sarda sarda* in the Alboran Sea

S. Saber*[1] , J. Ortiz de Urbina[2] , M.A. Puerto[1] , M.J. Gómez Vives[1] , L. Godoy[1] , M.J. Meléndez[2] , D. Macías[1]

*Corresponding author: samar.saber@ieo.es
[1] Instituto Español de Oceanografía, Centro Oceanográfico de Málaga, Puerto Pesquero, s/n, 29640 Fuengirola, Málaga, Spain
[2] Universidad de Málaga, Departamento de Biología Animal, 29071 Málaga, Spain and IEO

Keywords: Alboran Sea, Atlantic bonito, Gonadosomatic index, Reproduction, Spawning season

Introduction

Atlantic bonito (*Sarda sarda* Bloch, 1793), an epipelagic and neritic tuna species, is widely distributed in the Atlantic Ocean, the Mediterranean Sea and Black Sea (Collette and Nauen, 1983). Although this species has a high socio-economic relevance for a considerable number of local communities at the regional level, there is currently no stock assessment for this species and the stock status remains unknown. This species is caught using a variety of fishing gears. The average annual catch over the last 8 years (2010-2017) in the Mediterranean is 21599 t (https://www.iccat.int/en/accesingdb.html). On the Mediterranean Spanish coast this species is landed at 72 different ports, with Marbella (Alboran Sea, western Mediterranean Sea) being the port with the highest reported catches. The Atlantic bonito is a multiple spawner, i.e. it releases eggs over a long spawning period (weeks or months). Information on the life history traits of the species needs to be updated and used to develop better models for assessing fish stocks. Therefore the objective of this work is to study the reproductive biology of the Atlantic bonito, specifically the spawning season using the seasonal trends in the gonadosomatic index.

Material and methods

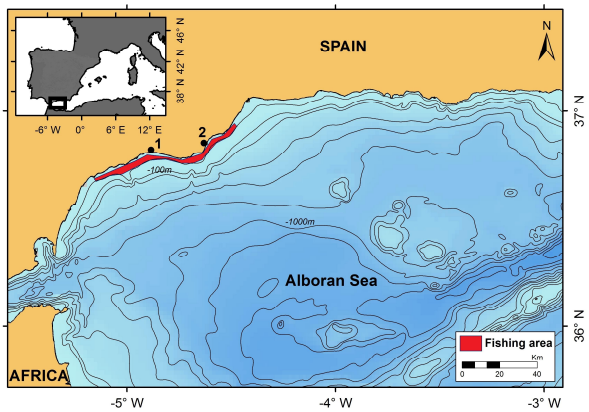
Atlantic bonito were collected between May 2018 and May 2019 from two ports, Marbella and Fuengirola, located in the Costa del Sol (Alboran Sea) (Figure 1a). The individuals were caught by coastal artisanal fisheries targeting Atlantic bonito: purse seine and a kind of trammel net called "solta". For each fish, fork length (FL) was measured to the nearest 0.1 cm and total body weight (W) was measured to the nearest g. The ovaries and testes (gonads) were removed and gonad weights (GW) were recorded to the nearest 0.01 g. The sex was macroscopically identified and classified as male or female. The gonadosomatic index (GSI) was calculated for males and females to examine the seasonal changes in the gonadosomatic index and therefore to estimate the reproductive status of individuals:

$$GSI = GW/W \times 100 \quad (1)$$

An exploratory analysis (not shown here) showed that the relationship among GSI, length and month is clearly non-linear, so the monthly relationship between GSI and FL was modelled by means of a generalized additive model (GAM) as a smooth interaction of the response variables length and month:

$$GSI = \alpha + s\,(FL, month) + \varepsilon \quad (2)$$

All statistical analyses were performed using R statistical software. For GAM modeling, package mgcv was particularly useful as well as other R packages, the ggplot2 and plotly.



a

Months	Date	n	FL (cm)	WT (g)	Fishing gear
January	31/01/2019	10	36.5-41.1	656-922	Solta
February	06/02/2019	3	36.1-38.1	593-751	Solta
March	11/03/2019	16	39.2-42.9	765-1071	PS
May	18/05/2018	10	42.3-54.0	1060-2592	PS
	06/05/2019	12	37.8-49.3	664-1567	PS
	23/05/2019	9	49.0-67.4	1723-4516	Solta
	28/05/2019	16	37.2-44.4	700-1323	PS
June	14/06/2018	12	41.2-53.2	996-2292	Solta
	25/06/2018	11	42.9-57.6	1075-2528	Solta
July	13/07/2018	16	39.2-45.3	769-1289	PS
September	03/09/2018	18	45.2-50.8	1311-1940	PS
	07/09/2018	14	46.3-51.8	1489-2087	PS
	12/09/2018	20	32.5-49.5	500-1770	PS

b

Figure 1: (a) Map indicating the fishing area where the artisanal boats targeting Atlantic bonito Sarda sarda and the localities where this species were collected. Sampling period: 2018-2019. Ports: 1, Marbella; 2, Fuengirola. (b) Number of Atlantic bonito sampled for spawning season estimation. Sampling period: 2018-2019.

Results and discussion

A total of 167 Atlantic bonito were sampled. The number of individuals, the sizes and weights ranges by sampling day are given in Figure 1b. The sizes of males ranged between 32.5 and 64.4 cm FL (n= 70; mean ± SD = 44.5 ± 5.7 cm) and females ranged between 34.6 and 67.4 cm FL (n= 97; mean SD = 44.6 ± 5.7 cm). The mean monthly GSI was calculated for both males and females. These values increased sharply from March, peaking in June, with a mean of 4.71 ± 0.7 and 4.85 ± 2.9 for males and females respectively (Figure 2a). The results of the GAM showed that GSI values are low (reproductively inactive individuals) for individuals below 42 cm FL independently of both the month and the size (Figure 2b). This result would point to the length at first maturity, which is consistent with the reported length at first maturity 36.8 - 42.5 cm FL (Kahraman et al., 2014). High GSI values (lighter colours) extend over a longer period of time for large fish (greater than 57 cm FL) than for medium-sized ones (45 - 56 cm FL). This result would suggest that smaller and younger fish have a shorter spawning season on average than larger and older fish (Farley et al., 2013). However, GSI values up to 4 have been found in regressing Atlantic bonito (Rey et al., 1984), suggesting that the use of the GSI alone would not be a good indicator of the spawning activity, that is, GSI should be calibrated through the use of macroscopic and/or microscopic techniques. The monthly variation in mean GSI values of males and females and the results obtained from the GAM suggests that the spawning season for Atlantic bonito in the Alboran Sea (Western Mediterranean Sea) takes place from May to July. Our findings are in line with other studies conducted in the Northeast Atlantic Ocean (Baibbat et al., 2017), the Strait of Gibraltar (Rey et al., 1984) and the Eastern Mediterranean and Black

Sea (Kahraman et al., 2014). Additional samplings covering the whole year should be included in the model to fully explore the reproductive biology of this species in the Alboran Sea.

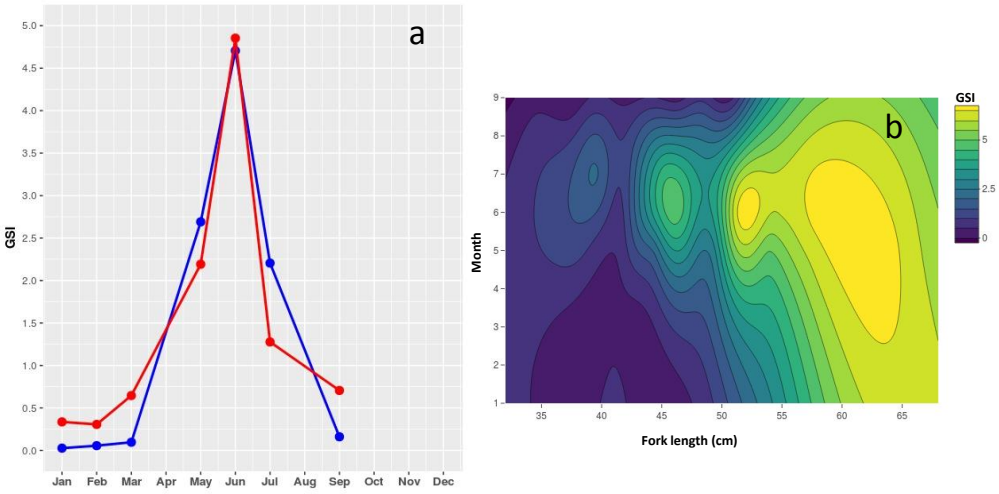


Figure 2: (a) Monthly average of the gonadosomatic index (GSI) estimated for females (red line) and males (blue line). (b) Contour plot of the fitted generalized additive model (GAM) for gonadosomatic index as a function of months and fork length. Fork length on the x-axis, month on the y-axis, and the values on the response scale (gonadosomatic index) given by the contours (isolines of constant gonadosomatic index values; lighter color indicates higher values).

Acknowledgments

We are grateful to all crews and workers from Marbella and Fuengirola fish market for their collaboration, as well as P. Bárcenas and T. Dobinson for their valuable contributions. This project has been partially funded by the EU through the European Maritime and Fisheries Fund (EMFF) within the National Program of collection, management and use of data in the fisheries sector and support for scientific advice regarding the Common Fisheries Policy; by the Instituto Español de Oceanografía (IEO) GPM 17-19 project and, the ICCAT small tunas year research program.

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Tidal dynamics effect on the connectivity patterns of the blackspot seabream (*Pagellus bogaraveo*) in the Alboran Sea

Irene Nadal Arizo*[1] , Simone Sammartino[1] , José Carlos Sánchez Garrido [1] , Jesús García Lafuente[1]

*Corresponding author: irenenadal@ctima.uma.es
[1] Physical Oceanography Group, University of Malaga

Keywords: Blackspot seabream, Early Life Stage, Hydrodynamic connectivity, Tidal dynamics, Alboran Sea

Introduction

Several researchers have reported that the blackspot seabream have been subjected to an excessive exploitation over the years (Gil Herrera, 2006). Experts in fishery ecology agree that for optimizing the fisheries as well as curbing the over-exploitation is necessary to study population dynamics and connectivity of the species according to the circulation and oceanographic variability (Cowen and Sponaugle, 2009). The objective of the present work is to analyse the blackspot seabream connectivity with a hydrodynamic numerical model, using early life stage (ELS) blackspot seabream virtual particles as passive tracers advected by dynamical tides.

The numerical model

The model used to carry out the numerical simulations is the MIT general circulation model (Marshall et al., 1997). The general configuration of the model, its advection and vertical mixing scheme is described in detail in Sammartino et al. (2014). To assess the dynamic interaction of the ELS blackspot seabream with marine environment, a lagrangian particle tracking (LPT) algorithm was used. Lagrangian simulations are an effective way to study the general pattern of connectivity between different sub-areas as a result of observing the advection of eggs and larvae (that, numerically, are considered as passive particles) under the effect of the current. For this particular case, LPT was based on the Runge-Kutta 4th order method. Lagrangian particles were simulated under different spatial and temporal combination of conditions to investigate how different tides affect them. First, to see the particles behaviour according to the release area, three starting areas were defined within the SoG: Tanger, TangerMED and Tarifa. On the other hand, ten possible destination areas were defined: Cadiz, Estepona, Malaga, Roquetas, Carboneras, Oran, Melilla, Alhucemas, Tetuan and Arcila. Secondly, to see the particles behaviour at several vertical levels, five release depths were considered, at 1m, 12m, 25m, 52m and 81m. Third, with the aim of estimating the effect of tides on the ELS dispersal patterns, an array of LPT simulations have been started under a set of eight tide combinations of the different conditions depending on the tidal phase [(H) High, (L) Low, (F) Flood, (E) Ebb tide] and the tide strength [(S) Spring, (N) Neap tide]. Finally, with the aim of obtaining a more robust estimation of the metrics used, a total of four replicas for each of the aforementioned condition, were implemented throughout the first two months of 2005. Each single LPT simulation lasted for 60 days.

Results

Figures 1 and 2 shows maps of the averaged maximum particles of ELS reached at all the connectivity boxes from Tarifa and TangerMED, respectively.

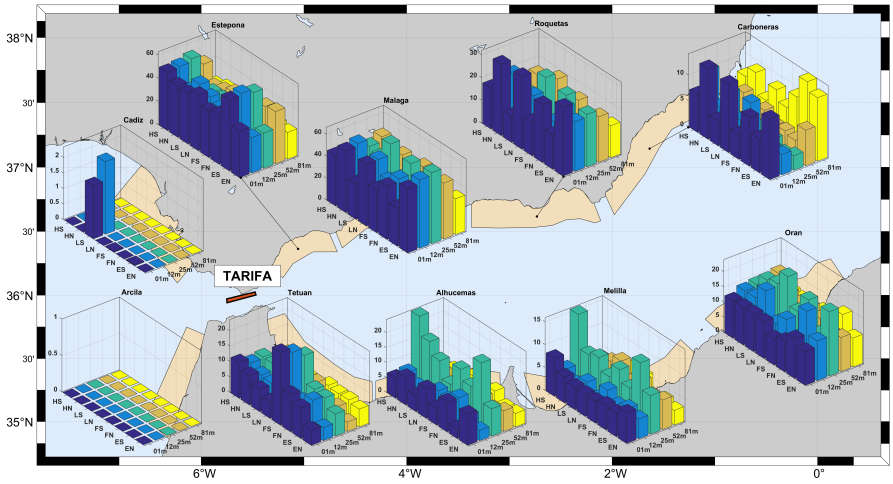

Figure 1: Map of the averaged maximum particles of ELS reached at all the connectivity boxes from the Tarifa area according to the 160 combinations and the 10 adjoining boxes.

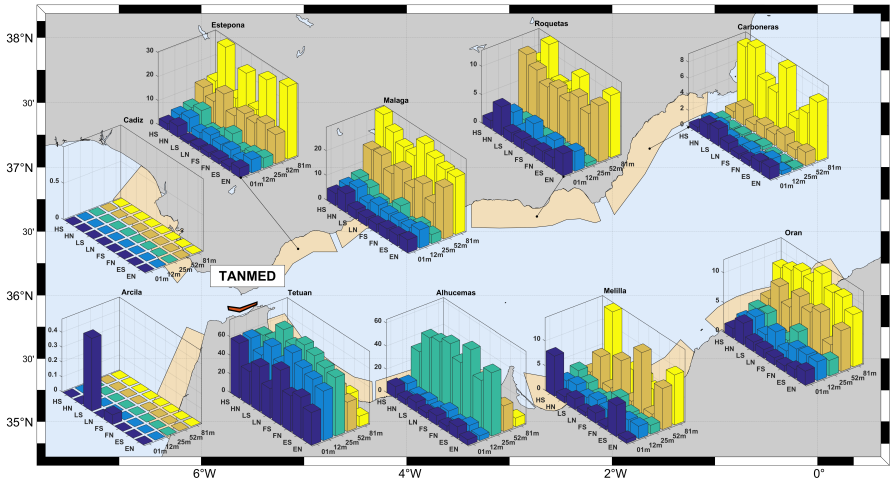

Figure 2: Map of the averaged maximum particles of ELS reached at all the connectivity boxes from the TangerMED area according to the 160 combinations and the 10 adjoining boxes.

For their location within the SoG, these areas are a good example of the latitudinal divergence of the connectivity patterns. Particles released in Tarifa are registered predominantly in the northern boundary of the Alboran sea, whilst particles released in TangerMED are registered in the southern boundary. Instead, few particles were registered at the Atlantic Ocean, demonstrating the Atlantic Jet predominance. Relative to both maps, the connectivity boxes Estepona and Tetuan, for its proximity to the Tarifa and TangerMED release areas respectively, presents the earliest maximum connectivity percentages, and a progressive drop of values from High Tide (HS) to Low Tide (LS), according to the general tidal modulation of the SoG exchange that prescribe the maximum (minimum) inflow and the corresponding minimum (maximum) outflow in ebb (flood) tide. The variability due to the tidal fortnightly modulation is also noticeable, especially

in terms of time shift of the connectivity maxima, which, in turn, are not sensibly broadened. In the rest of the connectivity boxes of the northern and southern boundary, the discrepancy driven by the fortnightly modulation starts to be more evident. This, prior to other calculations suggests a faster speed of the particles under spring cycle and, thus, a higher dispersion of the eggs and larvae in this tide condition. On the other hand, there are not big differences between the different tidal phases (high, low, ebb and flood tides) along the boxes, which suggests a major influence of the tidal strength for the particles released in this area. Figure 3 provides a clear example of the effect of fortnightly modulation. The percentage of ELS registered and the corresponding elapsed time were lower under the spring cycle, whilst the percentage registered under neap cycle and its corresponding time, were significantly higher, which affirmed the lower dispersion and velocity of the eggs and larvae under this cycle.

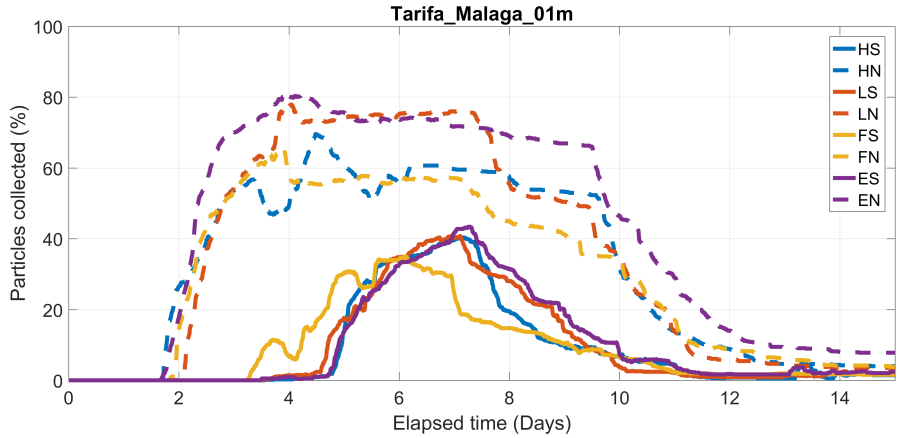

Figure 3: Percentages of ELS released at surface in Tarifa and found in Malaga box over the elapsed time under different tidal conditions. The four tidal phases combined with the spring cycle are displayed by solid lines, whilst the combined with the neap cycle are displayed by dashed lines.

Finally, the variability according to the depth level shown a higher energy and, thus, higher percentages on the most superficial levels.

Acknowledgments

Special thanks to Manuel Hidalgo and Juan Gil Herrera for sharing their expertise in fish connectivity.

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Identification and antimicrobial properties of cultivable bacteria associated with seaweeds from Cantabrian Coast

Susana Rubiño Campoy*[1] , Maria Hortós Bahi[1] , César Peteiro García[2] , Teresa Aymerich Calvet[1]

*Corresponding author: susana.rubino@irta.cat
[1] IRTA-Monells (Food Safety Programme), Finca Camps i Armet s/n, 17121 Monells (Girona-Spain)
[2] Seaweeds Centre, Marine Culture Unit

Keywords: Antimicrobial activity, Foodborne pathogens, Marine bacteria, Seaweeds, 16S rDNA sequencing

Introduction

Current emergence of antibiotic-resistant bacteria have led to investigate new habitats in the search of compounds with antimicrobial potential (Thilakan et al., 2016). Because seaweed-associated bacteria interact in multiple and complex ways, they constitute an interesting source of bioactive compounds (Goecke et al., 2013; Kartick and Mohanraju, 2018). In food industry they could be used as an alternative to traditional preservatives with promising potential against foodborne pathogens in order to achieve a high level of consumer protection (Pina-Pérez et al., 2017).

Materials and methods

Seaweed-associated bacteria were isolated from 11 different seaweeds, representing two of the main three classes (Phaeophyceae, brown algae; and Rhodophyceae, red algae) by culture-dependent methods, using general and specific medium prepared with destilled water and seawater. DNA extraction (Wizard® Genomic DNA Purification Kit) and 16S rRNA gene sequence amplification (Universal primers: 8F-1492) were carried out in order to identify the isolated strains. The PCR products were sequenced (Macrogen Inc., Spain; Primers: 785F-907R) and analized using EMBOSS and GenBank. The antagonistic activity of epiphytic bacteria were studied on solid media by double-layer and agar well diffusion method against 20 target strains, including foodborne pathogens and spoilage bacteria.

Results and discussion

Fifty-three epiphytic bacteria were isolated from the surface of the 11 seaweeds. 50 of them where identified up to genus level and 7 different genus (Figure 1) were obtained, being the most representative the genus *Bacillus* sp (54%) (Figure 2). From them, only 4 out of the 33 strains (Figure 1) that were able to grow in seawater, reported antimicrobial activity against 3 up to 5 test strains. All being active against *Listeria monocytogenes* (Figure 3). All the strains reporting antimicrobial activity were prove to be *Bacillus* sp. and were isolated from 3 different brown algae (class Phaeophyceae), belonging to 3 different families: Cladostephaceae (*C. spongiosum*), Fucaceae (*Fucus guiryi*) and Sargassaceae (*Cystoseira tamariscifolia*) (Ismail et al., 2016).

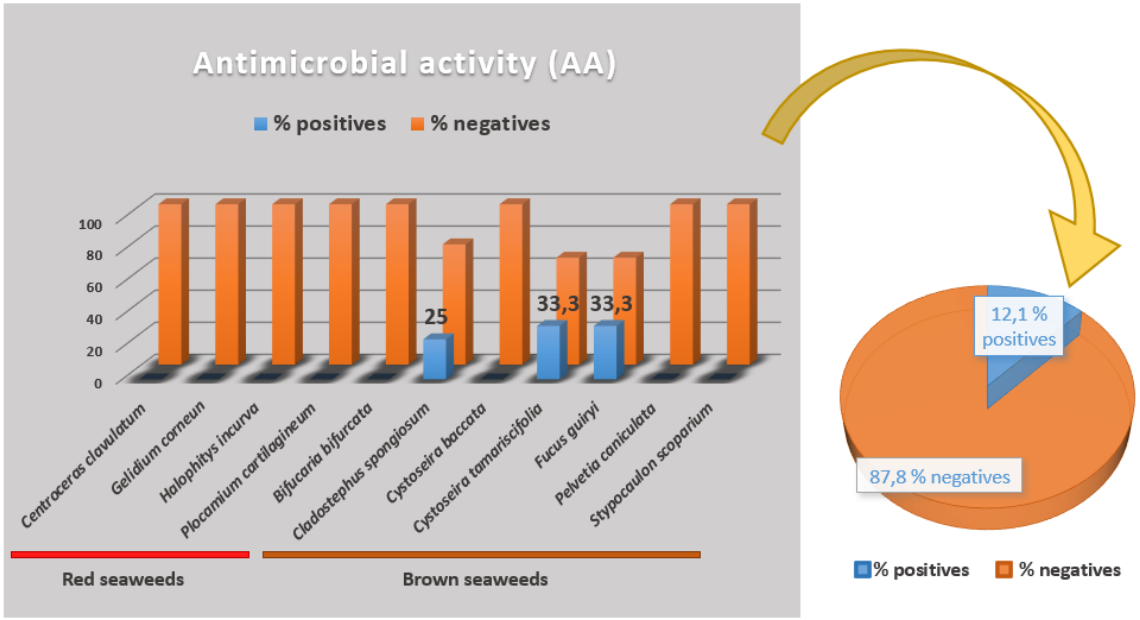

Figure 1: Antimicrobial activity in different species of seaweeds.

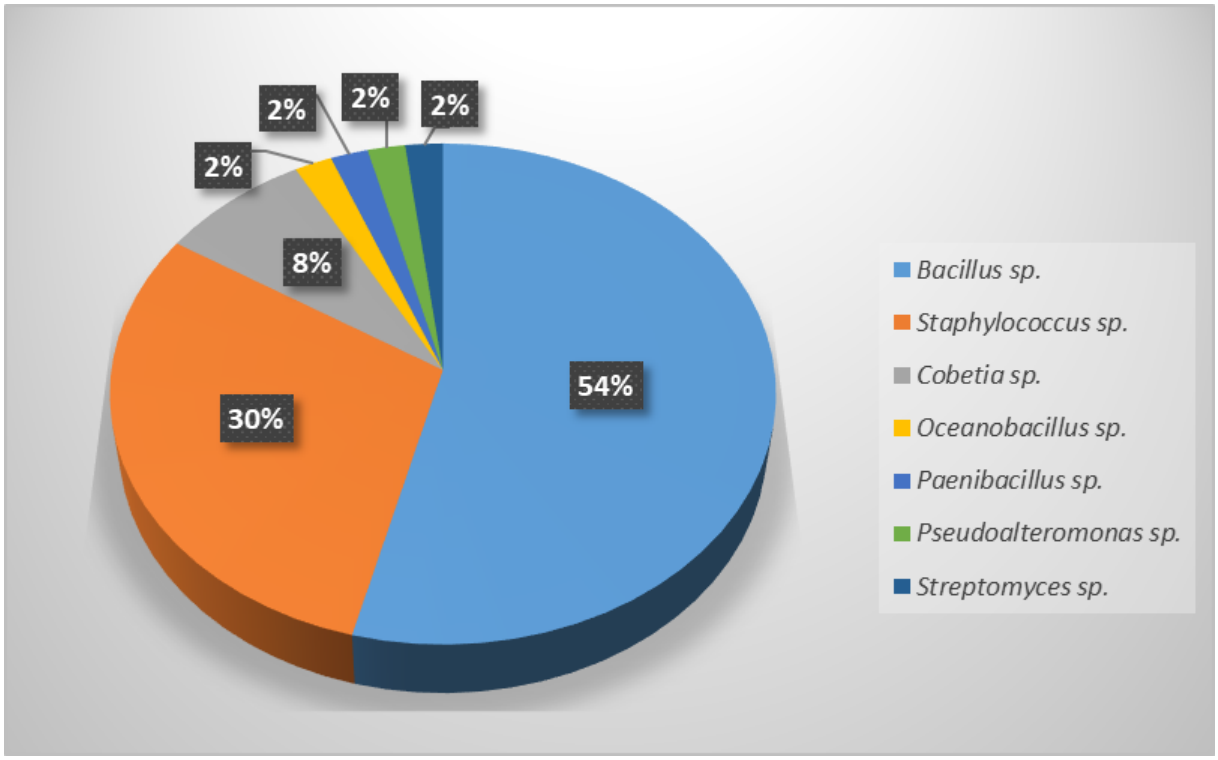

Figure 2: Percentage of differents epiphytic bacteria isolated from surface of 11 seaweeds.

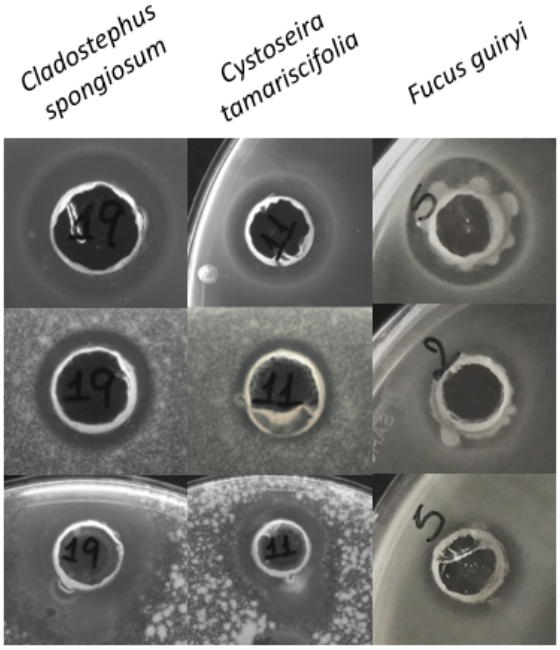

Figure 3: *Bacillus* sp. strains isolated from three different seaweeds.

Acknowledgments

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Development of a DNA vaccine against *Phdp* and evaluation of different nanoparticles as delivery systems

Catalina Fernández-Díaz*[1] , María Eugenia Zuasti[1] , Marian Ponce[1]

*Corresponding author: catalina.fernandez.diaz@juntadeandalucia.es
[1] Nutrition Laboratory, IFAPA Centro El Toruño, Junta de Andalucía

Keywords: DNA vaccine, Nanoparticles, Chitosan, Ulvan, Photobacteriosis

Introduction

Photobacterium damselae subsp. *piscicida* (*Phdp*) is a facultative intracellular, halophilic Gram-negative bacterium and is the aetiological agent of pasteurellosis (a bacterial septicaemia) in wild and farmed marine fish. It is a disease that causes severe economical losses in world aquaculture of marine fish due to its capacity for inducing massive mortality, ubiquitous distribution, widespread antibiotic resistance and the lack of efficient vaccines. DNA vaccines offer a number of advantages over conventional vaccines. They represent a simple and powerful concept: the coding sequence of an antigenic pathogen gene is incorporated into plasmid DNA, which will allow its expression in host cells. Thus, DNA vaccines circumvent the need for preparation, purification, and delivery of a pathogen or antigenic protein. Instead, they utilize the intrinsic machinery of host cells. Moreover, DNA vaccines may be able to engage immunological mechanisms that are not easily attainable with other approaches. Methods to produce, manipulate, and purify DNA are now standard in most biology and bioengineering laboratories, making the tools of DNA vaccine production widely accessible. DNA vaccines are safer than conventional vaccines. However, pDNA is susceptible to degradation in the extracellular environment and rather unable to transpose cellular barriers in its naked form. Encapsulating pDNA within degradable delivery vehicles such as nanoparticles (NPs) provides an effective way to protect the DNA from the surrounding environment prior to delivery. Several polymers have been used to encapsulate pDNA including poly D,L-lactide-co-glycolic acid (PLGA), chitosan (CS) and ulvan. Chitosan is a natural biodegradable polysaccharide extracted from crustacean shells. CS posses positive charge and can effectively bind DNA negatively charged. In addition, CS protects DNA from nuclease degradation. It is proved that the CS is non-toxic both in experimental animals and humans. Ulvan is an anionic sulphated polysaccharide and can easily forms NPs with cationic polymers such as CS. In this study, the *impdh* gene from *Phdp* was used to prepare a DNA vaccine (pPDPimpdh). CS-NPs, CS-ulvan-NPs and DLPLG-NPs were used to encapsulate pPDPimpdh. Characterization, encapsulation efficiency and stability of pPDPimpdh were determined.

Materials and methods

Recently, the gene *impdh* from *Phdp* has been identified as a promising antigen for the development of a DNA vaccine against photobacteriosis (Nuñez-Díaz et al., 2018). For this reason, genomic DNA was isolated from the strain *Phdp* Lg41/01 using the DNeasy blood and tissue (Qiagen). The gene encoding for *impdh* was amplified with a specific primer set using a proofreading enzyme. The forward primer contained a Kozak sequence just before the start codon to ensure proper initiation of translation of prokaryotic genes in eukaryotic cells. As we wanted to express our protein fused to GFP at the C-terminus, the reverse primer did not contain a stop codon. PCR amplified product was cloned into de expression vector pcDNA™6.2/C-EmGFP-GW/TOPO®

(invitrogen). The recombinant clone was selected by PCR with universal primers T7 Promoter and FP2 reverse and confirmed the presence and correct orientation of the insert by sequencing. Plasmid was named as pPDPimpdh. Then, plasmid was purified with the EndoFree Plasmid Mega purification kit. CS-NPs, CS-ulvan-NPs and DLPLG-NPs were prepared as described by Jiménez-Fernández et al. (2014), Fernández-Díaz et al. (2017) and Csaba et al. (2010), respectively. Size, polydispersity index and Z potential of NPs were then calculated by dynamic light scattering on a ZetaSizer Nano-ZS90 (Malvern, UK). The stability of pPDPimpdh in NPs was examined using DNase I (BioLabs).

Results and discussion

In this work, the *impdh* gene from *Phdp* was used to prepare a DNA vaccine (pPDPimpdh). The integrity of the plasmid was confirmed by agarose gel electrophoresis. The characterization of nanoparticles elaborated is depicted in Table 1.

Table 1: Nanoparticle characteristics: Particle size (nm), Z-potential (mV) and polydispersity index (PI).

	Particle size (nm)	PI	Z-potential (mV)
CS-NPs	168.4 ± 18.3	0.25 ± 0.02	+24.5 ± 2.33
CS-Ulvan-NPs	387.5 ± 6.6	0.17 ± 0.01	+33.0 ± 0.14
DLPLG-NPs	301.8 ± 8.4	0.19 ± 0.03	-46.0 ± 1.27

In the area of drug delivery systems a NP size ranging between 100 and 500 nm is accepted. In addition to the particle size, the charge of the particle is also an important characteristic to consider for efficient gene delivery. Those NPs that have a higher zeta potencial value, will have a better stability (Kole et al., 2018). The size of all of the NPs prepared in this work was lower than 500 nm. Moreover, zeta potential of CS-NPs and CS-Ulvan-NPs were found to be +24.5 and 33.0 mV, respectively. Zeta potential of DLPLG-NPs was -46.0 mV. DNase enzyme failed to degrade the NPs conjugated with pPDPimpdh so stability of the constructions was confirmed. All these results indicate that the NPs prepared in this study are suitable for pPDPimpdh delivery into host tissues.

Acknowledgments

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Antioxidant properties of algae of the genus *Cystoseira* from the coasts of Cadiz

María Mena*[1] , Carolina de los Reyes[1] , María J. Ortega[1] , Eva Zubía[1]

*Corresponding author: maria.menarodriguez@alum.uca.es
[1] Department of Organic Chemistry, Faculty of Marine and Environmental Sciences, University of Cadiz

Keywords: Antioxidant, *Cystoseira*, Macroalgae, Natural products

Abstract

Oxidative stress, or imbalance between the production of free radicals and the endogenous antioxidant defenses, is involved in aging processes and in the development of a number of diseases (Liguori et al., 2018). The excess of reactive oxygen species and of other free radicals has detrimental effects on cellular components, including lipids, proteins, and DNA, resulting into various disease states. Therefore, antioxidants that quench or inhibit radical reactions may have a key role in the prevention and even the fight against aging and oxidative stress-associated diseases (Nimse and Pal, 2015). In this line, the use of antioxidants in functional foods, nutraceuticals, cosmetics, and pharmaceuticals has been greatly extended during the last years. Moreover, the growing preference of consumers for compounds from natural sources over synthetics, has led to intense research on the antioxidant capacity of natural products. Macroalgae have been shown to produce an array of compounds provided of interesting biological properties including antioxidant activity (Balboa et al., 2013). In particular, brown algae have been the richest algal source of antioxidant natural products, mainly meroterpenoids and phlorotannins. Brown algae of the genus *Cystoseira* (family Sargassaceae) are widely distributed in the Mediterranean Sea and the adjacent Atlantic coasts, with several species thriving along the coasts of Cadiz. Our recent study of specimens of *C. usneoides* collected in Tarifa (Cadiz) led to the obtention of a series of natural products of the meroterpenoid class, which showed significant antioxidant and anti-inflammatory properties (De los Reyes et al., 2016). These results prompted us to investigate the antioxidative potential and the natural products profile of other species of the genus *Cystoseira* settled along the coasts of Cadiz. In the present study, samples of *C. barbata*, *C. compressa*, and *C. foeniculacea* were collected at different locations of Cadiz coastline and subjected to extraction procedures for the obtention of diethyl ether and *n*-butanol extracts of each species. The antioxidant activity of all extracts was evaluated by using the ABTS assay (Re et al., 1999). In this method, the ABTS is chemically or enzymatically oxidized to its radical cation, which is intensely blue-green colored, and the antioxidant capacity of a compound is measured as its ability to reduce the color by reacting with the radical. All extracts exhibited significant radical-scavenging activity although with different potency. Nonetheless, the levels of activity observed for the extracts of *C. barbata*, *C. compressa*, and *C. foeniculacea* were higher than those reported for other species of this genus. The extracts of *C. barbata*, *C. compressa*, and *C. foeniculacea* were also analyzed by nuclear magnetic resonance spectroscopy (NMR) and, in some instances, subjected to separation by using column chromatography, on silica gel or on sephadex LH-20, followed by repeated HPLC purification steps. These chemical studies have allowed to define the presence or absence of meroterpenoids in the analyzed algal species, to isolate pure meroditerpenoids whose structures were determined by spectroscopic means, mainly NMR and mass spectrometry, and to obtain phlorotannin enriched fractions which possess potent radical-scavenging activity.

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Antitumoral and antioxidant activities of sulfated polysaccharides from *Durvillaea antarctica*

Virginia Casas Arrojo*[1] , Mª de los Ángeles Arrojo Agudo[1] , Roberto T. Abdala Díaz[1]

*Corresponding author: virginiac@uma.es
[1] Malaga University

Keywords: Antioxidant capacity, Antitumoral activitie, Durvillaea antarctica, FT-IR spectroscopy, Sulfated polysaccharides.

Introduction

Algae are widely recognized for their nutritional value. For decades, seaweeds have been an important source of secondary metabolites attracting the attention of researchers and pharmaceutical companies around the world. Sulfated polysaccharides of marine algae are one of the most studied metabolites as they offer a wide range of physiological properties, finding diverse applications as anticoagulant agents and antihyperlipidemics, antivirals and antitumor (Jiao et al., 2011). The species *Durvillaea antarctica* is easy to find in rocky substrata of high levels of the sublittoral exposed to the waves of the South Pacific coasts. It is very appreciated from a gastronomic point of view, in countries like Chile (where it is known as “Cochayuyo") an important economic and commercial activity is developed around these algae.

Materials and Methods

Polysaccharides were extracted in EtOH and N-methylpyridiniumbromide at 2% were dialyzed and lyophilized (Abdala Diaz et al., 2011) The cytotoxic activity in the cell line of mouse macrophages (RAW 264) and antitumor in human colon cancer (HTC-116), human leukemia (U-937) and human breast cancer (MCF-7) cell lines was determined by the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Abdala Diaz et al., 2011). Antioxidant capacity was evaluated by the ABTS trial according to Re et al. (1999) with few modifications (Re et al., 1999). These polysaccharides were characterized by Fourier transform infrared spectroscopy (FT-IR).

Results and Conclusion

This study shows that these polysaccharides have anticancer activity mainly in the cell line HTC-116. The IC50 obtained in cell lines U-937, HTC-116 and MCF-7 are 496.5 μg mL-1, 19.99 μg mL-1 and 763.2 μg mL-1 respectively. The highest value of antioxidant activity of the studied concentrations was obtained at 500μg mL-1 obtaining an activity of 14.3 $\pm$ 0.5 μmol TE g-1 DW. The FT-IR spestrous of polysaccharides obtained from D. antarctica show different peaks corresponding to different groups that make up the polysaccharides (Figure 1). Other studies on these polysaccharides are necessary but could be considered for studies as nutraceuticals.

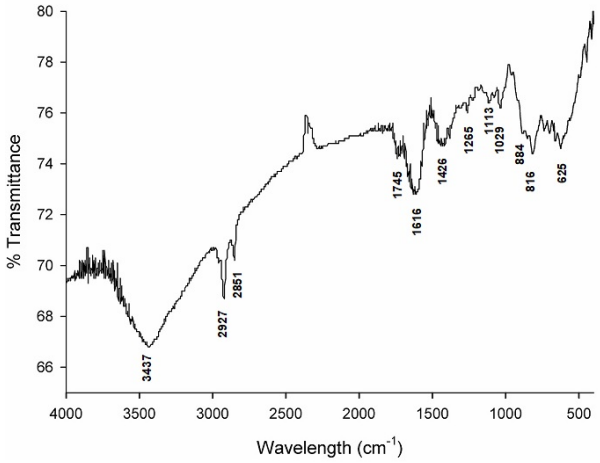

Figure 1: FT-IR spectroscopy of the polysaccharide obtained from D. antarctica.

Acknowledgments

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Laminaria ochroleuca brown seaweed as a rich edible source with high valuable compounds

Noelia Flórez-Fernández*[1] , María Dolores Torres[1] , Herminia Domínguez[1]

*Corresponding author: noelia.florez@uvigo.es
[1] Department of Chemical Engineering, Science Faculty, University of Vigo, Campus Ourense, 32004 Ourense, Spain

Keywords: Brown seaweed, Autohydrolysis, Bioactive compounds, Cytotoxicity, Rheological properties

Abstract

Marine seaweeds have a great nutritional value. Since hundreds of years, Asiatic countries have introduced seaweeds in their day a day diets. Nowadays, within the scientific field, seaweeds have been widely studied. Their compounds present a huge potential for different food and non-food industries. In this context, the extraction of the bioactive and gelling or thickening fractions has a dramatic relevance. Fucoidan, mainly composed by fucose and sulphate groups, and phenolic compounds have an important role in the pharmaceutical field (Venkatesan et al., 2015). A number of technologies have been studied to extract these potentially active compounds (Michalak and Chojnacka, 2014). Alginate from *Laminaria ochroleuca* brown seaweed is currently used in a wide range of products as stabilizer or thickener (Holdt and Kraan, 2011). This edible seaweed is found in the Atlantic coast, which makes the study of its bioactive and biopolymeric compounds very interesting for the Iberian Peninsula regions. The main aim of this study was the recovery of high valuable compounds from this edible seaweed using green extraction technologies. Autohydrolysis extraction was proposed as an environmentally friendly technology. A broad range of temperatures from 120 to 220 °C was studied with the purpose of optimising the optimal temperature for the extraction of the fucoidan fraction. The liquid:solid ratio was previously determined, being 30:1 (González-López et al., 2012). After the extraction process, liquid and solid extracts were separated by filtration process. Compounds from the liquid phase were analysed using spectrophotometric assays. Fucose content, antioxidant activity, phloroglucinol content and cytotoxicity in four human lines were analysed. Alginate fraction was extracted from liquid phase, adding 1% of $CaCl_2$. The rheological behaviour associated with the extracted alginate fraction was also analysed. To conclude, the obtained outcomes provided promising results, in terms of the recovery yield and quality of enriched liquid fractions in bioactive and alginate fractions. The mechanical properties of isolated alginates exhibited comparable features to those of the commercial biopolymers.

Acknowledgments

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Characterization of offshore wind in Spain and Europe

Marina Garrido Cabezas*[1] , Antonio Avilés Benítez[1]

*Corresponding author: marinagarkuk@gmail.com
[1] University of Málaga, Faculty of Sciences, Department of Ecology and Geology

Keywords: Wind turbine, Marine wind energy, Offshore technology, Floating wind power, Deep sea bottom

Introduction and Objectives

There are several factors that appear in the scene when it comes to distinguish between marine and terrestrial wind power, among which social, economic and even technological components stand out, since the creation of a marine wind farm is much more complicated than a terrestrial one. In recent years, there have been new and important advances in this field. Even so, this new model of renewable energy continues to come uphill for Spain. Spanish coast is quite deep, which makes it difficult to place fixed bases that mills have used until now; although, a new model of floating wind turbine has already come into operation in The Canary Islands. The European continent tops the world ranking marine wind farms, where the United Kingdom monopolizes 45 percent of them, followed by Germany with 35 percent.
The realization of this project involves achieving certain objectives, among which are: To learn and study in depth how marine wind turbines work and the technology that surrounds them. Understand in which area each type of lift should be used and which are the most suitable wind turbines to be used in the open sea. Explain briefly the different configurations that can present the wind farms and the problems they have.To see the evolution of this technology and its current weight both in European countries and in the rest of the world, as well as its future projections. Contextualize the progress of offshore in our country and the steps it has taken in this regard. Make a comparison with the traditional terrestrial wind. To know the environmental impacts that this type of mill generates in the marine environment. Draw conclusions about the future of this technology.

Current situation of offshore wind in Spain and Europe

Northern European countries have been in charge of developing and dominating the offshore wind market; is largely due to the fact that the depths of their seas do not exceed 50 metres, as well as a great wind resource as shown in Figure 1. Another factor that has made these countries so innovative is that they have established maritime industries (gas and oil), this makes them play with some advantage in this field (Azul, 2018) 2020, growth projections assume 25 GW. The development experienced by this technology arises thanks to the goal proposed by certain countries for the expansion and growth of this energy in their seas; there are more and more technicians, engineers and scientists capable of carrying out different successful offshore solutions, capable of guaranteeing and covering the needs of the marine environment; and that can allow the implementation of parks in the shortest possible time and with ever lower prices.

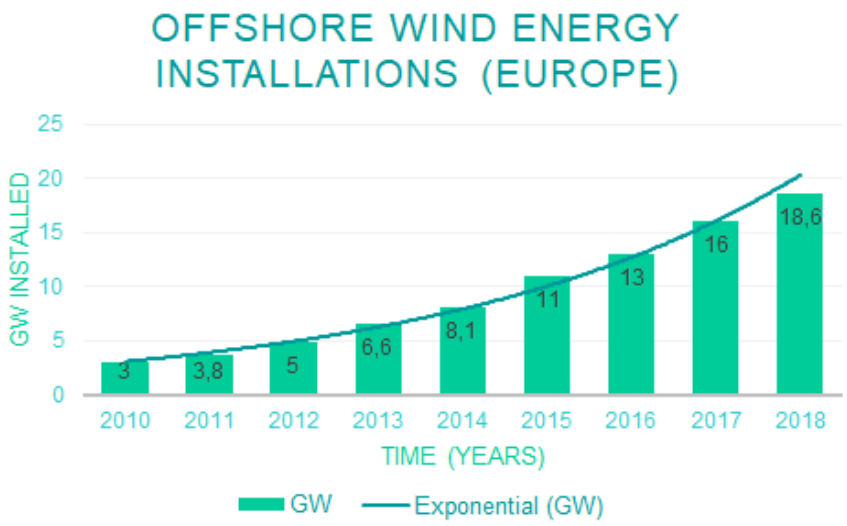

Figure 1: Evolution of offshore facilities in Europe since 2010 (Source: Prepared by the authors based on data extracted from Wind Europe's annual report).

It is this combination of factors that makes countries such as the United Kingdom, Denmark, Germany and Belgium top of the list of installed power (Virgilio, 2018). On the other hand, the wind resource of the Spanish coasts is lower than that found in Nordic countries is even lower than that which may exist in countries that are geographically closer, such as the United Kingdom or France. The first step forward taken by our country in the field of offshore wind power was the installation of a wind turbine at the Arinaga quay in Gran Canaria in July 2013. This mill, with a power of 5 MW, is subject to a marine wind regime, even though it is located on land. The start-up of this turbine had no other purpose than to serve as a preliminary step before the installation of a new hundred percent offshore wind turbine off the same coasts of Gran Canaria called ELISA, which in addition to being the first in Spain is also the first with fixed foundations in southern Europe. It is already connected to the grid and generating energy; its installation has marked a before and after for offshore wind, as it has been lifted without the need for cranes.

Conclusions

There is a growing awareness of the energy transition and a greater rejection of hydrocarbons by society; this transition goes hand in hand with renewable energies. Offshore wind energy has evolved impressively since its inception in 1991, although it is still in development. Europe, as a key power in the expansion of this new technology, has very stable future projections. This is due to the fact that, in general, the countries that have offshore wind farms have very realistic medium and long term challenges that are easy to meet, among them the United Kingdom and Germany, followed from a distance by Denmark. The progress made makes it possible to reduce costs and to place offshore in a very competitive way in the clean energy market.

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Sunfish buoy (International Patent for Oceanographic Buoy): a naval Engineering and oceanography combination

Daniel José Coronil Huertas*[1] , Juan Manuel Vidal Pérez[1] , José Juan Alonso del Rosario[2] , Santiago Pavón Quintana[1]

*Corresponding author: daniel.coronil@uca.es

[1] Navigation Sciences and Techniques and Ship-Building Department, School of Naval and Oceanic Engineering, University of Cadiz, Avda. República Saharaui s/n, Puerto Real, 11510, Cádiz, España.

[2] Applied Physics Department, Faculty of Marine and Environmental Sciences, University of Cadiz, Avda. República Saharaui s/n, Puerto Real, 11510, Cádiz, España.

Keywords: Oceanographic moorings, Drag force, Lift force, Drag coefficient, Computational Fluid Dynamics

Introduction

A number of oceanographic surveys are carried out every year to measure many oceanographic and meteorological variables. One of the main requirements for any mooring is its minimum possible excursion of its components as a reaction to the current. The measurements of oceanographic and meteorological variables in the ocean by means of oceanographic mooring systems report errors and deviations, especially in places with high dynamics (for example in the Strait of Gibraltar (Vidal et al., 2006), south of the Iberian Peninsula). Hence, for those places with high hydrodynamic regime, a very careful and detailed study is usually required. Vertical excursions of the mooring lines due to the drag force of the current is a critical factor because it causes errors in time series recording (Vidal et al., 2011). The key to improve this type of mooring is the buoys design with the maximum buoyancy and the minimum drag force possible (Zambrano et al., 2006).

Material and methods

There are many patents on the design of buoys but none of them could be classified as “optimum”. At the same time, many innovative projects have been developed concerning the oceanographic moorings in the last decades. In the frame of this study, a special designed and patented prototype of a buoy by INNOVA OCEANOGRAFIA LITORAL S.L. and the University of Cadiz has been chosen (National Patent number ES2645314; International Patent Number WO2017/207834). Its shape is quite close to an ocean sunfish (Figure 1). This allows a lower drag than an equivalent spherical buoy.

Figure 1: The Sunfish Buoy - Patented prototype of a special buoy.

The methods for designing and analyzing in Engineering are the numerical simulation and experimentation. The first one involves the application of partial derivatives equations (drag coefficient calculations made with numerical simulations in CFD - Computational Fluid Dynamics, solving the Navier-Stokes equations). The computational works use 2D and 3D numerical simulations by means of finite elements and finite volumes with the TDYN CFD+HT software (Figure 2).

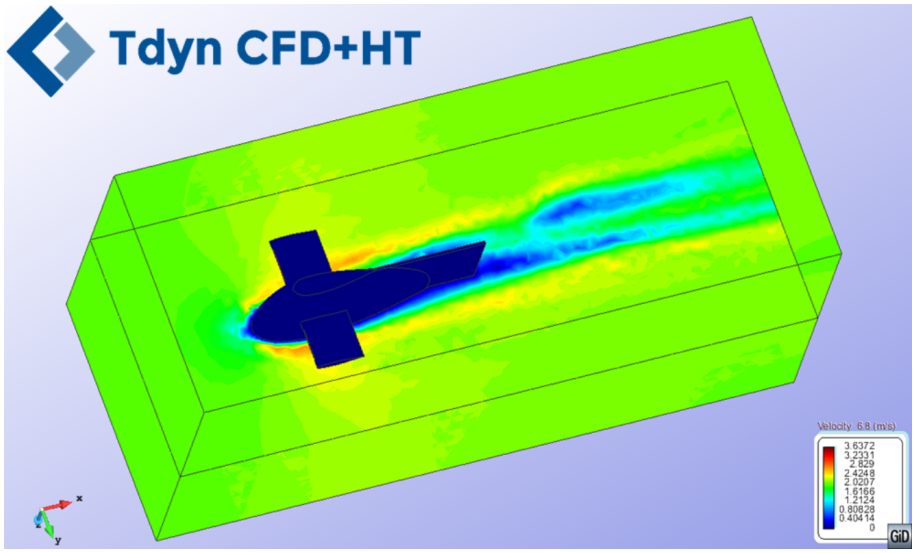
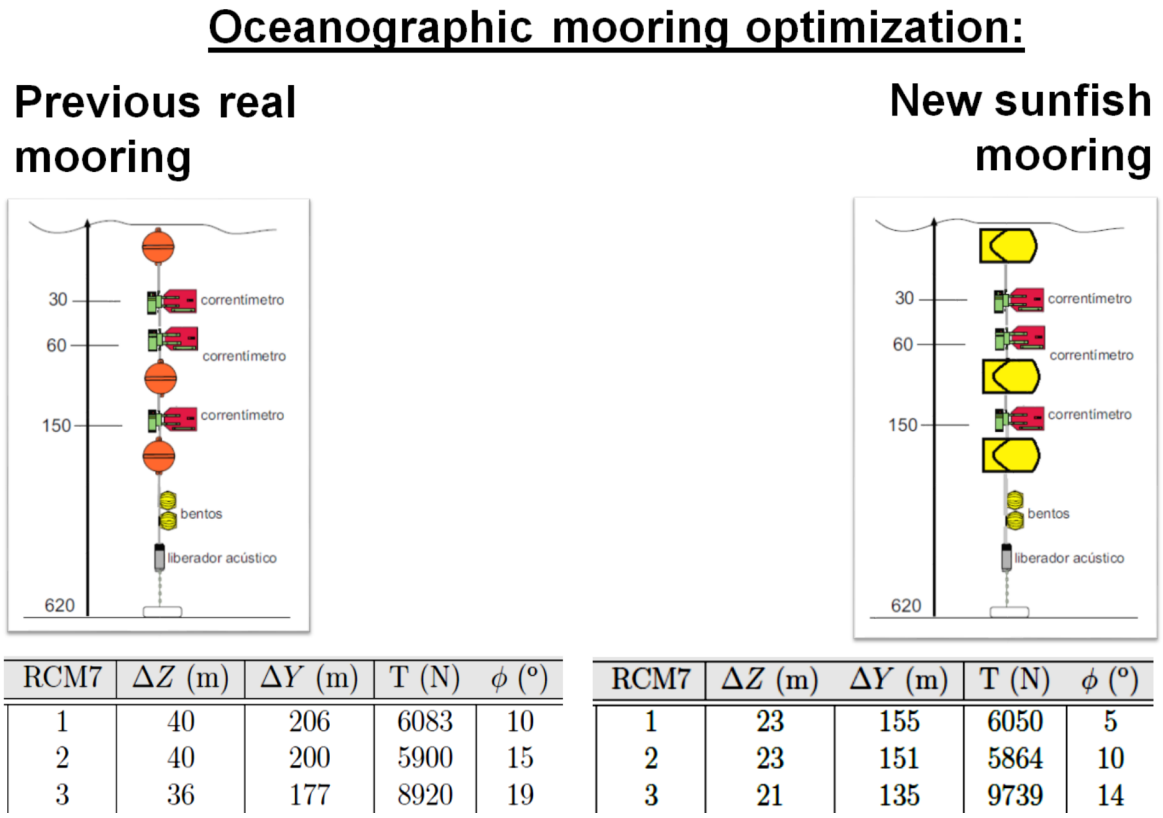


Figure 2: Numerical simulations in CFD with the TDYN CFD+HT software.

Results and conclusions

The comparison of the results of the numerical simulation of a sunfish buoy with the equivalent spherical buoy (Figure 3) has led to a reduction of about 56% of the drag force, vertical excursion (ΔZ), horizontal excursion (ΔY), wire tensions (T) and inclination (Θ).



RCM7	ΔZ (m)	ΔY (m)	T (N)	ϕ (°)
1	40	206	6083	10
2	40	200	5900	15
3	36	177	8920	19

RCM7	ΔZ (m)	ΔY (m)	T (N)	ϕ (°)
1	23	155	6050	5
2	23	151	5864	10
3	21	135	9739	14

Figure 3: Oceanographic mooring optimization with the Sunfish Buoy.

Naval Engineering calculations applied to an Oceanographic problem success to develop a patented oceanographic buoy, with a new geometry (a sunfish-like shape). Sunfish buoy reduces the mooring line inclination, and therefore, minimizes the errors in the recordings of the measuring instruments.

Acknowledgments

Special thanks to INNOVA OCEANOGRAFÍA LITORAL S.L. for the collaboration in this project.

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Study and ecosystem analysis of the coast of Tenerife: municipality of Granadilla de Abona

Rodrigo Barroso-Bolaños*[1]

*Corresponding author: rodrigo.barrosobolanos@alum.uca.es
[1] University of Cádiz

Keywords: Ecosystem-Based Management, Coastal management, Integrate Coastal Zone Management, Natural capital, Marine angiosperms

Introduction

Large urban cities are home to a large percentage of the world's population and settle to a greater extent near the coastline. They consume approximately three quarters of the world's resources and it is expected that 40% of the population growth estimated for 2027 will occur in these environments. Not only is there a preference for these environments linked to economic and social wealth, but 90% of the world trade transported by large ships depends on it. Likewise, the current economic model uses natural resources-capital without any kind of sustainable planning, surpassing the planet's limit to provide support and supply, and without taking into account the carrying capacity of the affected ecosystems (FAO, 2017). For this reason, after decades of imperative development, some of the impacts of overexploitation are visible, such as the progressive rise in sea level, the accentuation of the effects of natural disasters, the loss of natural and soil barriers, as well as the deterioration and destruction of habitats, biomass and biodiversity, as well as associated problems such as the presence of plastics and air and water pollution. All these impacts culminate in a common point: the total or partial loss or degradation of ecosystems and their ecosystem services (SE), which translates into a deterioration in the quality of life of people (Gret-Regamey et al., 2017). These losses are more pronounced in islands, known as hot spots of biodiversity and SE, due to their strong isolation and the presence of a significant number of endemisms, protected species and endangered species in their ecosystems. The Canarian archipelago consists of seven islands located northwest of the African continent. The study area is located in the southeast of the island of Tenerife and includes the municipality of Granadilla de Abona (Figure 1), which is subject to different pressures derived from anthropogenic activities. In addition, it presents several figures of protection as a natural park, a community interest place (c, d), a marine ecosystem with meadows of seagrass, *Cymodocea nodosa* (e) (Barberá et al., 2005), flanked by several population centers, marina, fishing and industrial ports, an industrial state and an airport (b, f, a), which translates into a number of activities that converge in space, making it difficult to manage it.
The aim of this work is to analyze and evaluate the existing SEs in the Maritime Terrestrial Public Domain (DPMT), (South end 28°01'40,97"N, 16°35'47'95"W and North end 28°65'38,69 "N, 16°29'62,40"O) in the Servitude Zone and one nautical mile offshore of the study area, to facilitate the decision-makers the implementation of an integrated management of coastal areas based on the SE.

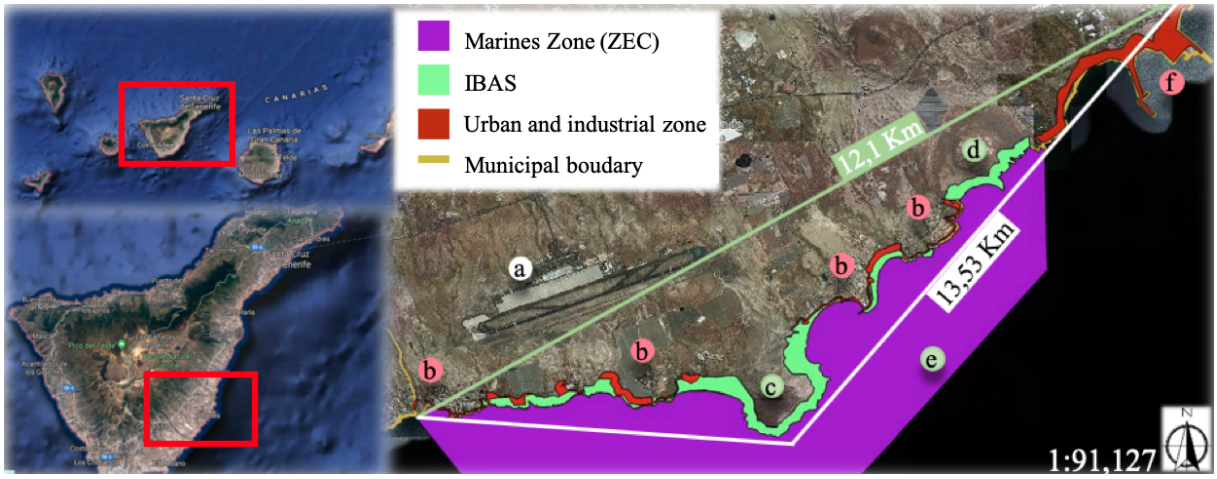

Figure 1: Cartography of the municipality of Granadilla de Abona: (a) Airport (b) Population center (c) Natural Park (d) Community Interest Place (e) Seagrasses (f) Industrial state.

Materials and methods

The methodology applied has been based on a literature review and the undertaking of fieldwork, through an adaptation of Scherer and Asmus (2016). A characterization of the study area has been carried out, taking into account the classification into 3 subsystems of the integrated management of coastal areas (physical-natural, socio-economic and administrative legal). A cartography has been made through the integration of 28 layers, to study the interactions between ecosystems, their services and population centers, using the free software QGIS 2.18.14 'Las Palmas'. The SEs have been quantified and evaluated through a panel of experts, based on the classification of Regulation, Support, Culture and Supply, given a value to the importance of the SE: (1) Low, (2) Medium and (4) High; and to the tendency of the subsystem against anthropogenic actions: (-1) Positive, (2) No modification and (3) Decrease. Multiplying the value of the trend by that of importance, vulnerability has been obtained. Finally, the existing problems were analyzed through the DPSIR model (Driving forces; Pressures; States; Impacts and Reponse).

Results and discussion

25 SEs have been identified within the 5 ecosystems that have been studied (Montaña Roja - MR, Montaña Pelada - MP, Arid Lands - RA, Dune-Beach System - DP, and marine ecosystem - EM, including a marine phanerogam zone), 7 of supply, 3 of cultural, 10 of regulation and 5 of support, which come into conflict in 41 points of the area under study.

Table 1: Vulnerability matrix calculation for ecosystems and SE.

	Supply							Cultural			Regulation										Support					4		
Ecosystems/SE	(A) Provision of Food	(A) Breeding and reproduction	(A) Nursery	(A) Water resource	(A) Genetic resources	(A) Raw materials	(A) Biomass production	(C) Landscape - Aesthetic Values	(C) Recreational activities	(C) Historical Heritage	(R) Climate balance	(R) Nutrient Cycle	(R) Pollution purification	(R) Dune system control	(R) Soil protection	(R) Producción de biomasa	(R) Morphosedimentary fixation	(R) Erosion regulation	(R) Carbon sink	(R) Coastal line protection	(S) Water cycle	(S) Habitat	(S) Navigability	(S) Effluent receiver	(S) Stock genético	Nº SE/Ecosystem	Σ values / SE	Vulnerability / Ecosystem
Montaña Roja (MR)	6	12	12		8			12	4	12		2		12	2	4				4		4			12	14	106	8
Montaña Pelada (MP)	3	6	6		6			12	4	8		12			2	8				2		6			4	13	79	6
Arid lands (TA)						4			2	12		2										2				5	22	4
Dunes - Beachs (DP)	3				3			6	8	12							12	12		12		12		4	3	11	87	8
Marine Ecosystem (EM)	6	12	12	4	12	2	12	6	2		4	12	8		12			12	12		2	12	4	4		19	150	8
Nº ecosystems / SE	4	3	3	1	4	2	1	4	5	4	1	3	1	1	3	2	1	2	1	3	1	5	1	2	3	62		
Nº SE / type of	18							13			18										12							
% type of SE / frequency	29,51%							20,97%			29,03%										19,35%							
Σ values / SE	18	30	30	4	29	6	12	36	20	44	4	28	8	12	16	12	12	24	12	18	2	36	4	8	19			
$\bar{x}$ / SE	5	10	10	4	7	3	12	9	4	11	4	9	8	12	5	6	12	12	12	6	2	7	4	4	6			
Σ valoues / type of SE	129							100			146										69							
$\bar{x}$ vulnerability / type of SE	7,17							7,69			8,11										5,75							
Global vulnerability / SE	0,3	0,5	0,5	0,1	0,5	0,1	0,2	0,6	0,3	0,7	0,1	0,5	0,1	0,2	0,3	0,2	0,2	0,4	0,2	0,3	0	0,6	0,1	0,1	0,3			

For a better understanding of Table 1, two graphs have been made (Figure 2) that show the vulnerability values for each ecosystem and for each SE identified.

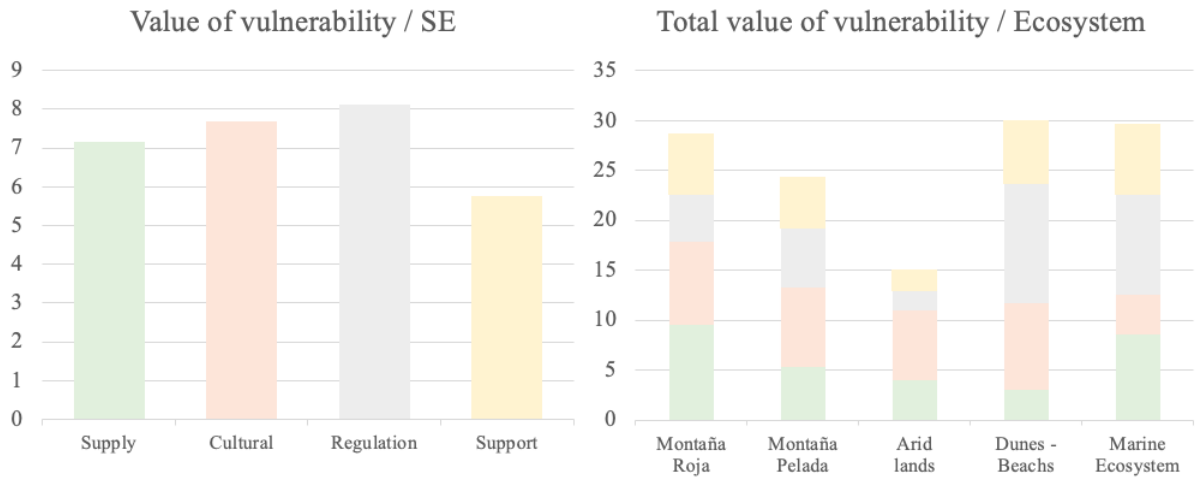

Figure 2: Vulnerability values by ecosystem and SE.

As it can be seen, the ecosystems with the highest vulnerability index are DP and EM, followed by MR, MP and TA. As for the SE, the greatest vulnerability is presented by the Regulation services, followed by the Cultural, Supply and Support services. Both in the marine ecosystem and in the dune-beach system, the regulation service registers greater vulnerability, this presents the same behavior when we study SEs in isolation and when we study them within each named ecosystem. Taking into account the rest of the ecosystems, the behavior of the proportion of the vulnerabity for each service varies with respect to the behavior presented by the SE as a whole.

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Study of the immune response in *Mitylus galloprovincialis* against ecotoxic particles of polystyrene nanoplastic

Marta Vila*[1] , Marta Sendra[2] , Pilar Yeste[2] , Ana Molina[1] , Rosa Leon[1]

*Corresponding author: marta.vila@dqcm.uhu.es
[1] Universidad de Huelva
[2] Universidad de Cadiz

Keywords: Nanoplastic, *Mitylus galloprovincialis*, Ecotoxic

Summary

Marine ecosystems are increasingly threatened by the presence of human-origin pollutants, mainly plastics. Polystyrene (PS) is one of the most abundant in the ocean, characterized by its versatility (food and industrial containers, disposable cutlery, compact discs, insulation of buildings, medical products and toys) and being a non-biodegradable polymer. Marine plastic pollution has increased in the last decade due to social consume model, where only a 6-26% of total plastic is recycled. The main fate of plastics are oceans which keep in between a 70-80% of plastic producedby manufacture (Alimi et al., 2018). The Mediterranean mussel is considered an outstanding ecological and economic value species in our country. It develops an immune response to these particles of polystyrene nano-plastic (NPs), through cell-mediated immunity of blood cells or hemocytes, as has been described in recent years (Canesi et al., 2012). The identification of the basic mechanisms of immunity and its modulation in mussels by studying different genes involved in the cellular response can provide important information and stablish this specie as an invertebrate model for studies on innate immunity and future immunotoxicological studies (Canesi et al., 2016).The present work studies the genes involves in the response immune triggered due to the presence of NPs particules of 50 nm, 100 mn, 1 μg.

Objetives

To study the genes involves in immune response in *Mitylus galloprovincialis* against ecotoxic particles of polystyrene nanoplastic (50 nm,100 mn, 1 μg). The selected genes had been: Chitinasa, IL-17, mTOR, p53, Lyso gene.

Results

Up to now, the results obtained show that most of the studied genes are expressed in the different tested conditions. However, the expression depends on the size of the particule and the exposure time to them. Moreover, the gen IL-17 does not expression in either condition studied. We conclude that the highest expression in most of the genes is found with the biggest NPs size.

Acknowledgments

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A new selective marker gene for the genetic transformation of microalgae

Ana Molina-Márquez*[1] , Marta Vila[1] , Javi Vigara[1] , Rosa León[1]

*Corresponding author: ana.molina@dqcm.uhu.es
[1] Departamento de Química. Facultad de Ciencias Experimentales. Universidad de Huelva. Campus de El Carmen. Avda. Fuerzas Armadas s/n. 21007 Huelva, Spain.

Keywords: Phytoene desaturase (CRTI), Phytoene desaturase (PDS), Phytoene synthase, *Chlamydomonas*, Norflurazon

Introduction

Microalgae are appreciated due to the wide range of compounds with an interesting application in different industrial sectors, from nutritional to pharmaceutical industry. Among the metabolites that they are able to produce are lipids, carotenoids, and bioactive products. Genetic manipulation is a necessary mechanism to further boost the productivity of microalgae-based compounds (Varela et al., 2015). However, the current microalgae selection methods are mainly based on the use of antibiotics, which have raised concern about the impact on human health and the environment. In this work, we propose an herbicide-resistance gene as a selectable marker and the bleaching herbicide norflurazon as a selective agent for the genetic transformation of microalgae. Phytoene desaturase (PDS) is a key enzyme in the carotenoid pathway in microalgae and plants, which catalyse the conversion of the colourless phytoene into chis-carotene. That intermediary is transformed into lycopene by chis-carotene (ZDS) and carotene isomerase (CRTISO). PDS of algae and plants are sensitive to norflurazon, and the carotenoid pathway is blocked, avoiding the conversion of the colourless carotenoid phytoene into lycopene. However, bacteria and fungi accomplish the conversion of phytoene into lycopene by a single enzyme, bacterial phytoene desaturase (CRTI), which is not affected by norflurazon and the carotenoid pathway is not blocked (Cunningham and Gantt, 1998; Breitenbach et al., 2001) In this study, a version of the *CRTI* gene adapted to the codon usage of *Chlamydomonas* (*CRTIop*) has been subcloned in a microalgae expression vector and has demonstrated to be a suitable selectable marker (Molina-Márquez et al., 2019)

Objectives

- To valuate the norflurazon minimal concentration inhibitory in *Chlamydomonas reinhardtii*.
- To design a synthetic *CRTI* gen version with the codon usage adapted to *C. reinhardtii* (*CRTIop*) and construction of plasmid PSI106-tpCRTIop.
- To analize *tpCRTIop* as a suitable selective marker gene in the genetic transformation of the model microalga *C. reinhardtii*.
- To examine phenotypically microalga transformants by HPLC.

Results and Conclusion

The microalga was transformed by the glass bead agitation with the plasmid PSI106-CRTIop method and selected in the presence of 1.5 μg mL-^{1} of norflurazon, concentration which was previously identified as inhibitory. Average transformation efficiencies of 550 colonies μg DNA-*1* were obtained. All the transformants incorporated the *CRTI* gene in their genomes, as shown by PCR, and most had a usual carotenoid composition, as shown by HPCL analysis.
We conclude that:

- It has been proved that*CRTIop* is a reliable selective marker in *Chlamydomonas*.
- Norf^{R}-*Chlamydomonas* obtained by transformation with *tpCRTIop* gene have normal pigments profiles in the presence of norflurazon, while in the control cells in presence of norflurazon there is a strong reduction of most of the colored carotenoids and accumulation of phytoene.
- Selection of transformants microalgae based on *tpCRTIop* is an interesting alternative to antibiotic-based transformants selection.
- Norflurazon sensitivity tests done in other microalgae species indicate that this could be a universal market for genetic transformation of microalgae

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Cross-Border Approach for Maritime Spatial Planning: Stakeholders engagement in a pilot transbounday Marine Protected Area between Spain and Portugal

Mónica Campillos Llanos*[1] , Cristina Cervera Núñez[1] , María Gómez Ballesteros[1] , Márcia Marques[2] , Adriano Quintela[2] , Lisa Sousa[2] , Fátima Lopes Alves[2] , Carla Murciano Virto[3] , Ana Lloret[3]

*Corresponding author: monica.campillos@ieo.es
[1] Instituto Español de Oceanografía
[2] University of Aveiro
[3] Centro de Estudios y Experimentación de Obras Públicas (CEDEX)

Keywords: Maritime Spatial Planing, Stakeholders, MPA, SIMNORAT, Transboundary cooperation

Introduction

The UE adopted a Directive to establish a common framework for Maritime Spatial Planning (MSP) among Member States (Directive 2014/89/EU), it claims the needs for an efficient and sustainable management of marine ecosystems and maritime activities, avoiding conflicts and promoting synergies between different uses of the sea at different scales (local, regional, national, transnational). According to article 9, Member States shall establish means of public participation by informing all interested parties and by consulting the relevant stakeholders and authorities, and the public concerned, at an early stage in the development of maritime spatial plans, in accordance with relevant provisions established in Union legislation. In this context, the European project "SIMNORAT: Supporting Implementation of Maritime Spatial Planning in the Northern European Atlantic" aims to support the implementation of the MSP Directive in the North Atlantic and to encourage cross-border collaboration on spatial planning issues. Specifically, the project includes a case study between Spain and Portugal to implement a Marine Protected Area in the Galicia Bank - Vigo and Vasco da Gama Seamounts.

Stakeholders context

The analysis and improvement of the stakeholder's engagement processes is essential in these kind of initiatives to involve stakeholders participation from early stages of MSP processes from each country and ensure the compliance of legal requirements from public participation of both countries. It includes public consultations and a transboundary stakeholder's workshop organized by the Spanish Institute of Oceanography (Spain) and the Universidade de Aveiro (Portugal). The workshop aimed to contribute to cross-border cooperation on MSP through involvement of stakeholders from different groups of maritime activities (Figure 1), in order to consider their interests in the area. For that purpose, participants discussed about potential interactions that could arise between activities, analyzing synergies, looking for conflicts, gaps and proposing solutions to face the challenge of a future creation of a cross-border MPA.

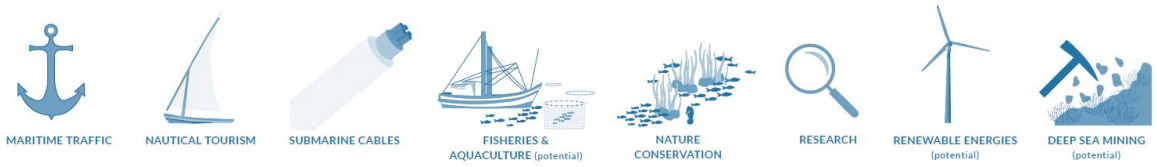


Figure 1: Maritime activities.

Stakeholders' perception

The general conclusions highlighted by the different working groups were synthesized in Figure 1 regarding synergies, conflicts, gaps and proposed solutions for the implementation of a cross-border MPA in the study area. According to this, there were obtained solutions summarized in Table 2.

Table 1: Stakeholders conflicts.

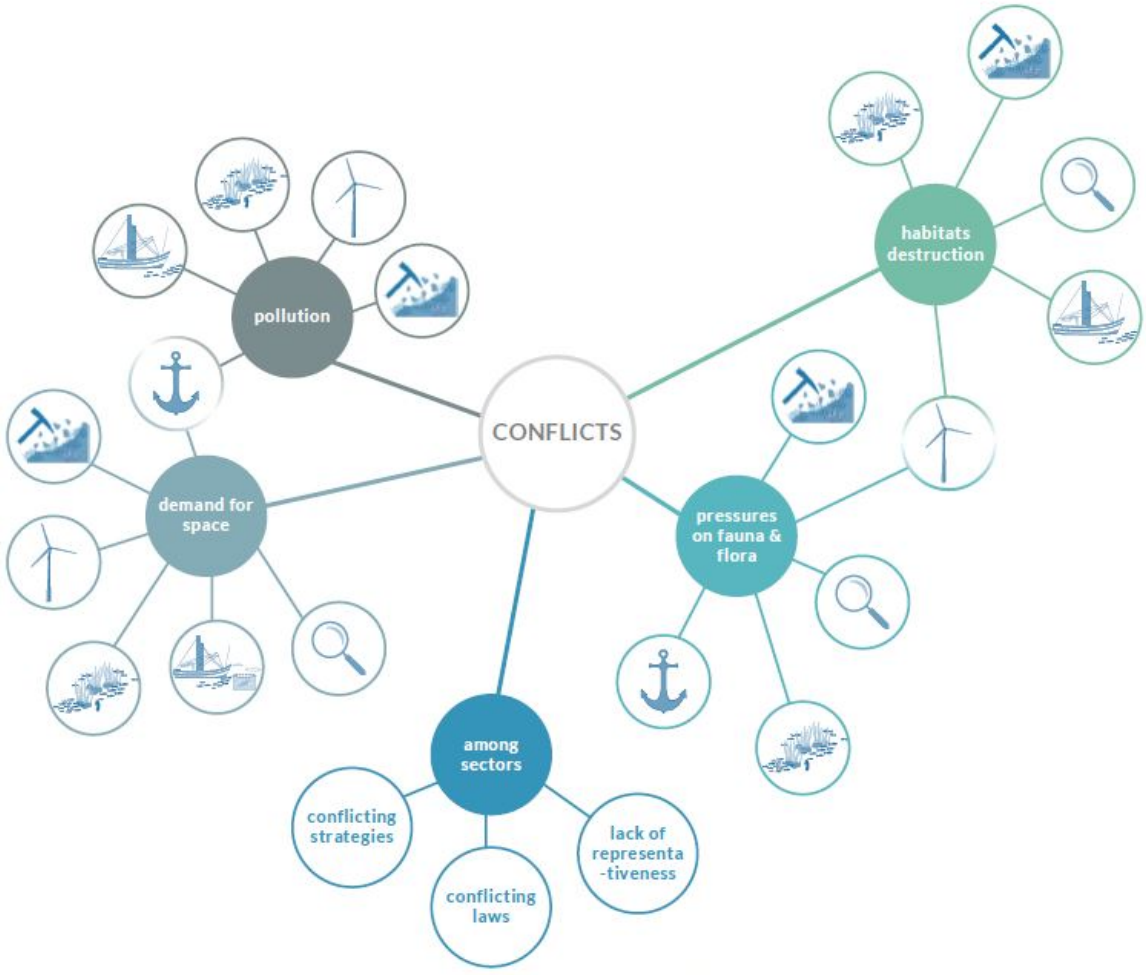
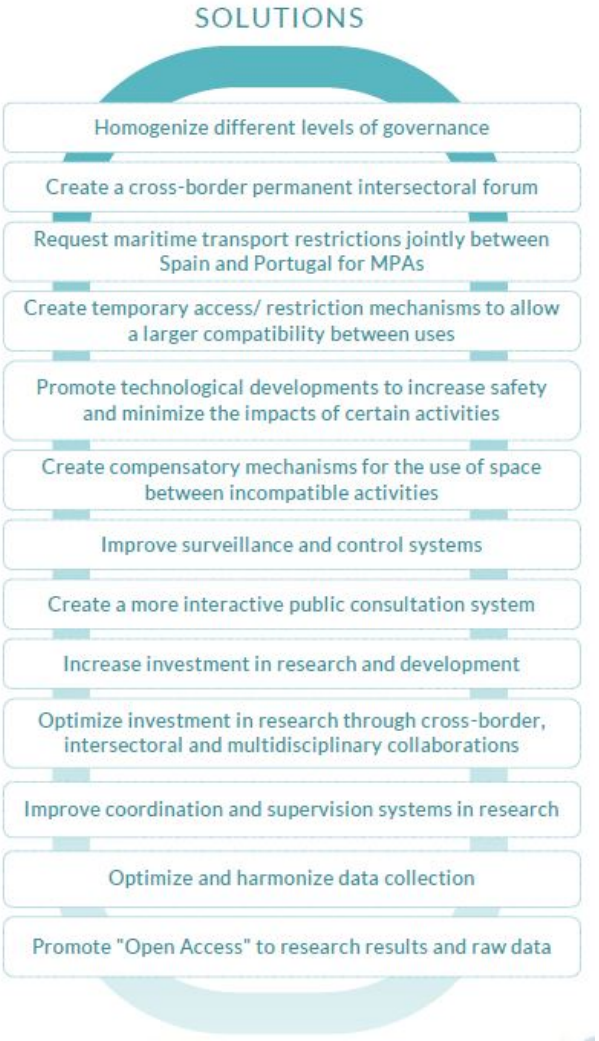


Figure 2: Stakeholders solutions.



Acknowledgments

This project was co-funded by the EC – DG Maritime Affairs and Fisheries (DG MARE). It was launched on 1st of January 2017 until 31st of January 2019, and involved Portugal, Spain and France based on a partnership of public bodies of the countries and one international organisation. It was composed of UAVR, CEDEX, IEO, AFB, CEREMA, Shom, and CPMR. SIMNORAT aimed to support the implementation of the MSP Directive in the waters of Portugal, Spain and France, as well as to establish cross-border cooperation mechanisms between these Member States, to contribute to the coherence of their marine spatial plans to be established in 2021.

Fishing for litter at the port of Fnideq (NW Morocco)

Soria Azaaouaj*[1] , Driss Nachite[1]

*Corresponding author: soriaazaaouaj@gmail.com
[1] Applied and Marine Geosciences, Geotechnics (LR3G) Laboratory, Department of Geology, Faculty of Science, Tetouan, Morocco

Keywords: Fishing for waste, Marine litter, Pollution, Plastic

Introduction

As part of the evaluation of sea floor pollution linked to marine litter at Fnideq area, a *Fishing for Litter* campaign was organized at the port of Fnideq on 11 November 2018 with the support of the National Laboratory of Pollution Studies and Monitoring and in collaboration with the Association of Scuba Diving and Environmental Protection *Campeónes Fnideq* and *Al Ahd Aljadid* Association for Development and Artisanal Fisheries.

Materials and methods

Three sites were sampled at 2.5- 3.5 m depth, with the participation of 6 divers and 4 artisanal fishing boats (Figure 1). The length of transects was 100 m, except for site 1 that was 37 m in length because it was limited by the entrance of the port (Cheshire et al., 2009). The methodology applied follows the UNEP / MED guidelines.

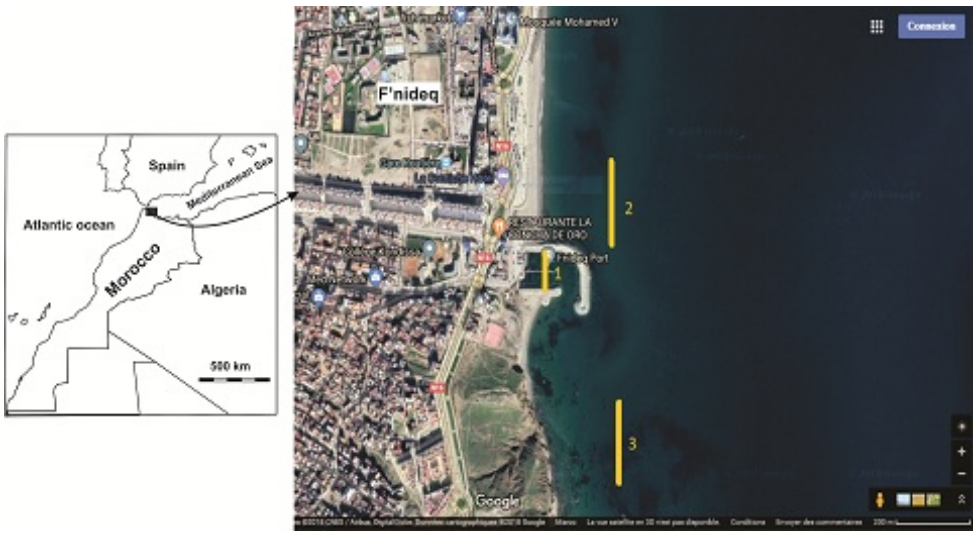


Figure 1: Location of the surveyed sites along the study area

Results and discussion

The results obtained show that in the 3 sites sampled, 881 items were collected with a total weight of 68.94 kg, which represents a density of 0.937 item/m^2 and 0.073 kg/m^2 (Table 1).

Table 1: Litter abundance and densities

Total collected items	Total weight (Kg)	Total of sea floor surface surveyed(m^2)	Items/m^2	Kg/m^2
881	68.943	940	0.977	0.073

25 types of items have been collected and grouped into 7 categories (plastic/polystyrene: 75.37%), cloth/textile (20.20%), metal (2.61%), rubber, paper/cardboard, processed/worked and sanitary waste with 0.45% each). The top 10 debris was represented by plastic bags, clothing/rags. The other categories of debris make up, respectively, 4.2% for plastic bottles, 2.27% for plastic food containers, 1.36% for cans, 1.02% for fishing lines, 0.91% for synthetic ropes and other textiles, 0.6% for metal food cans and 0.5% for plastic caps/lids. Debris distribution by surveyed site shows that sites 2 and 3 are the most polluted, with 1.005 items/m^2 and 1.003 items/m^2 respectively (Table 2).

Table 2: Litter abundance and densities in surveyed sites

Sites	Total items collected	Total weight (Kg)	Total of surface surveyed (m^2)	Items/m^2	Kg/m^2
Site 1: Port	77	3.180	140	0.55	22.714
Site 2: Oued jdid	403	32.432	400	1.005	81.079
Site 3: Kendissa	401	33.331	400	1.003	83.328

The density of benthic debris at Fnideq is high (0.937 items/m^2) compared to regional data recollected using the same methodology, for the Adriatic and the Ionian Sea (0.028 ± 0.034 items/m^2, Abu-Hilal and Al-Najjar (2009)). However, much higher values were noted in the Gulf of Alqaba in the Red Sea (2.8 items/m^2, Vlachogianni et al. (2017)). It should also be noted that these density values are higher than those recorded at the Moroccan Mediterranean beaches (0.06 ± 0.04 items/m^2, Nachite et al. (2018)). The general composition and origin of debris seem to be similar to beach litter, with a preponderance of plastic bags and other debris from land origin (Maziane et al., 2018). Marine debris (31 items), mainly related to fishing activities, does not exceed 3.52% of the total collected items.

Acknowledgments

We would like to thank the National Laboratory of Pollution Studies and Monitoring and the Association of Scuba Diving , Environmental Protection "Campeónes Fnideq" and Association (al ahd al jadid) for their participation and support.

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King crabs and squat lobsters in deep waters off Mozambique (Indian Ocean)

Isabel Muñoz*[1] , Eva García Isarch[1] , Jose A. Cuesta[2] , Enrique Macpherson[3]

*Corresponding author: eli.munoz@ieo.es
[1] Instituto Español de Oceanografía (Cádiz), Puerto Pesquero, Muelle de Levante, s/n 11006 Cádiz
[2] instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC) Calle Republica Saharaui, 4, 11519 Puerto Real, Cádiz
[3] Centro de Estudios Avanzados de Blanes (CEAB-CSIC) Carrer Accés Cala Sant Francesc, 14, 17300 Blanes, Girona

Keywords: Galatheoidea, Lithodoidea, Mozambique, Deep waters, Barcoding

Introduction

This work presents the preliminary results of the Ph.D Project "*Study of deep-sea decapod crustaceans communities in waters off Mozambique. Comparative with others African communities*". In this study, obtained results after the revision of the Lithodoidea and Galatheoidea superfamilies belonging to the Infraorder Anomura, are presented. As well as the distribution of the main species identified in the study area. This revision has been performed from a morphological and genetic point of view. In the area covered by this study, as well as the waters that surround Mozambique (mainly South Africa and Madagascar), there are works on certain families, such as chirostylid and galatheid crustaceans (Baba, 1989). Recently some inventories have also been published on decapods from various islands of the French tropical Indo-Pacific (Poupin, 2016) One of the most recent works is a checklist that encompasses all studies performed to date on decapods in the waters of Namibia, South Africa and Mozambique (Emmerson, 2016). However, these are very specific taxonomic studies, descriptions of species or genre review, but little or nothing regarding aspects such as the composition, abundance and distribution of the communities that make up the species of these groups previously cited.

Materials and methods

The specimens studied for this work belong to the Collection of Decapod and Stomatopod Crustaceans of the Oceanographic Centre of Cadiz (CCDE-IEO). Specifically, they come from three oceanographic survey carried out in waters of Mozambique by the IEO in 2007, 2008 and 2009. The methodology used to capture the specimens was through a bottom trawling gear ("baka" type). The studied area corresponded to the shelf and slope of Mozambique, at depths from 100 to 700 meters (Figure 1). The specimens belonging to two superfamilies (Lithodoidea and Galatheoidea) were reviewed and identified, at the lowest possible taxonomic level by morphology and genetic analysis through sequencing techniques. Molecular analyses were performed based on fragments of two mitochondrial genes, the Cytochrome Oxidase subunit I (COI) and the 16S rRNA. Distribution areas and depth ranges of the identified species were analyzed from the survey geo-referenced catch data.

Figure 1: Study are.a

Results and discussion

In the Mozambique surveys, 41 individuals of a single species of Lithodoidea were captured: *Lithodes mamillifer*. They were all collected at depths greater than 588 m and throughout the entire sampling area (from Nampula to the border with South Africa) by the three surveys. Numerous specimens of Galatheoidea were caught, belonging to 3 families (Figure 2): Munididae (*Agononida africerta*, *Munida benguela* and *Paramunida marionis*), Munidopsidae (*Munidopsis africana*) and Chirostylidae (*Uroptychus dentatus* and *Uroptychus* n. sp). Only 36 specimens are conserved in the CCDE-IEOCD and all of them were genetically studied by DNA analysis (16S and COI). All species were collected at deep waters. M. africana, *P. marionis* and both *Uroptychus* species were found up to 400 m and *A. africerta* and *M. benguela* up to 300 m. Regarding the geographical distribution, *A. africerta* and *M. africana* were found throughout the entire sampling area, while the rest of the species have only been captured from the southern half to the border with South Africa. The only specimen identified as *P. marionis* was captured near the Bazaruto Island. Galatheoidea is one of the most diverse group of decapod crustaceans, with more than 250 species known in the Indian Ocean (Emmerson, 2016) and a large number of new species are being described in recent years. Two of the 6 species found in the "Mozambique" surveys, were recently described (Cabezas et al., 2010; Poore and Andreakis, 2012) even after the date of the surveys and one is a new species in process of description (*Uroptychus* n. sp.). This reveals the importance of expanding the knowledge of this large taxonomic group.

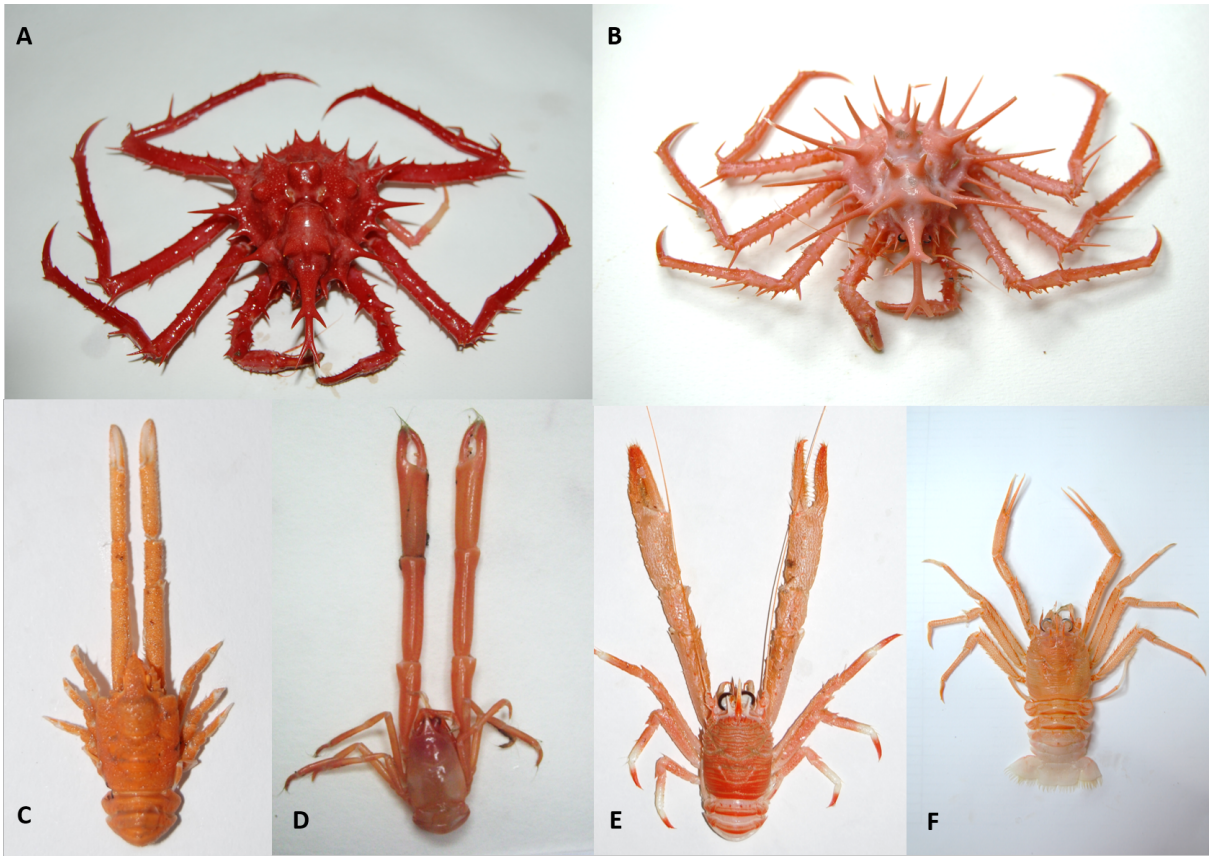

Figure 2: Specimens photographed on board (A: *Lithodes mamillifer*, adult B: *Lithodes mamillifer*, juvenil C: *Munidopsis africana* D: *Uroptychus* n.sp. E: *Munida benguela* F: *Agononida africerta*).

Acknowledgments

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When the microclimate does matter: differences in the demographic, morphometric and reproductive variables of *Fucus guiryi* from two nearby populations

Raquel Sánchez de Pedro*[1] , Andrea Nieves Fernández[1] , María Jesús García-Sánchez[1] , Antonio Flores-Moya[1] , Elena Bañares-España[1]

*Corresponding author: rsdpc@uma.es
[1] Universidad de Málaga, Departamento de Botánica y Fisiología Vegetal. Facultad de Ciencias. Campus de Teatinos sn. 29010 Málaga, Spain

Keywords: Canopy, Demography, Global warming, Seasonality, Thermal regime

Introduction

Marine forests and canopy-forming macroalgae provide essential ecosystemic services from cold to warm latitudes, whose positive effects cascade through their communities and sustain whole ecosystems. However, global change is leading to the retreat and extinction of many populations over the past decade (Riera et al., 2015). This process is particularly enhanced at southern-range and peripheral populations, which are more vulnerable to climatic stress due to their exposure to higher absolute temperatures, despite local conditions can ameliorate microclimatic conditions, thus providing climatic refugia (Lourenço et al., 2016; Melero-Jiménez et al., 2017). This study aims at comparing the demographic, morphometric and reproductive-ecological variables of two close populations of *Fucus guiryi* [Zardi, Nicastro, E.S.Serrão & G.A.Pearson] at the Strait of Gibraltar (Tarifa-TRF, Guadalmesí-GDM, ca. 8 km away).

Material and methods

Seasonal sampling surveys took place between spring 2018 and spring 2019, at diurnal low spring tides. All the variables were studied in spring and fall, while in summer and winter only individual length and reproductive status were recorded. Microclimate of each sampling site was characterized from direct observations, deployment of *in situ* temperature loggers during summer 2018, and retrieval of meteorological and oceanographic variables from public databases. For the demographic variables, non-destructive quadrats of 0.16 m^2 (n=4) were sampled randomly within the intermediate intertidal zone. Length and reproductive status (vegetative, incipient, mature apices) were determined for each individual. Cover was calculated from 5 equidistant transects perpendicular to the highest water mark, by recording the exact intervals where *F. guiryi* was present. At GDM, cover was estimated from pictures due to the low extent and cover of the population. Morphometric variables were measured at each site and sampling from 10 individuals (individual bushiness - IB, length of the frond between the holdfast and the first dichotomy - LF, frond width-WF and receptacle size (height-RH, width-RW, length-RL). Two-way factorial ANOVA was applied to test the main and interactive effects among sites and season, except when data lacked and one-way ANOVA or t-tests were performed. Reproductive status was analyzed by means of PERMANOVA.

Results and discussion

Microclimatic conditions differed greatly due to local characteristics of each sampling site. TRF presented a higher proportion of bare rock covered by algae, more abrupt rock surface with abundant tidal pools of 10-100 cm depth. This ambient prevents water loss and ameliorates abiotic stress during emersion. In contrast, *F. guiryi* at GDM is disposed as patches on flat or slightly angled surfaces of the flysch platforms, and outside their canopies, there is mainly acorn-barnacle-covered-bare rock. Maximum air temperature registered did not exceed 30°C, while at the upper intertidal distributional limits temperature raised up to 41.5±2.8°C at TRF and 44.3±1.0°C, at GDM. Nevertheless, no significant differences in mean temperatures (21-24.6°C) or mean values of maximum (32-27°C) and minimum temperature (17-18.3°C) were obtained. Canopy reduced temperature by 5-7°C and attenuated irradiance by 10-16%, corresponding to irradiances below 100 μmol m^{-2} s^{-1}, values highly consistent with those reported for several populations of *F. guiryi* from Portugal (Monteiro et al., 2019). On the other hand, TRF was exposed to higher waves than at GDM, due to the shore position in relation to the predominant wave direction (W). In turn, individuals of *F. guiryi* are exposed to higher environmental stress at GDM, due to the higher maximum temperature, less frequent watering, patchier distribution, and more direct wave impact that can lead to plant detachment. Regarding demographic variables, sampling site and season had a significant effect on the thalli length of *F. guiryi*, with no significant interaction among factors. *Fucus guiryi* from both sites experienced parallel seasonal dynamics in individual length, showing the expected growing season from spring to summer observed in many temperate macroalgae, when maximum size is reached (Figure 1). However, individuals from TRF were 1.4-1.8 times longer than at GDM (Figure 1). Both populations differed in their minimum (TRF 1.4±0.6 cm; GDM 0.8±0.2 cm), mean (TRF 5.8±1.0 cm; GDM 3.5±0.9 cm) and maximum (TRF 11±2 cm; GDM 7±2 cm) thalli length across seasons. As a result, both had different population structure and heterogeneity. TRF had a significantly higher percentage of individuals of size classes 6-12 cm than GDM, whereas at the latter 84% of individuals were below 4 cm ($p<0.01$). In addition, the percentage of new recruits (0-2 cm) at GDM has significantly increased during the study, probably due to the frond breakage by wave storms after summer 2018. *Fucus guiryi* from TRF had twice the population density and cover than at GDM (average density 55±26 *vs.* 26±15 individuals m^{-2}; average cover 14±7% *vs.* 7±3%). Population density has declined by 50% for both populations along the study, with no significant interactions among factors, whereas algal cover remained unaltered across seasons.

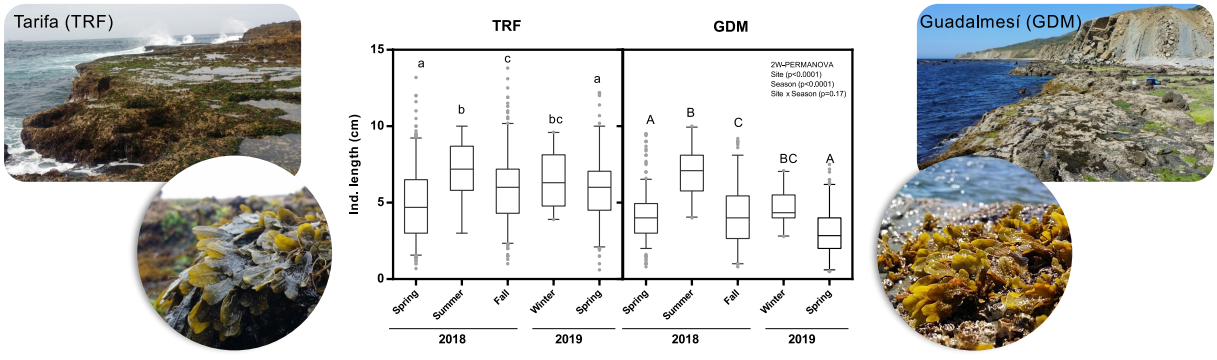

Figure 1: Seasonal changes in individual length of *F. guiryi* from two populations at the Strait of Gibraltar (TRF-Tarifa, GDM- Guadalmesí).

In spring 2018, *F. guiryi* from GDM presented lower bushiness than in TRF (GDM, 0.8±0.2). In fall, 25-52% of individuals from GDM experienced severe breakage of apical fronds, after a period of extreme air summer temperatures (up to 45.6°C in August) and an intense wave

action in early autumn, which constrained the measure of all the morphological variables. *Fucus guiryi* from both populations presented similar LF and WF across the study (averaged values, LF: 1.4±0.5 cm; WF: 1.1±0.2 cm). LF was 3 times lower than those reported for Portuguese populations, while WF was more similar to *F. guiryi* from the Canary Islands (Riera et al., 2015) or *F. spiralis* from Portuguese populations (Zardi et al., 2015). Receptacle size varied among sites and samplings with interactive effects. Receptacles became thicker from spring to fall for both populations, being 2-times thicker for GDM specimens (0.4±0.1 *vs.* 0.8±0.2 cm). RL and RW remained invariable within each site, and only in fall 2018, receptacles were longer and wider for TRF specimens. Also, RW from specimens of GDM declined by 50% from spring to fall, probably derived from the disruptive stress experienced by this population after summer 2018. Reproductive population increased throughout the study period, from 16-36% to 45-60%. Despite seasonality did not affect the percentage of reproductive individuals within each population, in spring 2019 TRF had a greater density of reproductive individuals than GDM (21±3% *vs.* 7±5, Figure 2). Minimum length of reproductive individuals neither experienced seasonal changes but differed greatly between sites, with 50% shorter individuals at GDM (2.2±0.8 cm) than in TRF (4.6±1.2 cm).

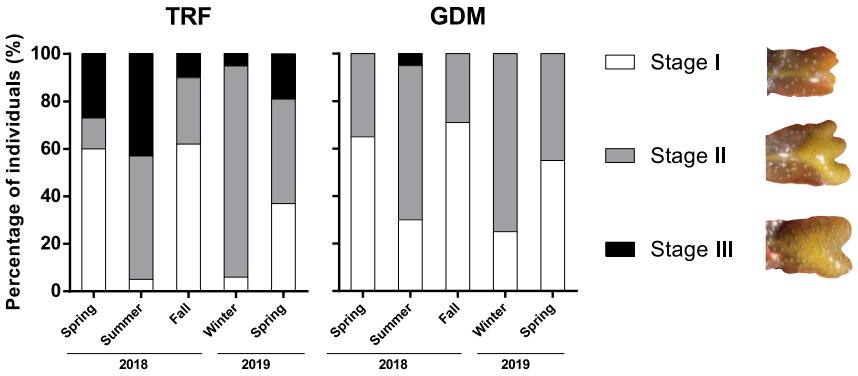

Figure 2: Seasonal changes in the percentage of individuals of *F. guiryi* at each reproductive stage (ST I- Vegetative; ST II- developing receptacles; ST III- Mature receptacles) from the two studied populations (TRF-Tarifa; GDM-Guadalmesí). Images of each stage are illustrated.

Table 1: Percentage of apical tips of *Fucus guiryi* from TRF at each reproductive stage (ST I: Vegetative; ST II: Developing receptacles; ST III: Mature receptacles) during the study period. Different letters indicate significant differences among seasons within each stage ($p < 0.05$).

	Spring	Summer	Fall	Winter	Spring'19
ST I	97.91 ± 7.25^{a}	50.95 ± 29.98^{b}	95.76 ± 10.13^{a}	69.5 ± 20.06^{c}	86.83 ± 19.92^{bc}
ST II	1.68 ± 6.71^{a}	38.53 ± 32.05^{b}	4.24 ± 10.13^{c}	30.25 ± 20.42^{b}	12.43 ± 19.69^{d}
ST III	1.03 ± 5.69^{a}	10.53 ± 19.29^{abc}	1.58 ± 7.72^{b}	0.25 ± 1.12^{c}	2.37 ± 7.05^{ab}

Site had a clear influence on the percentage of individuals reaching mature receptacles ($p<0.001$), with a significantly higher proportion of individuals at stage III at TRF (0-41%), for all seasons (Figure 2). *Fucus guiryi* at TRF exhibited a higher proportion of mature plants (27±19%), whereas at GDM it was only reported in summer'18 with less than 5% of the individuals (Figure 2), for which most of the individuals had developing receptacles (stage II, 35±11%). At TRF, proportion of individuals with mature receptacles increased by 15% from spring to summer 2018, when 41% of reproductive individuals reached stage III, with also a significant decrease in the % of vegetative apices (Table 1).

In conclusion, demographic, morphological and reproduction-related variables for the populations

of *F. guiryi* from the Strait of Gibraltar were in good agreement with those from rear-edge populations from southern Portugal (eg. individual length, bushiness) (Zardi et al., 2015). Interestingly, specimens from TRF deviated from the correlation more than those from GDM (eg. higher bushiness, individual length and fertility at the growing season, and higher population extent, cover and density), suggesting that Tarifa acts as a true climate refugia for *F. guiryi* (Lourenço et al., 2016).

Acknowledgments

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Aquatic environment determines the carbon acquisition and assimilation potential in seagrasses and submerged freshwater angiosperms

Sebastià Capó-Bauçà*[1] , Pere Aguiló-Nicolau[1] , Jeroni Galmés[1] , Concepción Iñiguez[1]

*Corresponding author: sebastia.capo@uib.cat
[1] Research Group on Plant Biology under Mediterranean Conditions, Universitat de les Illes Balears–INAGEA, Palma, Balearic Islands, Spain

Keywords: Aquatic photosynthesis, Carbon concentration mechanisms, Freshwater angiosperms, Rubisco, Seagrasses

Abstract

Seagrasses and submerged freshwater angiosperms are key 'ecosystem engineers', providing an array of ecosystem services. These organisms have to face low dissolved CO_2 levels, along with the slow CO_2 diffusion in water, which is a major limitation for CO_2 fixation by Rubisco and, therefore, productivity (Maberly and Gontero, 2017). To increase CO_2 concentration around Rubisco, the vast majority of aquatic macrophytes have shown evidences of carbon concentrating mechanisms (CCMs). However, there is a general concern that seagrass photosynthesis will be favoured by future increased CO_2 levels, suggesting less efficient CCMs than those found in seaweeds (Borum et al., 2016). The mechanisms of carbon utilization in seagrasses and freshwater angiosperms have been substantially less analysed than in algae, and their Rubisco kinetics are completely unknown (Larkum et al., 2017). Therefore, our study aims to discern the adaptations in the carbon acquisition and assimilation mechanisms of different phylogenetic clades of aquatic angiosperms. Considering that CCMs might be modulated by the physicochemical characteristics of the aquatic environment, the clades selected include species adapted to seawater and freshwater environments. As a result, four ecologically-relevant seagrasses (*Posidonia oceanica*, *Cymodocea nodosa*, *Zostera noltii* and *Zostera marina*) and four permanently submerged freshwater angiosperms (*Vallisneria gigantea*, *Potamogeton pectinatus*, *Ruppia cirrhosa* and *Ceratophyllum demersum*) were selected. In these species, the strength of CCMs were tested through different techniques including pH drift assays, the photosynthetic response to different dissolved inorganic carbon (DIC) concentrations and to different inhibitors of CCMs components (Figure 1) and the carbon isotopic discrimination of leaf tissue. Full characterization of Rubisco kinetic traits, including the Michaelis-Menten constant for CO_2 and O_2, the maximum carboxylation and oxygenation rate and the CO_2/O_2 specificity were also analysed together with leaf anatomical traits and N leaf concentration. We observed that cell wall thickness (T_{cw}) and the ratio of exposed chloroplasts to extracellular surface area (S_c/S_m) induce a mesophyll conductance to CO_2 too low to sustain the *in vivo* photosynthetic rates recorded (Figure 2). Moreover, *in vivo* photosynthetic apparent affinity for CO_2 is significantly higher than *in vitro* Rubisco apparent affinity for CO_2, suggesting co-evolution of Rubisco and CCMs. Despite the fact that our results suggest an efficient CCM operation as a wide adaptation in angiosperms to live in aquatic ecosystems, freshwater and marine species revealed differences in the type and strength of their CCMs, independently of their phylogeny, suggesting that the environmental variability in inorganic carbon availability has promoted an evolutionary convergence in their carbon acquisition mechanisms.

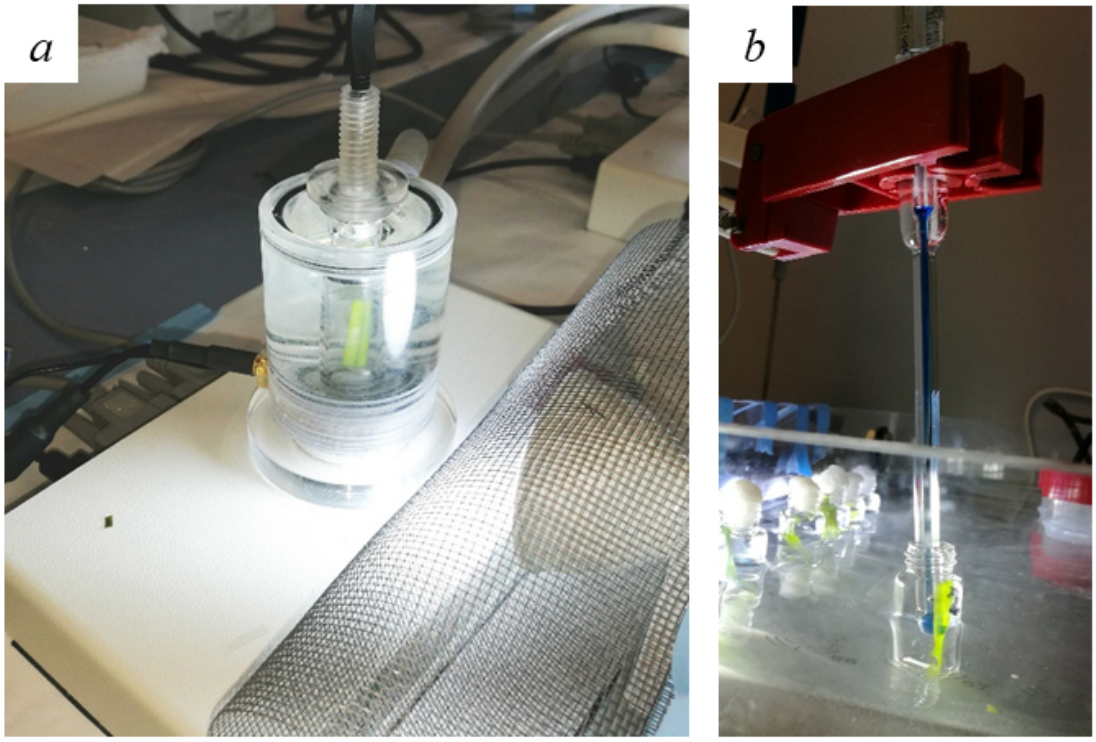

Figure 1: *a*, photographs measuring O_2 production with Hansatech O_2 electrode. *b*, photographs performing a pH dripht assay.

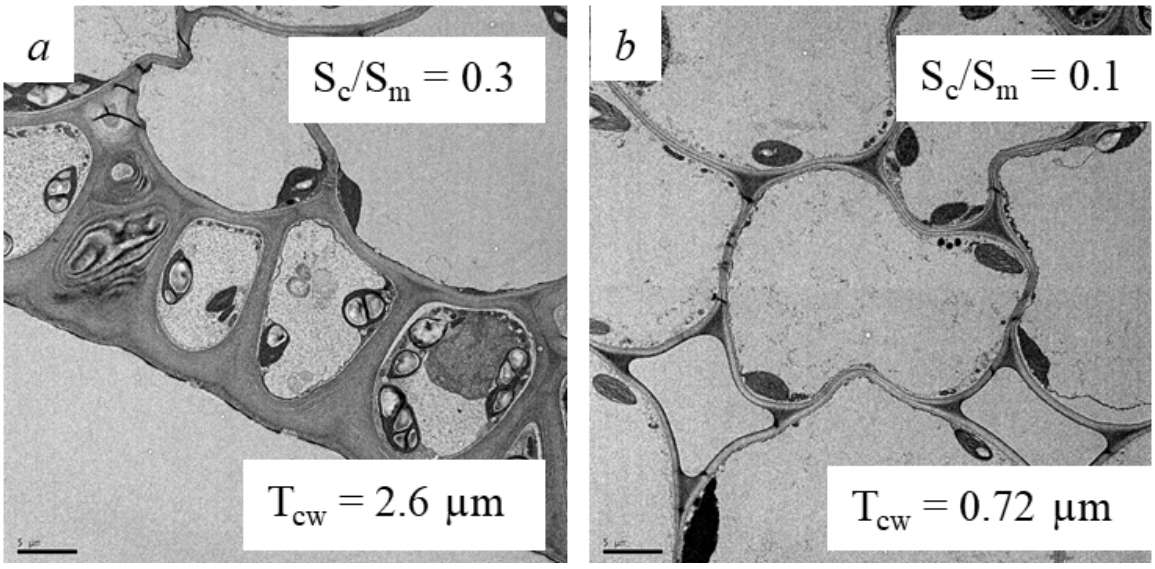

Figure 2: Electronic microscope images of *Posidonia oceanica* leaf. *a*, epidermic cells with their cell wall thickness (T_{cw}) and the ratio of exposed chloroplasts to extracellular surface area (S_c/S_m). *b*, internal mesophyll cells with their cell wall thickness (T_{cw}) and the ratio of exposed chloroplasts to extracellular surface area (S_c/S_m).

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Oxidative stress response in *Posidonia oceanica* meadows by the phosphorus and iron loading in the water column and sediment

Víctor Fernández-Juárez*[1] , Antoni Sureda-Gomila[1] , Aurora Rayo-Aguilar[1] , Nona Sheila Agawin Romualdo[1]

*Corresponding author: victor.fernandez@uib.es
[1] Universidad de las Islas Baleares

Keywords: Posidonia oceanica, Phosphorous, Iron, Oxidative stress

Introduction

Posidonia oceanica is marine phanerogam essential in the maintenance and sustenance of the Mediterranean Sea, standing out for its high primary productivity, as carbon sink, as habitat and nursery for a variety of micro- and macro-organisms, as sediment stabilizers, as buffers for ocean acidification, and as an important site for biogeochemical processes. It is well reported that *P. oceanica* meadows established high rates of nitrogen atmospheric (N_2) fixation, through the called diazotrophs or N_2-fixing bacteria (Agawin et al., 2016). The nitrogenase complex (nifH, nifD and nifK) reduce the N_2 in NH_3, inorganic nitrogen that are assimilable for the plant itself and the rest of organisms associated with. The nifH analysis showed the huge diversity of these bacteria in *P. oceanica* (Cyanobacteria, Proteobacteria, Firmicutes, Bacteroidetes and Arqueas) and have been detected on the surface of the leaves, roots and rhizomes (epiphytic population) or even inside of the roots (endophytic population) (Agawin et al., 2016). Biomarkers of oxidative stress are a useful tool to evaluate the physiology of the cells and to analyse the effect that different stimulus could have in these cells. Oxidative stress appears when the balance between the production of reactive oxygen species (ROS) and the antioxidant defences (superoxide dismutase [SOD], catalase [CAT], glutathione peroxidase [GPx] or glutathione reductase [GR]) are deregulated. The accumulation of ROS, especially the highly reactive free radicals, as the hydroxyl radical (OH·) react with cellular compounds (DNA, proteins and lipids) leading to oxidative damage and if stimulus does not disappear can cause cell death.

Materials and methods

Here, we performed three different experiments to study the effect of phosphorus (P) and iron (Fe) loanding. These two elements are catalogued as the main limiting factors for N_2 fixation, and nothing is known about their effect in *P. oceanica*. We studied the effect of P and Fe in the water column, in clean water from the Faro de Ses Salines (Mallorca, Spain), and in polluted water from near of the commercial and touristic harbor (Calanova, Mallorca, Spain). In addition, we studied the effect of P and Fe in sediment that we performed in Faro de Ses Salines (Mallorca, Spain). All these experiments were done during summer of 2017-2018, setting four conditions in triplicate: -P-Fe (control), -P+Fe, +P-Fe, +P+Fe. Water column experiments were done in acid clean aquariums, and sediment experiment was performed in situ with fertilising parcels with the conditions above mentioned.

Results and conclusions

Leaves of *P. oceanica* (not discriminating between old and new leaves) were homogenized and supernatant were used to measured SOD, CAT, GPx and GR activity. Lipid peroxidation was measured through detection of malondialdehyde (MDA). All the assays were conducted with n=6, following the procedures by Sureda et al. (2017). Overall, results show that P in the water column had a positive impact reducing the activity of antioxidant enzymes and MDA levels, indicating that P-limitation column is a stressing stimulus for the plant. Increasing Fe levels enhanced the response against oxidative stress. Contaminated water reduced stress oxidative response, probably because of their eutrophication character. However, results in sediment experiments were totally opposite. Generally, P enhanced a response against oxidative stress, meanwhile Fe, reduced this response. Phosphorous could let arsenic more available for the plant, taken into account the deleterious effect of this element. Fe is capable to trap sulphites harmful to the plant, and is a chelator of P, reducing their deleterious effect, as we show here. In conclusion, we present the first insights of the effect of P and Fe in water could and sediment in *P. oceanica*, and how depending where is the nutrient co-limitation, the biochemical response could be totally different.

Acknowledgments

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Influence of Tectonics and erosive processes in the physiographical configuration of the middle shelf of the Gulf of Cadiz (NE sector)

Julia Tadeo Legorburu*[1] , Olga Sanchez Guillamon[2] , María del Carmen Fernandez Puga[1] , Juan Tomás Vázquez Garrido[2]

*Corresponding author: jultadeo@gmail.com
[1] University of Cadiz
[2] Spanish Institute of Oceanography

Keywords: Geology, Continental shelf, Gulf of cadiz, Cartography, Marine Geology

Introduction

The continental margin of the Gulf of Cadiz (GoC) is a very complex region from the geology and oceanographic point of view, due to its location close to the boundary between the African and Eurasian plates as well as to the interaction of different water masses that are exchanged through the Strait of Gibraltar. The main objective of this paper is to characterize a sector of Gulf of Cadiz continental shelf from a morphological and structural point of view, identifying and correlating the morphologies with geological and/or oceanographic processes related to the evolution of the continental shelf. The study area is located on the northeastern continental margin of the GoC, between the coordinates 6°21' W and 6°20' W and 36°30' N and 36°10' N, along the internal and middle continental shelf, in a bathymetric range between 16 and 87 m depth.

Material and Methods

High resolution reflection seismic profiles with boomer source system have been used to carry out this work. The data set has been retrieved from oceanographic cruises in 1994 by the MOPTMA (Ministry of Public Works, Transportation and Environment). A total of 189 seismic profiles have been interpreted in the continental shelf area between the city of Cadiz and the Trafalgar Cape. The total length of the high resolution seismic profiles collected is approximately 2000 km and its direction is ENE-WSW with an average penetration of 21m bsf. The profiles were collected at depths ranging from 30 to 87 m and a lateral separation of 300 m. The method used in this work consisted in scanning and digitalizing the original analog seismic records and convert in a sgy format to be later interpreted with the HIS Kingdom Geoscience software. The main morphological types and geological structures were mapped with ArcGis software.

Results and Discussion

In this section is made a morphological and structural characterization of the continental shelf from north (from the city of Cadiz to Roche cove), central (from Roche cove to cape Roche) to the south sector (from cape Roche to cape Trafalgar) (Figure 1).

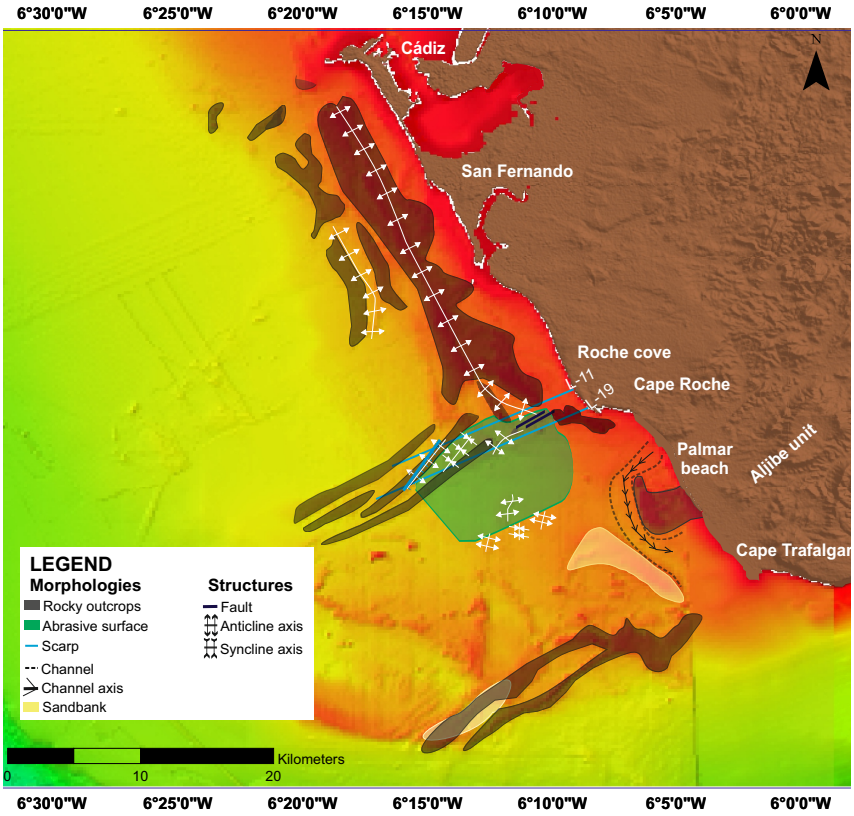

Figure 1: General map of the study area with the morphologies and structures interpreted.

Morphological characterization of the continental shelf

Rocky outcrops, scarp, abrasive surfaces and channels and sandbanks are identified throughout the study area after the bathymetry interpretation. Rocky outcrops are found throughout the study area with irregular and elongated shapes. In the northern sector seven outcrops are found. The three located further north are small and without a preferred orientation. They are located 5 km away from the city of Cadiz at 55, 65 and 78 m depth respectively. The four outcrops located further south in the north sector follow NW-SE direction. They are located at a range of 40 and 80 m depth. One of them is 29.4 km length, located in a depth range of 30 and 45 m and it begins in front of the city of Cadiz and extends parallel to the coastline to the Cape Roche. Parallel to this one at a depth range between 50 and 80 m, the other three outcrops are found with approximately the same 9 km length. In the central sector a set of elongated outcrops is identified following NE-SW orientation and located in a depth range of 40 to 45 m. In the south sector two outcrops without a preferential orientation are identified. The first one is located in a range between 25.5 and 30 m depth and the second between 37.5 and 45 m. Scarp form a positive relief with a steep slope towards one of its sides. In the study area have been identified a scarp with step to the E located in the central zone at a depth range of 35 to 40 m. Its length is 4.4 km and its orientation is NE-SW. In the area closest to the coast, the height of the step is 1.5 m, increasing towards the south between 3 to 4.5 m. Previously named and described by other authors, such as the Palmar and Barbate sandbanks (De Castro et al., 2017) are partially identified. The Palmar sandbank extends in NW-SE direction and is located 4 km to the southwest of Cape Trafalgar, between 8 and 25 m depth. It is 7 km long and 2.6 km wide and covers an area of approximately 20 km^2. The Barbate bank is elongated and extends in the NE-SW direction. It is located 14 km from Cape Trafalgar at a depth between 18 and 29 m. It is 6 km long and 2 km wide and occupies an area of 15 km^2. In the central sector an abrasive surface is found in a depth range between 30 and 40 m. It is located in front of the Cape Roche at

a distance of 8 km. It covers an area of 15 km^2 (Figure 1). A channel is identified, in front of the Palmar beach bordering an outcrop located eastward. It is 6 km long and 2 km wide. Its form is in U shape and its axis is asymmetric and it is displaced towards the west. It has a greater slope in the east.

Structure

Faults and anticline and syncline folds are identified in the study area. The faults are located in front of Cape Roche at a depth of 15 m. Their length is 200 m and 160 m and their orientation is NW-SE. The vertical displacement of these fractures is 15 m. Both faults are superficial but only one of them is associated with an elevation (Figure 2).

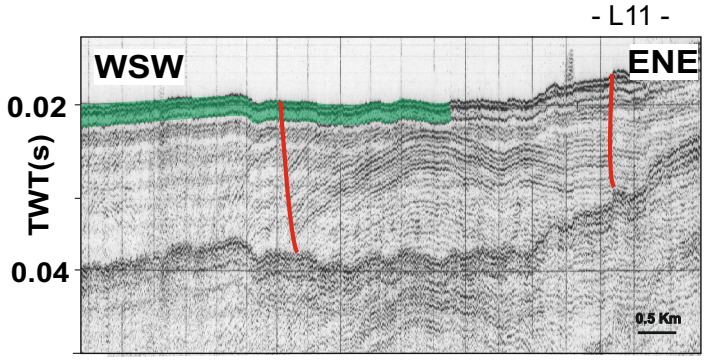

Figure 2: Seismic profile showing faults at 15 m depth and the abrasive surface.

The seismic records show a high deformation related to the tectonic activity of the area, which translates into a complex system of anticline and synclinal folds. In the north sector have been interpreted two anticline folds. Both present an NW-SE axis orientation. The longest anticline is about 29 km long and 7.5 km wide. It presents an asymmetric geometry with the eastern flank being narrower and steeper than the western flank. The other anticline structure has an extension of 8.6 km and is located between 60 and 66 m deep. In the central sector, a set of anticlines and synclinal have been identified. They present an axis with an NE-SW orientation. They are approximately 3 km long and they are found in a deep range of 20 to 40 m. In the south sector, anticline and synclinal structures are identified. They present an axis with an NNE-SSW orientation. They are 1 km long and are found in a deep range of 40 to 60 m.

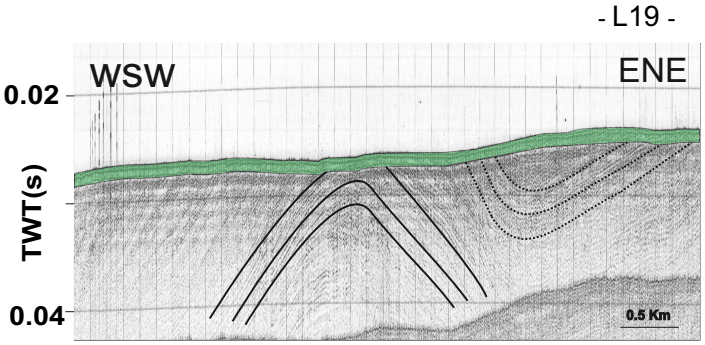

Figure 3: Seismic profile showing anticline and syncline folds eroded by the abrasive surface at 30 to 40 m depth.

The actual physiographic configuration of the studied area of the continental shelf of the Gulf of Cádiz is largely controlled by the occurrence of rocky outcrops. These also have a direct influence on the oceanographic dynamic bounding the mass water Surface Atlantic Water (SAW) direction. Large outcrops identified in the North sector are related to internal structures such as anticline which axes outcrop with NW-SE orientation. In the central sector outcrops are also associated with anticline and syncline folds axes in a NE-SW direction. The change in the outcrop orientation is possibly due to the transition southward transition from the Sub-Betic Domain to the Flysch Units The outcrop identified in the south sector, in front of Palmar beach, is the prolongation of an elevated onshore system called Aljibe unit whose southern end was located onshore when the sea level was approximately between 35 and 20 m bellow present sea level. The channel next to this outcrop is also the continuation of an onshore stream, the Salado River. We clearly see that these last two structures are simply a continuation of land morphologies, modified after by erosion and sedimentation processes that nowadays appear offshore due to the sea level rise. In this same area, sedimentation processes occur, the incoming water is channelled and changes its direction following the channel, in the areas where its velocity decreases sediments deposit forming the Palmar sandbank. The recent tectonic activity has an influence on scarps, other authors (De Castro et al., 2017), associate them with normal faults. We agree on that but it has been impossible to identify it with the available profiles. Oceanographic processes have controlled the existence of abrasive surfaces mainly in central and south sectors that appears to be highly eroded by them.

We conclude that the current configuration of this shelf sector is greatly influenced by recent tectonic processes, oceanographic dynamics linked to the SAW and erosive processes related to sea level variations during Holocene times.

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Morphological features of the Mallorca Channel, Balearic Islands (Central Western Mediterranean)

Olga Sánchez Guillamón*[1] , Juan Tomas Vázquez[1] , Desirée Palomino[1] , Patricia Bárcenas[1] , Maria del Carmen Fernández Puga[2] , Luis Miguel Fernández-Salas[3] , Olvido Tello[1]

*Corresponding author: olga.sanchez@ieo.es
[1] Instituto Español de Oceanografía
[2] Universidad de Cádiz. Facultad de Ciencias del Mar y Ambientales
[3] Instituto Español de Oceanografía.

Keywords: Morphology, Seamounts, Pockmarks, Bioconstructions, Mallorca Channel

Introduction

The Mallorca Channel (MC) corresponds to a seaway between the Ibiza and Mallorca islands, at the southwest sector of the Balearic Promontory. This promontory, of structural origin, is located in the middle of the Western Mediterranean (Figure 1a). It could be described as an asymmetric channel, whose width varies between 100 and 200 km, narrowing towards the north, and deepens up to 1050 m below sea level (mbsl) (Figure 1b).

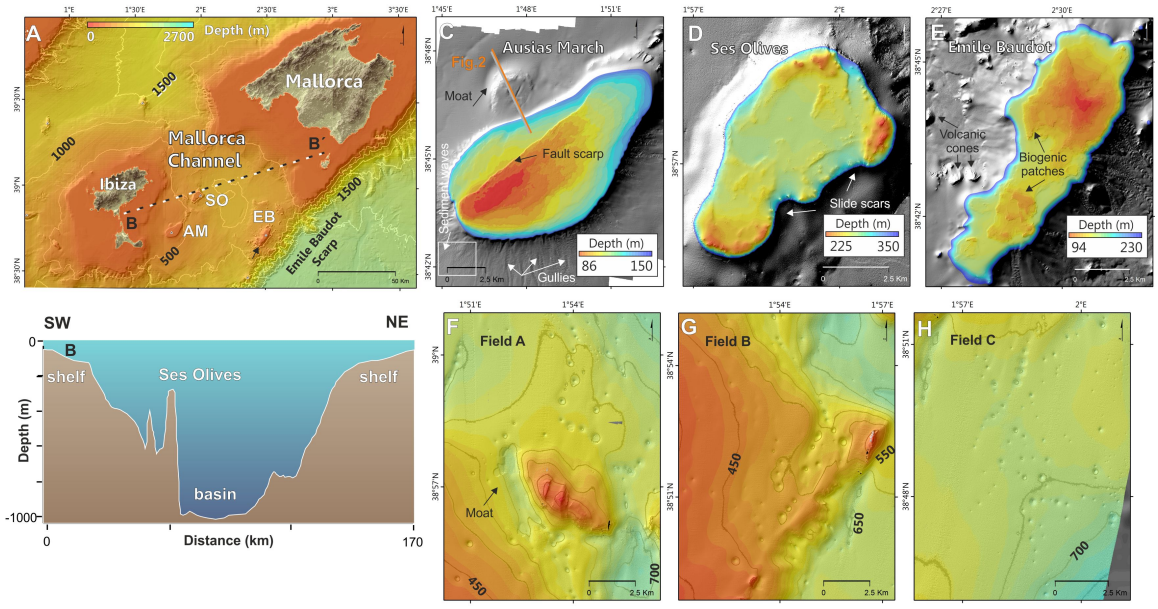

Figure 1: Bathymetric maps of the study area A) Location of the Ses Olives (SO), Ausias March (AM) and Emile Baudot (EB) seamount in the Mallorca Channel and situation of the bathymetric profile of B, B) Bathymetric profile of the Mallorca Channel, C-E) Location of the main morphological features in the seamounts: guillies, sediment waves, moats and slide scars and situation of the parametric profile of Figure 2, and F-H) Pockmarks fields A, B and C distributed across the study area.

It is characterized by the presence of a variety of morphological features namely seamounts, scarps and depressions (Acosta et al., 2003; Vázquez et al., 2015). Through the Mallorca Channel,

four main water masses have been differentiated: the Modified Atlantic Water, which spans from the surface to 200 mbsl, the Levantine Intermediate Water, between 200 to 400 mbsl, the Western Mediterranean Deep Water, between 400 to 800 mbsl and lastly, the Bottom Water, that flows roughly from 800 to up to more than 3000 mbsl (Millot, 1999). The main aim of this work is to characterize the seafloor morphology of the MC and to study their relationship with the oceanographic and geological processes. To carry out this work, multibeam echosounder and seismic datasets were compiled during the INTEMARES0718 expedition aboard the R/V Ángeles Alvariño. It has yielded bathymetric maps with a resolution ranging from 2 to 8 m and high resolution parametric profiles that have allowed the identification of new morphological features and their detailed description.

Morphological features

Ten main morphological features characterize the seafloor of the MC as follows: i) Three seamounts called Ses Olives, Ausias March and Emile Baudot, located between 86 and 1005 mbsl. They are 375, 264, 600 m high, respectively, 10 to 17 km long and up to 77° of slope. They have tabular summits and irregular basal geometry, remarkably elongated in NE-SW trends (Figure 1). ii) A volcanic cone field has also been identified on the flanks and adjacent seafloor of Emile Baudot seamount between 215 and 915 mbsl (Figure 1). This comprises at least 120 spike and flat-topped conical edifices that rise from 25 to 420 m, with maximum widths and lengths of 140 to 1785 m. They are mostly circular, although some of them have irregular geometries. iii) A linear fault scarp is located on the summit of Ausias March seamount at 86-150 mbsl. It is 8.6 km long, up to 64 m deep in its SW edge and 23 m in its NE edge with 32° of slope and a NE-SW trend pattern (Figure 1 and Figure 2). iv) Three pockmarks fields are identified between 450 to 700 mbsl, mostly with circular shapes (Figure 1). Field A includes 70 randomly distributed depressions, 20 to 400 m long and up to 20 m deep, in which 15 of them are N-S and NW-SE aligned. Field B contains 30 depressions, reaching up to 500 m in length and 40 m in depth. They are chained in NW-SE and N-S trends. Field C includes 110 chained depressions, with lengths of about 10 to 300 m and depths of 3 to 25 m of N-S and E-W orientation. v) Elongated depressions are located around seamounts and could be classified as contourite moats. They have so different lengths (1 to 5 km) and widths (150 to 500 m). They show around 10 m of incision and are mainly NE-SW oriented. In addition, a major 2 km long and 35 m deep moat is identified locally, associated with the western edge of Ausias March seamount. It is asymmetric, half-moon shaped and NE-SW oriented (Figure 1). vi) Various contourite drifts are identified associated with the moats, depressions and the seamounts. They are mainly mounded drifts located at the edges or bases of these features (Figure 2). vii) Small scale sediment waves are identified at the south of Ausias March seamount at 300 to 400 mbsl. They comprise slightly sinuous crests separated between 50 to 100 m, heights of 1 to 2 m, lengths of 300 to 500 m and NE-SW to N-S trends. They occupy a 2.5 km^2 of total area (Figure 1). viii) Gullies are located in the upper sector of the eastern flanks of Emile Baudot and Ausias March seamounts. They are 1 to 5 km long and have NW-SE and NE-SW to N-S trends, respectively, and an irregular spatial distribution (Figure 1). ix) Mass transport features as slide scars are identified on the eastern flanks of the Ses Olives and the western flank of Emile Baudot seamounts as well as in the adjacent seafloor (Figure 1). They have amphitheatre geometry, lengths of about 1.5 to 2.2 km in Ses Olives and up to 5 km long in Emile Baudot seamounts. Mass transport deposits are present along the MC slope, mainly at the foot of slope of the seamounts at different depths generating scarps of up to 20 m high at the seafloor. In seismic profiles, the buried deposits are up to 50 ms thick (Figure 2). x) Bioconstructions are identified in the summits of Ausias March and Emile Baudot seamounts. They are up to 2 to 15 m high, around 200 m long and have circular shape (Figure 1). Biogenic patches are formed by accumulations of fossil and contemporary coralline algae (rhodoliths) and cold water corals.

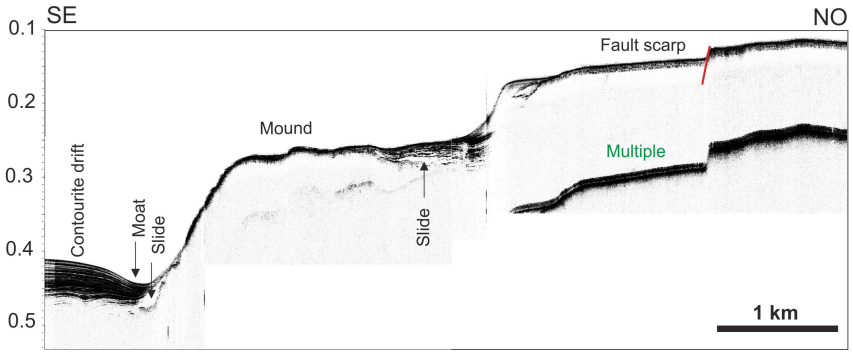

Figure 2: Example of high resolution parametric profile showing some morphological features and sedimentary deposits: mounds, fault scarp, contourite drift, moat and slide deposits.

Discussion

The great variety of morphological features of the MC can be explained by means of several structural, fluid venting/volcanism, gravitational, depositional and erosive, and biogenic processes and grouped into five main groups: (a) seamounts and minor cones have volcanic or structural origin and are the most significant edifices in the MC (Acosta et al., 2003) A linear scarp divide the Ausias March, that is generated by a normal fault (Acosta et al., 2003) (b) pockmarks are related to fluid flow venting although they could have been reworked by the action of bottom currents; (c) slide scars, gullies and mass transport deposits are related to gravitational instabilities (Vázquez et al., 2015) associated to downslope processes affecting the seamounts and adjacent areas; (d) bottom currents related features have been differentiated, they are mainly produced by depositional processes such as the contourite drifts and sediment waves, but also contourite moats are present around the seamounts; (e) Finally, the formation of biogenic patches at the shallow summits of the seamounts (<200 mbsl) could be related to the effect of the Modified Atlantic Water (MAW) (Acosta et al., 2003)

Acknowledgments

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Mares de Andalucía, the first oceanographic campaign from CEI·MAR

Leonardo José Pozzo-Pirotta*[1] , Bárbara Sellés-Ríos[2] , Lucía Rodríguez-Arias[3] , Teresa Conejo-Orosa[1] , José Antonio Caballero-Herrera[4] , Juan Diego Castro-Claros[4] , Lidia Pino-de la Torre[4] , Candela García-Gómez[5] , Simone Sammartino[6]

*Corresponding author: leopozzo@hotmail.com
[1] Ecology and Geology Department, University of Málaga
[2] Centre for Ecology and Conservation, University of Exeter
[3] Ecology Department, University of Cádiz
[4] Animal Biology Department, University of Málaga
[5] Centro Oceanográfico de Málaga, Instituto Español de Oceanografía
[6] Physical Oceanography Group, University of Málaga

Keywords: Internal waves, Alboran Sea, Phytoplankton, Nitrate, Phosphate

Introduction

CEI·MAR (International Excellence Campus of the Sea) constitutes the largest scientific and business aggregation in the marine environment of Spain. The principal aims are to create an academic, scientific, entrepreneurial and innovative environment, and to promote education and research in different specialities concerning the sea. To achieve this goal, CEI·MAR provides the universities a singular infrastructure network, such as several specialized labs, sea classrooms, and the R/V UCADIZ (Moreno-Ostos et al., 2018). The UCADIZ, 25 meters length and 7.5 beam, supports scientists with a dry and wet laboratory apart from top tier instruments for oceanographic campaigns. The first oceanographic campaign organised by CEI·MAR, as a summer school for young marine scientists, was *Mares de Andalucía*, developed between 18-26th July of 2018. From Motril to Cadiz, selected students from universities of Malaga, Cadiz, Granada, Huelva and Almeria had the opportunity to improve their biological and physical oceanographic skills in field. The present document summarizes the tasks carried out during the second leg: Malaga – Algeciras, through which samples were collected from seven different stations (Figure 1).

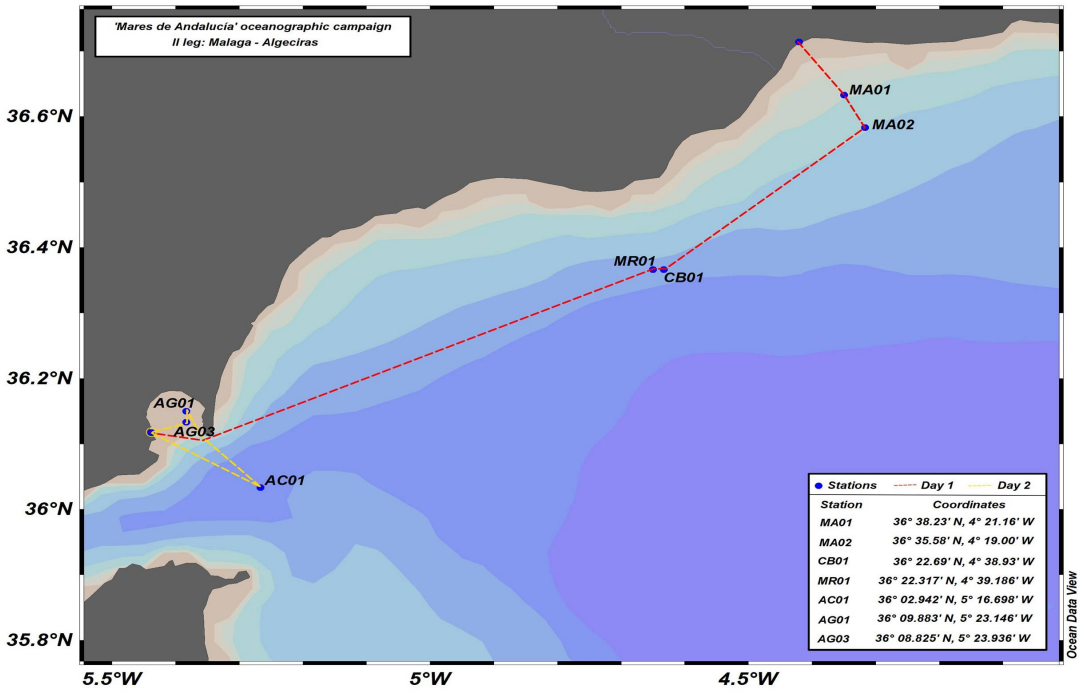

Figure 1: Navigation route carried out for the second leg from 'Mares de Andalucía' oceanographic campaign (Malaga - Algeciras).

Materials and Methods

For each sampling station, the rosette equipped with Niskin bottles and CTD probe was deployed, defining at which depths should the bottles be closed in the upcast. Abundance of *Synechococcus*, *Prochlorococcus*, Picoeukaryotes, Nanoeukaryotes, Cryptophytes and bacteria was determinate collecting 3 replicates of seawater from every sampling station and fixed with 1% paraformaldehyde and 0,5% glutaraldehyde. The samples where then analysed with a FACScalibur flow-cytometer, following the procedure indicated in Gasol (1999). Determination of nitrate, nitrite, phosphate and silicate concentrations was carried out taking 2 replicates of seawater from each depth, immediately frozen at -20 °C. The nutrients were then analysed by segmented flow analysis using a Bran-Luebbe AA3 autoanalizer, following the methods described in Ramírez et al. (2005). Chlorophyll-a concentration was determinate by filtering 3 litres of seawater from each depth and extracting the pigment with 6 mL of acetone 90% for 24 hours, for later analysis with a spectrophotometer according to the methodology described by Jeffrey and Humphrey (1975).

Results and Discussion

Station AC01, located out of the Bay of Algeciras, shows the clear baroclinic structure of the water column at the eastern margin of the Strait of Gibraltar. The T-S diagram in the upper part of Figure 2b presents a mixing line between Atlantic Surface Water (SAW) and Mediterranean waters, notably bowed towards temperatures and salinities lower than expected. This is clearly due to the presence of the North Atlantic Central Water (NACW), which progressively replaces the SAW, mixing with Mediterranean waters. If we look at the T-S diagram centred on the Mediterranean water bodies (lower part of Figure 2b), we can notice how the diapycnal mixing between these and the Atlantic water changes progressively to isopycnal from 400 m depth downwards. This isopycnal mixture is characterized by a progressive decrease in temperature and salinity, caused by the presence of Western Mediterranean Deep Water (WMDW) which mixes

with the Intermediate Levantine Water (LIW) maintaining a nearly constant density. Isopycnal mixture produces a high instability of the water column reflected by low N^2 values from 250 m onwards, as can be observed in the corresponding profile (Figure 2a).

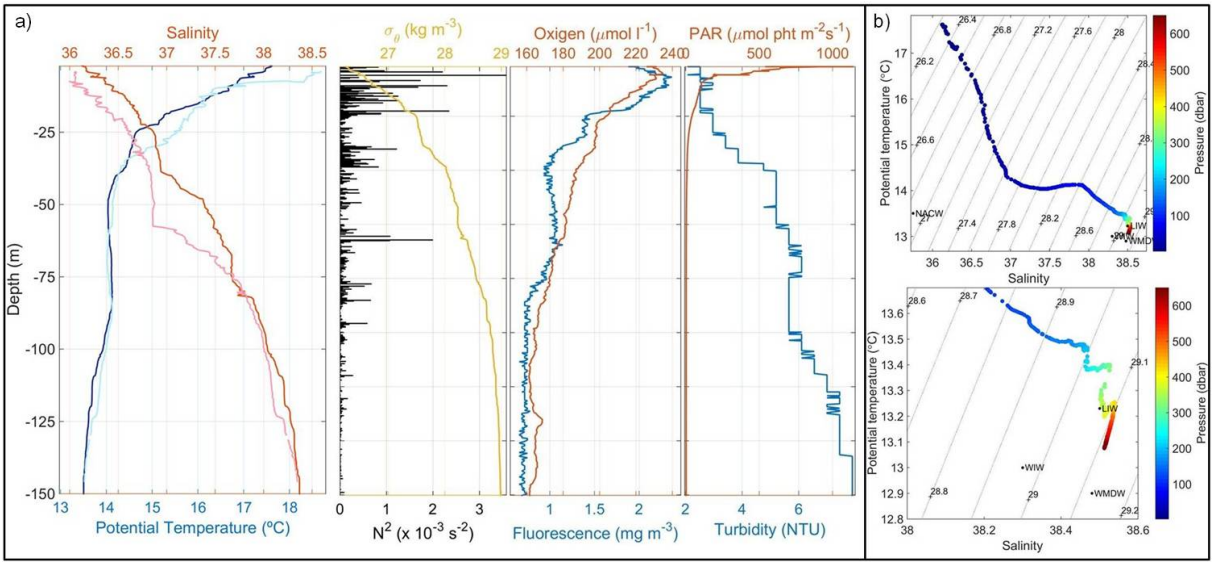

Figure 2: Vertical profiles recorded by the CTD in station AC01. Panel a): downcast (dark colours) and upcast (light colours) of potential temperature and salinity, and vertical profiles of Brunt-Väisälä frequency, potential density, dissolved oxygen, fluorescence, PAR and turbidity. Panel b): T-S diagram (top) and zoom onto deeper Mediterranean waters (bottom). Notice that panel a) profiles are limited to the first 150 m depth.

The first profile on Figure 2a shows the downcast and upcast vertical profiles displayed by the CTD. Between 40 and 60 m depth, a notable vertical displacement of the salinity profile is observed. Its origin could be the pass of internal waves which would cause that halocline displacement in such a short time lapse (Naranjo et al., 2015). As can be seen in Figure 3, nitrate concentration followed the same dynamics in both stations, increasing as it deepens in the water column. In the surface, these concentration values were roughly the same; 3.3 μg/L in MA01 and 3.8 in AG01, but from 20 meters to below, those in the Algeciras station were higher, reaching a maximum of 11.7 μg/L. Phosphate is a scarce nutrient in the Mediterranean Sea and its vertical profiles remained almost the same along the water column, with values below 1 μg/L in both stations.

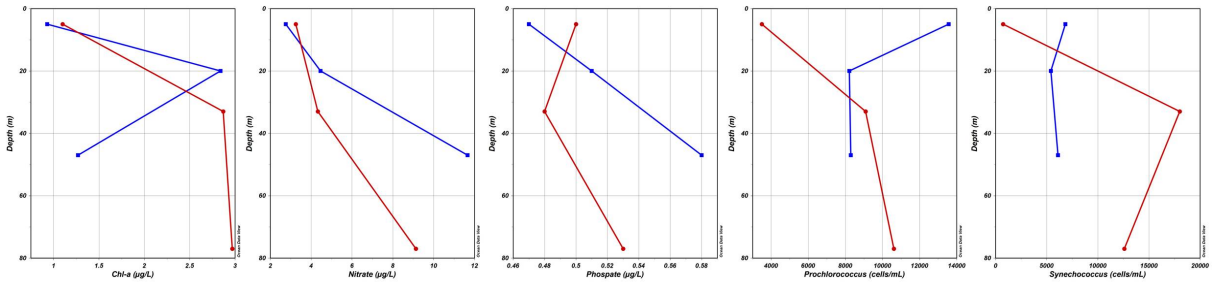

Figure 3: Vertical profiles for chlorophyll-a, nitrate, phosphate, *Prochlorococcus* and *Synechococcus* in MA01 (red) and AG01 (blue) stations.

Chlorophyll-a surface concentrations were similar in Malaga and Algeciras, but they followed different dynamics at greater depths (Figure 3). In MA01, chlorophyll-a increased with depth, remaining almost constant from 30 meters, from where only increased 0.1 μg/L. In AG01, the deep-maximum chlorophyll (DMC) was observed at 20 m reaching 2.84 μg/L. From this depth, chlorophyll-a concentration decreased to values similar to those in the surface, around 1 μg/L.

DMC in station MA01 matched with the lowest nutrients concentrations and maximum value of *Synechococcus* abundance, possibly due to the uptake of these microorganisms and the prevention of nutrients injection because of the stable structure in the water column. This trend could not be seen in station AG01.

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Extreme Environmental Conditions: the study of a Mediterranean hypersaline coastal lagoon.

Maximiliano Szkope-Cobo*[1] , Leonardo José Pozzo-Pirotta[1] , José Luis Díez García[1]

*Corresponding author: carlosmszkope@gmail.com
[1] Department of Ecology and Geology, University of Málaga

Keywords: *Artemia sp.*, Hypersaline lagoon, Cyst, Phytoplankton, Floods

Introduction

On the Mediterranean coast, Guadalhorce′s river mouth is constituted by several permanent lagoons presenting different degree of salinity and depth. With an anthropic origin linked to the extraction of aggregates in the mouth of the river, the building of Guadalhorce′s dam ended with the floods and marshes existing in the mouth of the river. Dredging extraction during the 70s, leaded to a situation where the phreatic level of the alluvial aquifer came to the surface, simulating the original natural landscape of the area. Its operation during high rainfall is associated with an increase in flood level and a decrease in salinity, maintaining most a continuous phase of murky waters with phytoplankton and cyanobacteria (Nieto Gil et al., 2013). One of the major grazers of phytoplankton presented at hypersaline lagoons in Guadalhorce mouth is *Artemia sp.* (Nieto Gil et al., 2013). *Artemia sp.* is an anostraceous, adapted to the harsh conditions imposed on hypersaline environments. Adaptation to these conditions has taken place at different functional levels or domains such as a highly efficient osmoregulation system (Post and Youssef, 1977). Such extreme conditions allows *Artemia sp.* grow without other potential macro-planktonic predators (Gajardo and Beardmore, 2012). Under extremely critical environmental conditions like dying out, *Artemia* takes refuge by producing highly resistant cyst withstanding severe dehydration, enabling an escape from population extinction. Their occurrence is due to the capacity of *Artemia* females are able to switch reproductive mode, producing either cysts (oviparity) when environmental conditions become deleterious or free swimming nauplii (ovoviviparity) under suitable conditions (Gajardo and Beardmore, 2012). The major aim of this experiment focuses on the studying of the physico-chemical and biological conditions at Limícolas lagoon depending on the water level. Also, the production of *Artemia*′s cyst under stressful conditions and its viability were tested.

Materials and methods

Sediment samples (24 Kg) were collected from the first 5 cm sediment layers, both from the near-shore flooded sediments (16.5 Kg) and from the outer edge dried sediments (7.5 Kg) in Limícolas lagoon. Also, water samples (60 L) were collected from the same place before sediment samples were collected, without filtering the water assuring the presence of bacteria, phytoplankton and zooplankton communities. Sediment samples were then placed in three methacrylate aquariums (31x50x25 cm), provided with a drainage system, exposed to a constant photoperiod of 16 hours of light / 8 hours of darkness in chamber with a temperature set at 25°C. Control treatment (aquarium C) maintained the original conditions of the sampling site, seasonal water level variations were reproduced in aquarium WL, while the dynamics of dried sediments from the outer edge of Limícolas lagoon was reproduced in aquarium D. Aquariums C and WL were filled with unfiltered water in the same conditions as was collected in the lagoon (biota included) while the aquarium D, was filled with water filtered by 50 μm net (avoiding the introduction

of *Artemia sp.* individuals and cyst) to study the presence and viability of cyst present at the sediment by the emerge of nauplii and adults. Three times per week, determination of chlorophyll *a* (as universal photosynthetic pigment) was carried out by filtering 3 replicates of 5 mL from each aquarium with a Swinnex Filter Holder (0.5 μm /μg cellulose membrane filter), keeping it 24 hours in darkness with 2 mL of acetone 80 percent for chlorophyll a extraction. Then it was analyzed using a spectrophotometer (Thermo−SCIENTIFIC GENESIS 10S VIS) and determinate concentrations following the equations described in Wellburn (1994). Zooplanktonic organisms were determinate by direct observation on a magnifying glass, where *Artemia sp.* was the only genus found. In order to determinate the viability of *Artemia*′s cysts collected from the aquarium's walls, these were placed in a 500 mL conical flask with sea water sterilized by filtration and continuous oxygenation at 25°C. Also, physicochemical variables such as pH, NaCl concentration and redox potential (Eh) were measured with the same rate.

Results and discussion

The knowledge of chlorophyll a (Chl-a) and physicochemical variables altogether allows to interpret the abundance of microalgae community. Through the changes on the variables cross the time, could be assume as changes on these communities that may affect brine shrimps environment. As it is shown in Figure 1, the negative redox potential measured on the aquariums and the evidence of a bottom-up bubble flux during the whole sampled period suggest the presence of important respiration processes associated with bacteria activity. Despite the acidification produced by respiration processes, pH increased (Figure 1) in all aquariums until the day 28 of the sampled period, because of huger photosynthetic processes.

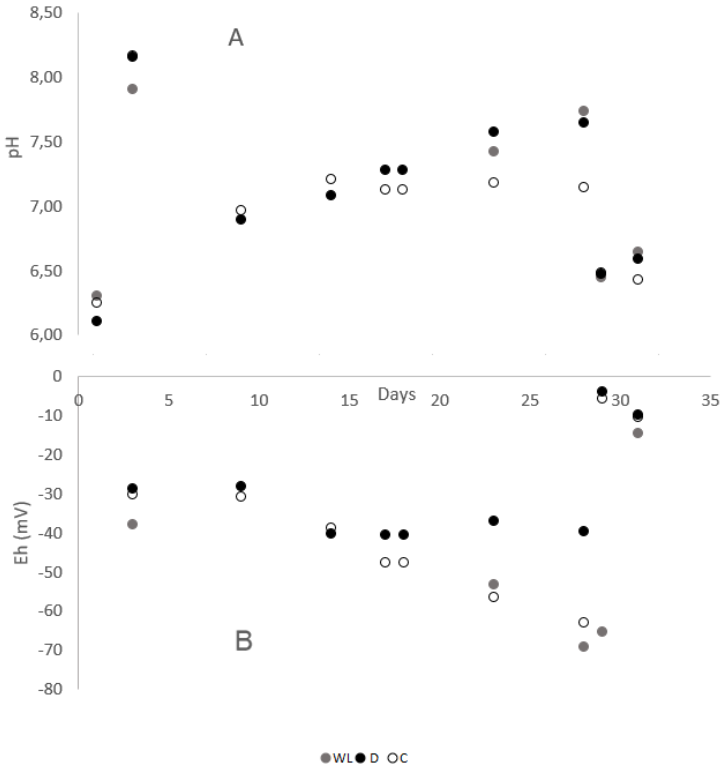

Figure 1: Variations of pH (A) and Eh (B) in each aquarium throughout sampling period.

The NaCl concentration measured during the experiment in the three aquariums comprise a range (90 - 180 g/L) in which the hatching of Artemia cysts is viable (Post and Youssef, 1977)

(Figure 2). Due to thick salt crushes on the collected dry sediment at aquarium D, the highest NaCl concentration were found.

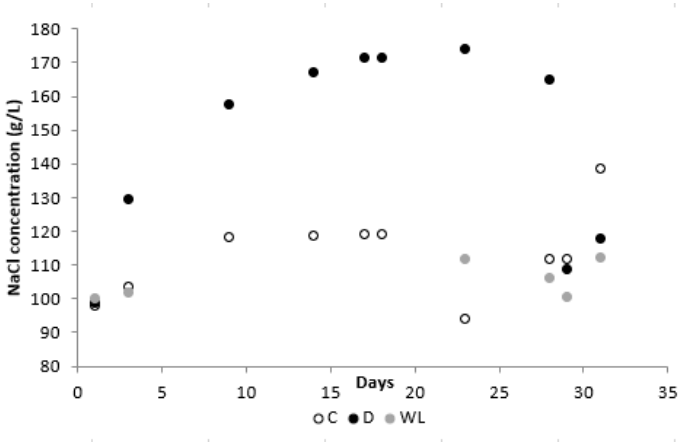

Figure 2: Variations of salinity in each aquarium throughout sampling period.

As it is shown in Figure 3 when the aquariums where set, phytoplankton community suffered an acclimatization period supported by the decrease on chl *a* concentration the first days of the treatment, whereupon, chl-a concentration increased in every aquarium the next few days. As NaCl concentration in aquarium D increased, chl *a* concentration went the other way, these high concentrations of salt limited the number of phytoplankton species capable to grow under this conditions (Chakraborty et al., 2011) although the absence of grazers bias large variations in chl *a* concentration along the experiment. On the other hand, aquarium C go through larger variations in chl *a* concentration due to continuos presence of *Artemia* individuals, regulating the abundance of phytoplankton by increasing their population. Finally, variations in chl *a* concentration in aquarium WL can be assumed as a consequence of the dissecation−rewetting treatment, showing a gap between chl *a* concentration decrease and the increase of *Artemia* population. Phytoplankton communities and physicochemical water conditions determinate the generation and growth of individuals of *Artemia sp.* (Post and Youssef, 1977).

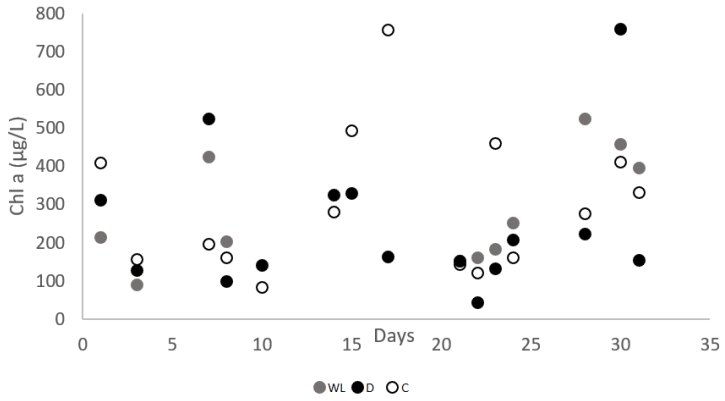

Figure 3: Variations of chlorophyll *a* concentration in each aquarium throughout sampling period.

The viability of the *Artemia* cysts present in the aquariums tested by exposing them to optimum hatching conditions concluded that the cysts produced recently in the C and WL aquariums are viable for the generation of new populations due to their rapid hatching of most cysts, while cysts located in aquarium D were not viable, so the presence of *Artemia* was not observed in that tank.

Acknowledgments

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Interactive effects of temperature and salinity in the seagrass *Cymodocea nodosa*

José Miguel Gil Fernández*[1] , Raquel Sanchez de Pedro[1] , Elena Bañares-España[1]

*Corresponding author: jomigilfe@gmail.com
[1] Universidad de Málaga. Departamento de Botánica y Fisiología Vegetal. Facultad de Ciencias. Campus de Teatinos sn. 29010 Málaga, Spain.

Keywords: *Cymodocea nodosa*, High salinity, Global warming, Mediterranean sea, Sea phanerogams

Abstract

Mediterranean seagrass meadows are one of the most important targets for marine biodiversity conservation. They form complex ecosystems which supply important ecosystem services such as protection and support of different marine species, "blue carbon" sequestration and coastal hydrodynamism reduction (Cullen-Unsworth and Unsworth, 2013). The main effects of global warming that are expected in the Mediterranean Sea are the increase of temperature and salinity (Calvo et al., 2011). Although, the effects of water warming and the increase of salinity on seagrasses are relatively well known, studies of their joint effect are still scarce. In this sense, an experimental crossed design of two factors was established: temperature (25ºC *vs* 30ºC) and salinity (36 *vs* 48), using *Cymodocea nodosa* as a model specie. Four combined treatments were established being the specimens exposed to 25ºC and 36 of salinity, the control group.
Undamaged healthy *C. nodosa* shoots (including rhizomes and roots) were carefully collected by hand from three different zones (0.5-1 m deep) in Punta de la Torre Nueva beach (Málaga) in late May. The shoots were cleaned from dirt and epiphytes before the exposure to the acclimation period at 20ºC, 150 μmol photons m^{-1} s^{-1} and 12:12 (light:dark) photoperiod for 9 days. Then, one plagiotropic shoot with 3-6 orthotropic shoots were placed into 500 mL flasks with GF-C filtered natural sea water and aerated with an air pump. Control temperature (20ºC) were provided by a temperature-controlled growth chamber (EGCS 501 LED, Equitec©) and increased temperature treatments were achieved placing a heater into an aquarium containing the flasks (EHEIM, 25W). Control salinity (36) was measured by means of a refractometer and high salinity treatment were obtained by adding NaCl to the flasks to a final concentration of 48 mg L^{-1}. Three flasks were exposed to each treatment for 14 days (n=3). After the treatments, changes in the photosynthetic responses were analysed by pulse amplitude modulated fluorometry (PAM), carrying out electron transport rates - light curves (rapid light curves, RLCs). Moreover, the absorptance of the leaf, the growth and the survival of the shoots were also analysed.
Preliminary results showed that shoots exposed to 30ºC grew more than control plants. Moreover, shoots exposed to high salinity and high temperature showed clear symptoms of deterioration (e.g. decline of photosynthetic capacity and an increment of necrotic tissue incidence). The results suggest that the combined effect of warming and high salinity could compromise the persistence of *Cymodocea nodosa* especially in those populations inhabiting the intertidal range of closed bays. Nevertheless, more studies should be performed in order to evaluate the implications of this in the climate change predictions and its consequences.

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Analysis of molluscan assemblages from different habitats of the Gazul mud volcano (Gulf of Cadiz)

Olga Utrilla*[1] , Serge Gofas[1] , Javier Urra[2] , Pablo Marina[2] , Ángel Mateo-Ramírez[2] , Nieves López-González[2] , Tatiana Oporto[2] , Marina Gallardo-Núñez[2] , Carmen Salas[1] , José Luis Rueda[2]

*Corresponding author: olutrilla@gmail.com

[1] Departamento de Biología Animal, Universidad de Málaga, Campus de Teatinos s/n, 29071 Málaga, Spain.

[2] Centro Oceanográfico de Málaga, Instituto Español de Oceanografía, Puerto pesquero s/n, 29640 Fuengirola, Málaga, Spain.

Keywords: Molluscs, Gazul, Mud volcano, Gulf of Cadiz, Biodiversity

Introduction

Gazul is one of the mud volcanoes (MV) of the Site of Community Importance (SCI) "Volcanes de fango del golfo de Cadiz" (code ESZZ12002), and it is located in the Spanish margin of the Gulf of Cadiz (Díaz del Río et al., 2014). This MV stands out for the presence of different vulnerable deep-sea habitats and the high complexity of its bottoms, which potentially increase the biodiversity of this area. On the other hand, molluscs are one of the most diverse faunistic groups in marine environment and represent a good indicator for the evaluation of benthic biodiversity in particular areas (Appeltans et al., 2012). For this reason, molluscan assemblages from Gazul MV were studied and characterized in order to improve the knowledge on the biota of this area. This may contribute to its protection and future management under the framework of the Natura 2000 network and the LIFE IP PAF INTEMARES and ATLAS projects.

Materials and methods

Samples were obtained in different areas of Gazul MV during the INDEMARES/CHICA 0610, INDEMARES/CHICA 0412 and ATLAS/MEDWAVES 0916 oceanographic surveys (Figure 1). Sampling was carried out using different sampling methods such as box-corer and shipek grab (single points), benthic dredge and beam-trawl (linear transects) from 362 to 495 meters depth. Molluscs, as well as other faunistic groups, were separated from the sediment in each sample and preserved in 70% ethanol, being identified and quantified. Different statistical analyses were performed in order to identify and characterize molluscan assemblages associated with different habitats and to assess whether these assemblages could correspond to the different areas of Gazul MV: summit and flanks, seafloor depression and adjacent bottoms.

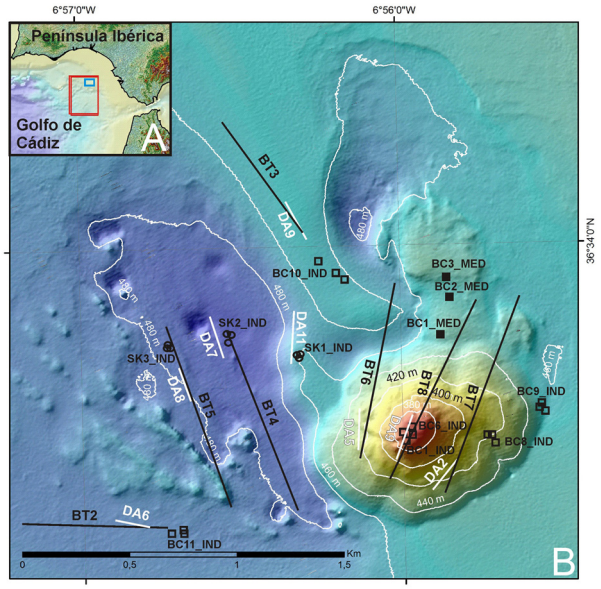

Figure 1: A: Location map of Gazul MV (blue square) within the Shallow Field of Fluid Expulsion in the Gulf of Cadiz (red square). B: Detailed map of Gazul MV with the stations sampled with beam-trawl (BT) (black line), benthic dredge (DA) (white line), box-corer (BC) (square) and shipek grab (SK) (circle) during the INDEMARES/CHICA 0610, 0412 and ATLAS/MEDWAVES 0916 oceanographic surveys.

Results and discussion

266 species and three main molluscan assemblages have been identified. The Assemblage I was found at the summit and flanks where abundant bioclasts, methane-derived authigenic carbonates (MDACs) and deep-water coral reefs and sponge aggregations predominate (Figure 2A, B). The most frequent species in the taxocoenosis were *Bathyarca philippiana*, *Limopsis angusta*, *Danilia tinei*, *Hiatella arctica*, *Karnekampia sulcata* and *Dacrydium* sp., and in the thanatocoenosis were *Alvania cimicoides*, *Parvicardium minimum*, *B. philippiana*, *Bittium watsoni*, *Limopsis aurita*, *Heteranomia squamula* and *Bathyarca pectunculoides*. High evenness, species richness and diversity values were generally found in this assemblage. The high diversity found in this area of Gazul MV may be due to the presence of MDCAs, which are hard substrates that can be colonized by sessile colonial organisms which provide substrate and shelter to other species (Palomino et al., 2016). The Assemblage II was found in the seafloor depression, where detrital bottoms with bioclasts, solitary corals and some MDCAs colonized by some gorgonians and sponges are predominant (Figure 2 C, D). The most frequent species in the taxocoenosis included *Asperarca nodulosa*, *B. philippiana*, *Leptochiton* sp., *Pseudamussium peslutrae*, *Limea crassa* and *L. aurita*, whereas the thanatocoenosis included *P. minimum*, *B. philippiana*, *A. cimicoides*, *A. nodulosa* and *Alvania tomentosa*. Molluscs found both in the summit-flanks and in the depression were mainly associated with hard bathyal substrates and with other invertebrates of these substrates (e.g. *Iphitus tuberatus* associated with corals, *Emarginula* spp. with sponges, *Curveulima devians* with echinoderms), in which they inhabit, find shelter and feed. These molluscs were in low abundance because they are very restrictive species to their host. Finally, the Assemblage III occurred in adjacent bottoms where sedimentary soft bottoms predominate with some sessile cnidarians at low densities (Figure 2E, F). The most frequent species in the taxocoenosis were *B. philippiana*, *Nucula sulcata*, *A. nodulosa*, *Astarte sulcata*, *L. angusta* and *Similipecten similis*, and in the thanatocoenosis were *P. minimum*, *B. watsoni*, *L. aurita* and *L. crassa*. These results match other studies in which a higher species richness and diversity are found in areas with

complex substrates such as corals than in adjacent areas without them (Henry and Roberts, 2007). The presence of MDCAs in the Gazul area and the hydrodynamism of this part of the Gulf of Cadiz may promote the differences between the molluscan assemblages found in the summit-flanks and in the depression with the assemblage found in the adjacent bottoms, which is in line to observations made for the biodiversity associated with cold seep ecosystems along the European margins (Vanreusel et al., 2009).

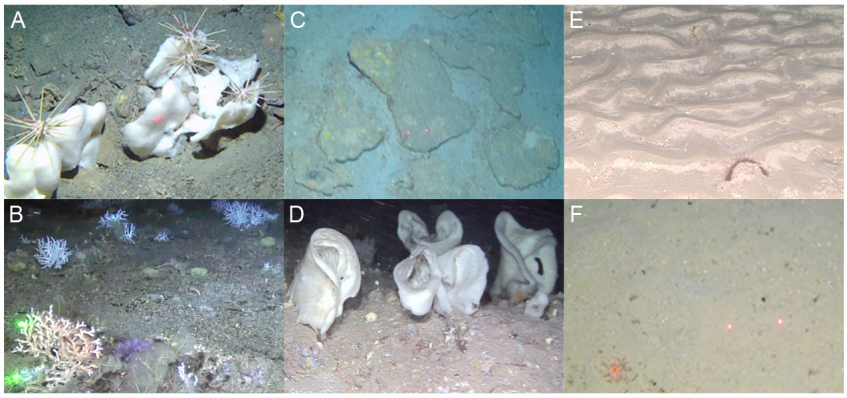

Figure 2: Some of the Gazul MV habitats at the summit and flanks (A-B), seafloor depression (C-D) and adjacent bottoms (E-F). A: Methane-derived authigenic carbonates (MDACs) with large sponges (*Phakelia endi*spp.), gorgonians (*Bebryce mollisendi*) and echinoderms (*Cidaris cidarisendi*); B: Deep-water coral reefs (*Lophelia pertusaendi*, *Madrepora oculataendi*) and black corals (*Antipathella subpinnataendi*). C: Soft bottoms with MDACs; D: MDACs with large sponges (*Asconema setubalenseendi*) and gorgonians (*Acanthogorgia hirsutaendi*). E: Soft bottoms with ripples and anthozoans (*Flabellum chuniiendi*); F: Soft muddy bottoms with anthozoans.

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Ecophysiological and molecular involvement of extracellular-regulated protein kinases (ERK 1/2) in the response of *Dunaliella viridis* to heat stress

Belén González Pastor*[1] , Armando Palma Olmo[1] , Guillermo Ortiz Charneco[2] , Carlos Jiménez[1] , María L. Parages[1]

*Corresponding author: begonzalezp@hotmail.com
[1] Department of Ecology, Faculty of Sciences, University of Málaga, Málaga 29071, Spain
[2] Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland

Keywords: MAP kinases, ERK, Environmental stress, Microalgae, Climate change

Abstract

The eukaryotic green microalga *Dunaliella viridis* has shown an outstanding capacity to face a broad range of environmental stressors such as, high irradiance, UV radiation, salinity, and temperature, among others. The lack of a rigid cell wall, as well as, its unique ability to respond, adapt and grow under stressful conditions makes *Dunaliella* an excellent model to study stress signal transduction in eukaryotic unicellular organisms. Mitogen-activated protein kinases (MAPKs) are highly conserved serine/threonine kinases that convert extracellular stimuli into a wide variety of responses at both cellular and nuclear levels. In eukaryotic cells, MAPKs are involved in both environmental stress responses (JNK and p38 pathways) and cell proliferation and differentiation (ERK pathway) through protein kinase cascades (Charneco et al., 2018). Previously, two different ERK proteins, ERK1 and ERK2 (ERK1/2) have been identified in *D. viridis* (Jimenez et al., 2007). Based on the non-direct implication of the ERK-like proteins in the acclimatization process against environmental stress proven by the specific blockade of the cascades, different short-term heat-shock (SH) experiments have been conducted in this work to uncover molecular mechanisms underlying the role of ERK1/2 in heat stress. The evolution of the maximum quantum yield of *D. viridis* after non-lethal SH, together with protein immunodetection by ERK1/2 western blotting and qRT-PCR experiments revealed that the ERK1/2 proteins are not directly involved in the response to heat stress, and that they are rapidly deactivated after stress, leading to a transient inhibition of cell division. Behaviour of MAPK-like proteins in algae is largely unknown at present. The analysis of their mechanism of action, as well as their function in this model microalga, will allow us to decipher the fate of unicellular eukaryotic organisms in aquatic ecosystems subjected to environmental stress derived from the conditions prevailing within a framework of global climate change.

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Physiological responses of adults and juveniles of *Cystoseira tamariscifolia* to projected warming scenarios along Alboran sea populations

Ana Campos Cáliz*[1] , Andrea Nieves Fernández[1] , Raquel Sánchez de Pedro[1] , Antonio Flores-Moya[1] , Elena Bañares-España[1]

*Corresponding author: anacamposcaliz@gmail.com
[1] Universidad de Málaga, Departamento de Botánica y Fisiología Vegetal. Facultad de Ciencias. Campus de Teatinos sn. 29010 Málaga, Spain

Keywords: *Cystoseira*, Early-developmental stages, Global warming, Marine heatwaves, Mediterranean

Introduction

Marine ecosystems are fragile habitats exposed to multiple environmental and anthropogenic stressors facing global change. Over the past decades, sea water temperature has risen worldwide, but at the onset of 21^{st} century, extreme temperature events in the ocean, known as marine heat waves (MHWs), have increased in frequency and intensity (Hobday et al., 2016). The ecological consequences of global warming and MHWs could be particularly detrimental for habitat-forming species, which have a key role in structuring benthic communities, provide shelter and sustain food webs. In the Mediterranean bioregion, *Cystoseira tamariscifolia* (Hudson) Papenfuss is one of the main canopy-forming species in the lower eulittoral and upper-sublittoral ecosystems. To manage the conservation of these communities, it is essential to understand how current and future projected warming scenarios will affect the thermal physiology of different life stages of these species (Smale et al., 2019). The aim of this study is to assess the thermal resilience of adults and juveniles of *C. tamariscifolia* from three populations located along an Atlantic-Mediterranean gradient in the Alboran sea, to predict their responses to future climate change scenarios. We hypothesize that juveniles will be more vulnerable to thermal stress than adults, and that thermal resilience will decrease towards atlantic-influenced populations.

Materials and methods

In this study, we chose three photophylic communities of *C. tamariscifolia* located on three rocky intertidal ecosystems, along a natural Atlantic-Mediterranean gradient of temperature and eutrophy: Calaburras (CLB)(36°29'24.0"N. 4°41'41.0"W), location with a higher Atlantic influence and the most euthropic; La Araña (MLG) (36°42'41.7"N. 4°19'39.3"W), intermediate location, with oligotrophic waters; and Marina del Este (MDE)(36°43'25.2"N, 3°43'36.36"W), the most mediterranean with ultraoligotrophic waters. Physiological thermal resilience was determined by growth and photosynthetic responses of juveniles and adults of *C. tamariscifolia*, by exposing them to three temperature levels (20°C, 24°C, 28°C). Thermal treatments were based on the previous climate analysis of the MHWs and the predictions of Bio-Oracle with climate change scenarios, under the representation RCP 8.5 in the three locations. Surface sea temperature (SST) data were retrieved from NOAA/NCEI (data set OISST-V2-AVHRR Daily 1/4 degree), from January 1982 to December 2018, for the detection of marine heat waves (MHWs) using the R package *heatwaveR*, as described by Hobday et al. (2016). In this way,

we obtain a categorization and classification of the extreme temperature events in the three sampling stations. Thanks to this analysis, we know that the maximum temperature reached during an MHWs was 28°C. Average SST data were consulted from Bio-ORACLE, in a present, near future (2050) and distant future (2100) climatic scenarios of RCP 8.5. Mean temperatures of approximately 20°C and 24°C were obtained for the study area, which indicates the actual temperature levels to be used in the experimental design. For growth in adult thalli, 3 apical fragments (2.5 cm) were cut off 12 individuals and were assigned to 4 experimental units (R1-R4) for each temperature (20°C, 24°C, 28°C). Zygotes were isolated following the protocol described by Falace et al. (2018). Four independent stocks were retrieved and inoculated in a 24-multi-well plate and in disks of epoxy-resin for photosynthetic measurements (Brawley and Johnson, 1991). Relative growth rates (RGR) were estimated by relative changes in length. Adults and juveniles were cultured with GF-C filtered natural seawater (NSW, sterilized), at 150 μmol photons m^{-2} s^{-1}, photoperiod 12:12 (L:D). Photosynthetic responses were determined by pulse amplitude modulated fluorometry (PAM), which measures the fluorescence of chlorophyll *a* associated with photosystem II. Optimum quantum yield (F_v/F_m) was used as a measure of physiological fitness, and photosynthetic parameters (ETR_m, α, E_k, NPQ_m) were determined from the electron transport rates- light curves (rapid light curves, RLCs). To each variable within each stage (adult/ juvenile), a crossed design 2 way repeated measures ANOVA was applied, with site (CLB, MLG, MDE) and temperature (20, 24, 28) as factors. Non-parametric Kruskal-Wallis ANOVA by ranks was applied when Levene's test for homogeneity of variances was not passed. All analyses were performed in STATISTICA 7.

Results and discussion

Temperature or location did not have a significant effect on adults growth but did on juveniles (Figure 1). Growth of juveniles from MDE was unaffected by temperature, while those from MLG had a significantly lower growth at 28°C. Nevertheless, juveniles from MDE had 10-times greater RGR than MLG, even though not statistically significant. This suggests that local acclimation of *C. tamariscifolia* to warmer waters (MDE, Mediterranean location) increases thermal resilience of juveniles. Since adult thalli of *C. tamariscifolia* from CLB were not reproductive, juvenile data from that location were not available. The dense algal epiphyte cover found at this location may be on factor influencing their capacity to reach maturity.

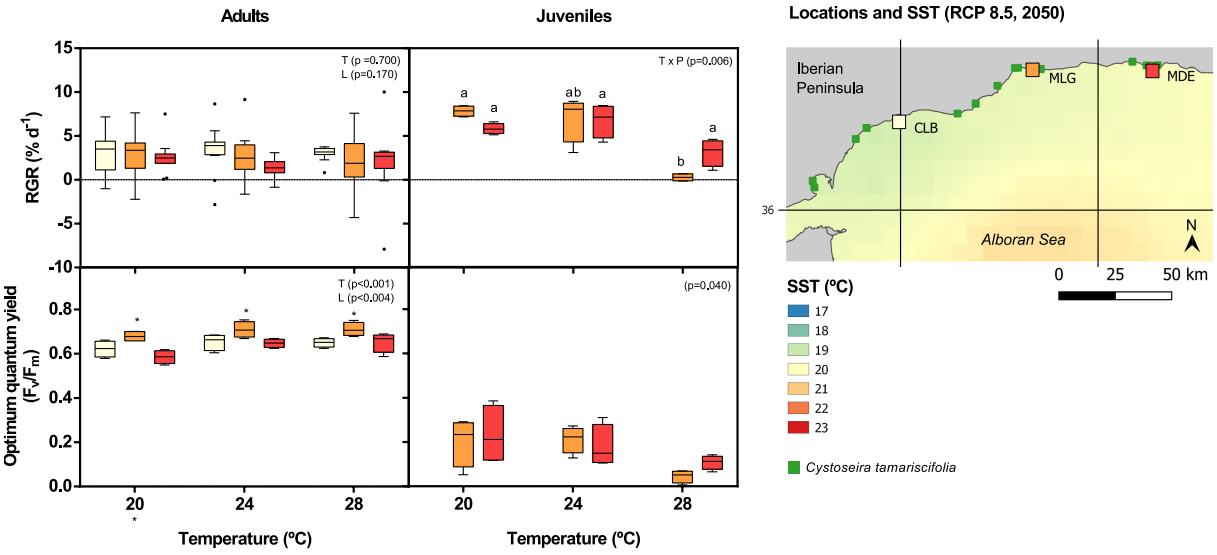

Figure 1: Growth and physiological fitness of adults and juveniles of *C. tamariscifolia* from three populations from the Alboran Sea, measured after two weeks of culture at 20, 24, 28°C. Note that colour symbology for the locations map indicates location levels in the graph. Sea surface temeprature (SST) is expressed in °C.

Location and temperature explained 44% and 18% of the total variance of F_v/F_m of adults, respectively. Individuals from MLG represent a significantly higher value of F_v/F_m compared with CLB and MDE. Also, F_v/F_m was lower at 20°C, suggesting that optimal range could be between 24-28°C, but in contrast, juveniles were unaffected (Figure 1). ETR_m and NPQ_m were unaffected by location and temperature, although the latter tended to be higher at 24°C in adults, and 20°C in juveniles (Table 1). Location influenced photosynthetic efficiency (α) but not in light saturation point (E_k) in adults. Adults from CLB presented higher photosynthetic capacity than those from MLG or MDE, which could be due to the higher water turbidity in that eutrophic environment (Celis-Plá et al., 2017) and/or the dense algal epiphyte coverage over their thalli. In the case of juveniles, E_k varied among locations, were MLG presented higher light saturation point than MDE. Differences in photosynthetic efficiency among locations and temperature levels, were only significant between MLG-20°C and MDE-28°C. These differences in E_k and α could be explained by their differing habitat and morphological characteristics. For instance, *C. tamariscifolia* from MLG inhabits tidal pools and have lower individual bushiness than that from MDE (subtidal pools, denser canopies), which implies lower self-shading and more light availability for understory juveniles in the case of MLG.

Table 1: Photosynthetic parameters obtained form rapid light curves (RLCs) of adults and juveniles of *C. tamariscifolia* from three populations of the Alboran Sea measured after two weeks of culture at 20, 24, 28ºC.

		Adults			Juveniles		
Variable	Location	20ºC	24ºC	28ºC	20ºC	24ºC	28ºC
ETR_m	CLB	180 ± 55	214 ± 23	180 ± 55	nd	nd	nd
	MLG	212 ± 139	136 ± 17	159 ± 29	90 ± 27	96 ± 14	114 ± 18
	MDE	231 ± 123	150 ± 37	187 ± 50	105 ± 13	112 ± 31	136 ± 54
α	CLB	0.43 ± 0.06	0.44 ± 0.05	0.43 ± 0.06	nd	nd	nd
	MLG	0.37 ± 0.05	0.35 ± 0.09	0.32 ± 0.06	0.06 ± 0.02	0.06 ± 0.01	0.07 ± 0.01
	MDE	0.36 ± 0.01	0.35 ± 0.05	0.42 ± 0.06	0.13 ± 0.06	0.09 ± 0.01	0.12 ± 0.02
E_k	CLB	414 ± 86	489 ± 54	414 ± 86	nd	nd	nd
	MLG	545 ± 280	415 ± 127	523 ± 163	1557 ± 41	1599 ± 103	1625 ± 217
	MDE	639 ± 335	441 ± 125	440 ± 65	974 ± 466	1273 ± 310	1107 ± 437
NPQ_m	CLB	0.52 ± 0.39	0.89 ± 0.53	0.41 ± 0.31	nd	nd	nd
	MLG	0.90 ± 0.34	0.81 ± 0.29	0.96 ± 0.28	0.33 ± 0.24	0.22 ± 0.17	0.02 ± 0.01
	MDE	0.65 ± 0.28	0.83 ± 0.07	0.54 ± 0.22	0.39 ± 0.39	0.14 ± 0.23	0.02 ± 0.02

Photosynthetic capacity (ETR_m) in μmol electrons m^{-2} s^{-1}; photosynthetic efficiency (α) in μmol electrons μmol photons^{-1}; light saturation irradiance (E_k) in μmol photons m^{-2} s^{-1}; maximum non-photochemical quenching (relative units).

This study demonstrates that adults of *C. tamariscifolia* from the Alboran sea have a more plastic physiological response to warming than their juveniles, based on the lack of effect of projected SST temperatures and differences between locations. This result remarks the ecophysiological homogeneity of this species within the Alboranic region. In contrast, juveniles of *C. tamariscifolia* were more sensitive to warming conditions, particularly those from a location with less mediterranean influence (MLG), whose growth dropped at 28°C. This is in good agreement with the higher vulnerability of microscopic stages of many other macroalgae (Brawley and Johnson, 1991; Falace et al., 2018), and it suggests that thermal resilience of juveniles of *C. tamariscifolia* declines towards the Atlantic, being those populations the most vulnerable to climatic stressors. On the other hand, differences in the photophysiology of both adults and embryos, particularly E_k and α evidenced that they are exposed to different light environments, derived from local environmental conditions and demographic and morphological variables (i.e. individual density and bushiness). This new understanding should help to improve predictions of the impacts of MHWs on these communities in the next future (Smale et al., 2019).

Acknowledgments

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Submarine geomorphology of the continental Caribbean margin adjacent of Urabá Gulf

Ana Maria Osorio Granada*[1,4,5] , Bismarck Jigena Antelo[2,4] , Juan Manuel Vidal Pérez[3,4]

*Corresponding author: anmosoriogr@unal.edu.co

[1] University of Cadiz, International Doctoral School in Sea Studies (EIDEMAR). Puerto Real. Cadiz, Spain

[2] University of Cadiz, College of Marine, Nautical and Radioelectronic Engineering (EIMANAR). Puerto Real, Cadiz, Spain

[3] University of Cadiz, College of Naval and Oceanic Engineering (EINO). Puerto Real, Cadiz, Spain

[4] Department of Nautical Sciencies and Naval Construction. Puerto Real, Cadiz, Spain

[5] Centre of Hydrographics and Oceanographics Research (CIOH), Cartagena, Colombia

Keywords: Acoustic technologies, Multihaz, Western Caribbean Sea, Deep submarine

Abstract

In this work, we study the morphology of the shallow and deep submarine environments along the western continental margin adjacent to the Urabá Gulf in the Colombian Caribbean Sea (CCS) using multibeam bathymetry data. Bathymetric dataset used were obtained recently by the Centro de Investigaciones Oceanográficas e Hidrográficas (CIOH) in 2018. Initially, the original data was pre-processed in the specialized Teledyne Caris software in order to make a quality control of the information and eliminate the raw depth soundings. After we create a Digital Terrain Model (DTM) with a spatial resolution of 35 m. We carried out standard morphometric calculations that include hillshade, slope, aspect, and curvature models. The morphometric analysis let us identify substantial differences in features submarine along continental Caribbean margin adjacent of Urabá Gulf. The continental shelf is considered here to be a major geomorphic division characterized by being a relatively large and extensive, locally interrupted by mud volcanoes, pockmarks, and reef structures. The continental slope reveals channels, narrow and deep canyons partially destroyed by extensive mass transport deposits and small landslides. Most of the landslides occur within the larger canyons and suggest that these canyons have acted as an efficient conduit of sediment to the deep basin, transporting large quantities of material to the deep sea.

The present morphology of the study area is the result of complex sedimentary processes that mainly include sediment transport and deposition, and sediment instability. These analysis are key to advance in the basic knowledge on the geology and geomorphology of the Caribbean region and have direct implications for future research on the characterization of deep ecosystems, geohazards, natural resources, and development of coastal plans and management strategies (Yonggang et al., 2016).

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Crustal Structure of the Cayos Basin, Western region of the Colombian Caribbean Sea

Ana María Osorio Granada*[1,4,5] , Bismarck Jigena Antelo[2,4] , Juan Manuel Vidal Pérez[3,4] , Orlando Hernández Pardo[5]

*Corresponding author: bioana331@hotmail.com

[1] Universidad de Cádiz. International Doctoral School in Sea Studies, EIDEMAR, Puerto Real. Cadiz, España

[2] Universidad de Cádiz, College of Marine, Nautical and Radioelectronic Engineering. Puerto Real, Cádiz, España

[3] Universidad de Cádiz. College of Naval and Oceanic Engineering. School of Marine, Nautical and Radioelectronic Engineering. Puerto Real, Cádiz, España

[4] Universidad de Cádiz. Department of Nautical Sciencies and Naval Construction. Puerto Real, Cádiz, España

[5] Universidad Nacional de Colombia, Department of Geosciences. Bogotá, Colombia

Keywords: The Caribbean Sea, Marine geophysics, Gravity and magnetic anomalies, Oceanic crust

Abstract

In last years, the study of Colombian maritime jurisdiction areas has been focus of attention by scientific, technical, industrial and governmental institutions. We have many projects focused on improved geological knowledge of our seas especially the Colombian Caribbean Sea (CSS). However, the exploration of the marine and insular areas of the country is limited due to logistical, economic and technical factors that difficult to obtain data and information base. In marine areas, the study of the structure of lithosphere through the interpretation of anomalies of potential fields (gravimetric and magnetic anomalies) plays an important role in the early stages of geological exploration, providing insight into regional features and a record of the properties and structure of the crust and its geodynamic evolution (Kearey et al., 2009). This work focus attention on The Cayos Basin which corresponds to a marine and insular frontier basin, represents a vast 144.755 km2 under-explored area in the western region of the CSS, in the Lower Nicaraguan Rise (LNR). Its geometry and location being determined by the proposal of the National Hydrocarbon Agency (ANH, Agencia Nacional de Hidrocarburos) where the sedimentary basins of Colombia are delimited including those of a marine type (Barrero et al., 2007). Within the area that covers the basin, there is a database of geophysical studies of gravimetric and magnetic, obtained through the Universidad Nacional de Colombia (UNC) and the United States Geological Survey (USGS) respectively. These data have been compiled and processed in OASIS MONTAJ software of GEOSOFT in order to perform regional interpretations of the crust and basement structure of the basin, modeling contrasts of density and magnetic susceptibility of the rocky bodies of the crust underlying, identify lineaments, structural paleohighs and give a possible approximation of the crust-mantle interface. The analysis and interpretation of these geophysical data allowed us to identify the geometry of the main structural features in the Cayos Basin, including the Hess Escarpment,the Pedro Bank Fault Zone, San Andrés Rift, the Nutibara Rift, and other depressions,submarine valleys , hills, and seamounts scattered throughout the area. Finally, the generated maps and the two-dimensional proposed models, an irregular geomorphology is suggested for the basin basement. This basement is characterized by present typical features

of distensive tectonic environments such as horts and grabens, where the tectonics has affected and uplift blocks of the basement reaching a depth that varies between 2 and 6 km. In the forward proposed model, the MOHO discontinuity reached an average of 23 km. These results are consistent with the seismic predictions of an oceanic crust which an anomalous thickness that extends throughout the Los Cayos Basin to the Colombia Basin.

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Obtaining and maintaining sea bass monoclonal cellular lines (*Dicentrarchus labrax*): Improving gene-transfer techniques

Rodrigo Barroso-Bolaños*[1] , Alba Vergès-Castillo[2] , José Antonio Paullada-Samerón[2] , José Antonio Muñoz-Cueto[2] , Águeda Jimena Martín-Robles[1] , Carlos Pendón[1]

*Corresponding author: rodrigo.barrosobolanos@alum.uca.es
[1] Department of Biomedicine, Biotechnology and Public Health, Area of Biochemistry and Molecular Biology. University of Cádiz, Puerto Real (Cádiz)
[2] Department of Biology. University of Cádiz, Puerto Real (Cádiz)

Keywords: Monoclonal cell lines, Gene transfer, Lipotransfection, Electroporation, Photoperiod

Introduction

Aquaculture is one of the productive activities that has grown the most in the last decades. However, despite the great economic investment dedicated in recent years to this industrial activity, it is still far from the progress made in other productive processes such as agriculture and livestock. One of the main obstacles that exist in the research lines dedicated to fish aquaculture is the availability and maintenance of embryos, larvae, juveniles and adult specimens. These difficulties, together with the new 3R policies (reduce, replace and refine) (Schaeck et al., 2013), make it necessary to search for new experimental models such as primary cultures or cell lines for in vitro studies, which can be extrapolated later to living organisms. Faced with the use of individuals, cell lines derived from them are a valuable tool in research, which may have applications in many areas such as marine biotechnology, nutrition or immunology, as well as to clarify the cellular response to light and temperature. At present, numerous freshwater fish cell lines are available, but the number of marine fish cell lines such as sea bass (*Dicentrarchus labrax*) is still very low. The European sea bass, *D. Labrax*, is a species of high commercial interest worldwide and, particularly, in Spain, with great importance for the aquaculture sector. Since this industry began to settle as an economically relevant activity, the production of sea bass has experienced an upward trend and it is expected that its demand will continue growing in the coming years (APROMAR, 2017). Therefore, a greater study of the production process is required, which allows the growing demand to be profitable through the optimization of the diets, the quality of the water used, the control of diseases and problems such as precocious puberty or the disproportion of sexes , aspects for which there is greater interest and to which the research devotes more resources. Other important factors in the optimization of production are: the temperature of the water where the specimens develop, the duration (photoperiod), the spectrum and the intensity of the light to which they are exposed. It is known that these two environmental factors are crucial in the synchronization of daily and seasonal rhythms, through their action on molecular clocks, so that their physiological functions, biochemical and behavioral processes can be affected by their variation (Martín-Robles et al., 2011). Therefore, in the present work two objectives were raised. Firstly, obtaining and maintaining embryonic monoclonal sea bass cell lines (DLEC) in different light conditions. Secondly, the use of these lines for the optimization of gene transfer, as a means to carry out studies of gene expression and its regulation.

Materials and Methods

To obtain the embryonic monoclonal cell lines, the DLEC (Buonocore et al., 2006) cells were cultured at 22°C in Leibovitz's medium (L-15) supplemented with fetal bovine serum and NaCl (complete L-15). When the cell confluence was approximately 80%, cultures were passaged for routine maintenance. From a culture in exponential growth phase, a limit dilution was made in a 96-well plate to obtain cell lines derived from a single cell, thus achieving monoclonal cell lines. The gene transfer assays were performed in one of the monoclonal lines obtained, in two environmental conditions: darkness (DD) and light photoperiod (LD) of 12 h of light and 12 h of darkness, transfecting 6 horas after the beginning of light period. To compare the efficiency of transfection by electroporation and lipofection, $1x10^5$ cells and the pDsRed and pEGFP plasmids were used. For this, the cells were detached with trypsin and counted by an automated cell counter TC20. Electroporation was performed by mixing 100μL of cells in L-15 medium per cuvette with 1μg of each of the plasmids. Subsequently, it was brought to 1 mL with complete L-15 medium and the cells were transferred to 12-well plates. The cells were maintained under the same culture conditions as the initial cell line. The lipofection was performed with the VIROMER RED reagent, following the instructions indicated by the manufacturer. Throughout the assay, the transfection efficiency was evaluated in a fluorescence inverted microscope, visualizing the fluorescent cells with the GFP filter to identify the EGFP protein, and the Red filter for the DsRed fluorescence. The health and cell morphology were visualized in a bright-field and by phase contrast.

Results and discussion

We obtained 21 cell lines derived from a single cell with different morphology. The cells derived from a monoclonal line were cultured in both photoperiod conditions (DD and LD), transfected with the pEGFP and DsRed plasmids and their expression was analyzed at 72 h post-transfection, with new transfected cells being found until at least 14 days after (Figure 1).

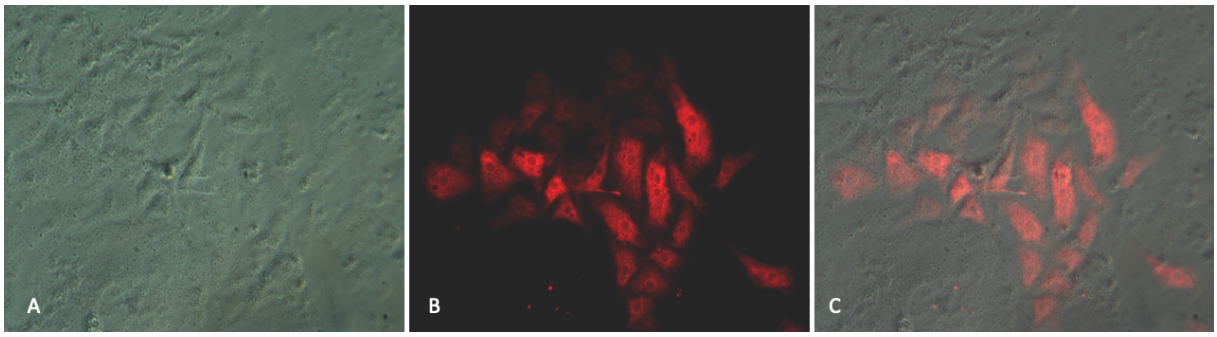

Figure 1: Fluorescence of DsRed transfection: (A) Bright-field (B) Red filter (C) Merge images A and B.

Comparing the efficiencies of the transfection in the different conditions tested, the electroporation of cells maintained in LD photoperiod produced the highest rate of transfected cells. It was also observed under these conditions, a high rate of transferential DNA integration, producing stable fluorescent cell sublines, endorsing the use of these cell lines as a model for the study of gene expression and its regulation.

Acknowledgments

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Phytoplanktonic seasonal cycle in Alboran Sea

Elena Pérez-Rubín*[1] , Maria del Carmen García Martínez[2] , Francina Moya[2] , Andreas Reul[3] , María Muñoz[2] , Manuel Vargas-Yáñez[2]

*Corresponding author: elenaprubin@gmail.com
[1] Universidad de Málaga/Instituto Español de Oceanografía
[2] Instituto Español de Oceanografía, C. O. Malaga (Fuengirola). Puerto Pesquero s/n, Fuengirola, 29640 Málaga, Spain.
[3] Andalucía Tech, Departamento de Ecología y Geología, Universidad de Málaga, Campus de Teatinos s/n, 29071 Málaga, Spain.

Keywords: Alboran Sea, Oceanography, Phytoplankton

Introduction

We present and discuss comparing with literature, the description of a seasonal cycle of phytoplanktonic communities in the NW region of the Alboran Sea, which is one of the most productive areas in the Mediterranean Sea.

The data analyzed in this work proceed from the project funded by the Instituto Español de Oceanografía (IEO): RADMED, which, systematically, monitors the Spanish Mediterranean waters on a seasonal basis. IEO started in 1992 the monitoring project ECOMalaga, in which a number of fixed stations perpendicularly to the coast were sampled on a seasonal basis, in order to get seasonal patterns and detect changes and trends. In 2007 all these projects were unified in one called RADMED (Radiales del Mediterráneo). RADMED project can be considered an Operational Oceanography activity, which in a systematic and long-term way, samples and takes measurements on the seas, oceans and atmosphere. The obtained observational data must be transferred in the shortest period of time to the assimilation centers, where they are treated and used in models whose final products may be disseminated producing statistics and alerts among others. In the waters of the study zone, a set of processes as primary production, Strait of Gibraltar dynamics and cyclonic circulation, give as result a high phytoplanktonic biomass abundance (micro and pico- and nanophytoplankton) throughout the year, being the most productive waters in the Spanish Mediterranean (García-Martínez et al., 2019). This high biomass values are located in very shallow waters, which indicates the input of nutrients to the surface or subsurface depths. This enrichment could be caused by the passage of the Mediterranean Outflow Waters (MOW) rich in nutrients, and the upwelling which takes place in Cabo Pino, due to the circulation of cyclonic cells that occurs in this area (Reul et al., 2005).

Material and Methods

The selected stations for this work located in the Alboran Sea are: Cabo Pino (P), Malaga (M) and Velez-Malaga (V) (Figure 1). In this work, data from Spring (2015), Summer (2017) and Fall (2016) RADMED surveys are analyzed. The samples were taken at standard depths (0-10-20-50-75-100m). For microphytoplankton determination, samples of 150 ml of sea water at each depth were fixed with lugol solution, and they were analyzed by microscopy following Uthermöhl metodology (Utermöhl, 1958). For the analysis of pico- and nanophytoplanktonic

communities by flow citometry, 5 ml of water sample at each standard depth were fixed with glutaraldehide and immediately frozen in liquid nitrogen on board until its laboratory.

Results

In Figure 2, integrated relative seasonal abundances of microphytoplankton are shown, at each location (stations P, M, V). The highest abundances are detected in summer. García-Martínez et al. (2019) analyzed the complete time series of RADMED data. In the case of the Alboran Sea, phytoplankton statistics were calculated using data from 1994 to 2015. These authors described the highest concentrations in Cabo Pino (P) station in fall, while our data show this maximum in summer. This is an example of the high variability that can be found in the phytoplankton abundance that is dependent on the oceanic conditions.

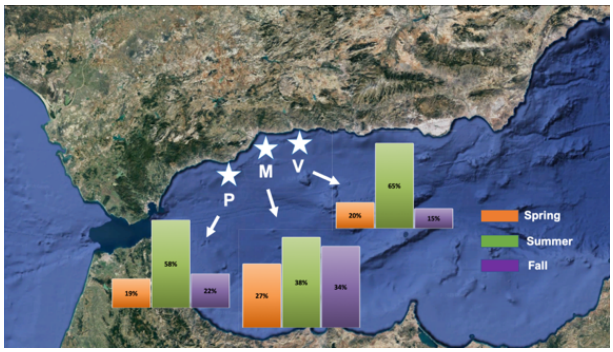

Figure 1: Integrated seasonal microphytoplankton abundance (cells/m^2) in Alboran Sea (RADMED station samples included).

In the pie charts shown the results obtained in each sampling station and its seasonal cycle are presented, integrating the concentrations obtained at the different depths. In spring similar profiles in the most distant stations are obtained (P and V). However, in Malaga the abundance is "balanced" between Diatoms and Small flagellates (almost 50%). Similar results are obtained in fall in Cabo Pino (Figure 2C). Again, these results are in contradiction to those found in García-Martínez et al. (2019), where the Diatoms become dominant in the entire water column.

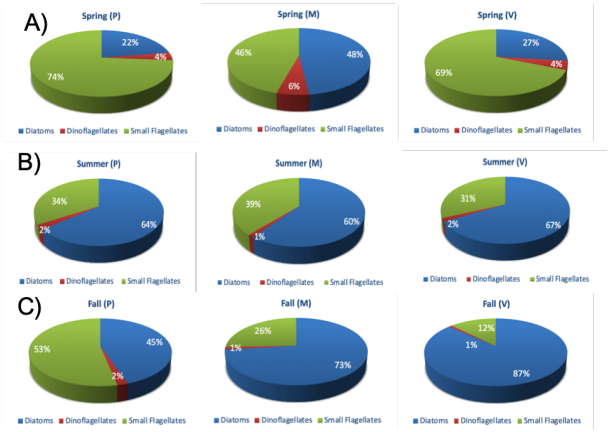

Figure 2: Integrated seasonal microphytoplankton abundance (cells/m^2) in Alboran Sea in Spring: Diatoms (blue), Small flagellates (green), Dinoflagellates (red).

In summer (Figure 2B), a general stratification appears in the three stations, obtaining very similar results where the dominant group is diatoms (about 60%) in accordance to García-Martínez

et al. (2019) As shown in the Figure 2C a diatom increasing concentration gradient from west to east is found in fall, being this pattern contrary to the one described in literature. On the whole at global level, only in two occasions a certain balance between the Flagellates/Diatoms abundance (M in spring and P in fall) is detected. Mainly the Diatoms were the most abundant group and just in the spring sampling in Pino and Velez, the Small flagellates were the dominant group. The Dinoflagellate's presence has remained stable in all the stations and during the seasonal cycle, in summer and fall between 1-2% and in spring 4-6%. All the groups (Diatoms, Small flagellates and Dinoflagellates) were most abundant in the surface layers (having the highest peak at 20 m). Concerning to pico- and nanoplankton, Figure 3 shows the integrated seasonal abundance. As shown, the three stations coincide that the highest abundances take place in spring, while the lowest are in fall. These results are contrary compared with the ones from García-Martínez et al. (2019) where in fact, generally the minimum abundances occurred in spring.

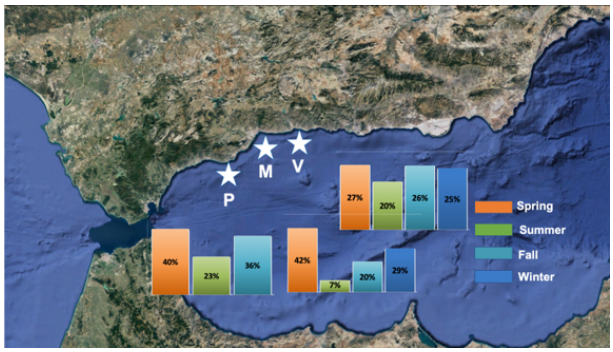

Figure 3: Integrated seasonal pico- and nanophytoplankton abundance (cells/mL) in Alboran Sea (RADMED station samples included).

In the Alboran Sea, the highest picophytoplanktonic abundance usually takes place under stratification conditions (from spring to fall). We have found important differences about their distributions in the water column: *Synechoccocus* abundance were maximum at the surface layers (especially in the first 20 m of the water column), decreasing with depth (except in Malaga in summer). This behavior has been described before, showing their preference for well-lit waters and their adaptation to nutrients-poor waters. On the contrary, the *Prochlorococcus* usually present a maximum depth from 25 to 75 m, showing their preference to poorly lit waters. However, the Prochlorococcus maximum at 10 and 20 m depth in summer (in P, M and V) and at 10 m depth in fall (P), suggests the existence of a shallower nutricline, with a high nutrient availability in the most superficial layers, even in the stratification period. The picoeukaryotic group has been found mostly in winter, when the input of nutrients to the photic layer is higher. Nanoeukaryotic has been the minority group in all stations throughout all the seasons. About the nutrients results, the highest abundances can be detected in fall for all the stations (except nitrites in Cabo Pino). Furthermore, it should be noted a gradient existence which decreases towards East in Alboran Sea, coinciding with Reul et al. (2005) and García-Martínez et al. (2019). In conclusion, the annual cycle for phytoplankton abundance described in this work must be considered the result of only one survey per season, and as it has been reported, it doesn't coincide in most cases with the mean values described in literature. This fact is due to the huge variability of the area, affected for different oceanographic structures: upwelling, exchange of Atlantic and Mediterranean waters, etc.

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Molecular studies on *Plocamium cartilagineum* complex (Plocamiales, Rhodophyta) suggest cryptic diversity in the Antarctic region

Nerea Sanchis Collado*[1] , Rafael Martín Martín[2] , Jordi Rull Lluch[2] , Amelia Gómez Garreta[2] , Daniel Vitales Serrano[1]

*Corresponding author: nereasanchisc@gmail.com

[1] Institut Botànic de Barcelona; Laboratori de Botànica, Facultat de Farmàcia i Ciències de l'Alimentació, Institut de Recerca de la Biodiversitat (IRBio) and Centre de Documentació de Biodiversitat Vegetal (CeDocBiV), Universitat de Barcelona

[2] Laboratori de Botànica, Facultat de Farmàcia i Ciències de l'Alimentació, Institut de Recerca de la Biodiversitat (IRBio) and Centre de Documentació de Biodiversitat Vegetal (CeDocBiV), Universitat de Barcelona

Keywords: *Plocamium*, Cryptic diversity, Barcoding, Nuclear content, Antarctica

Abstract

The red algae *Plocamium* (Plocamiales, Rhodopghyta) is a cosmopolitan genus, constituted by approximately 40 species (Wynne, 2002) that are widely distributed throughout the world's oceans. Morphological and anatomical traits, as well as biogeographical criteria have been traditionally employed to define most of the species. However, recent molecular systematic studies (e.g. Cremades et al. (2011); Saunders and Lehmkuhl (2005); Yano et al. (2006)) indicate that classical morphological-based delimitations have failed to accurately discern diversity and evolutionary relationship within this group of seaweeds. In this context, the type species *Plocamium cartilagineum* (Linnaeus) P.S. Dixon, with a worldwide distribution and showing considerably morphological variability, represents a particularly complex study case from a taxonomic and systematic point of view. Using a combination of morphological, anatomical and molecular information, Cremades et al. (2011) proposed a new taxonomic framework for North Atlantic *Plocamium* entities formerly considered *P. cartilagineum*. These and other authors also reported cryptic diversity in specimens originally described as P. cartilagineum from other distant areas of the world (e.g. Asia, Yano et al. (2006); or Oceania, Cooper and Wynn (2017)). However, other oceanic regions – such as Antarctic continent – have been relatively overlooked in previous works devoted to study the taxonomic and evolutionary diversity of *P. cartilagineum*. Here, our main aim is to study the diversity of *P. cartilagineum* from the Antarctic region using morphological and molecular information to compare with samples from other points of the world. In addition, we will investigate the variability of certain cytogenetic traits, as potential alternative markers for discriminating between taxonomic and evolutionary units within this species. The Antarctic samplings were carried out in South Bay and False Bay of Livingston Island (South Shetland Islands), during the austral summer of 2019. The Mediterranean samples were collected at the north coast of Catalonia (Spain), at Ras Cape (Colera, Girona) and La Trona (Roses, Girona). Samples were divided in two types of conservation and pre-processing methods. For molecular analyses, fresh samples were immediately conserved in silica gel just after collection from the field. For nuclear DNA content and morpho-anatomic study, collected algae were frozen (-20°C) until their arrival to our laboratory at the University of Barcelona. Once there, the specimens were defrosted for their determination. Little fragments of the specimens were fixed with Carnoy solution (3:1 of 95% ethanol-glacial acetic acid) during 24 hours and stored in 70% ethanol

at 4°C for subsequent nuclear DNA content analysis. Reference specimens were pressed and deposited in the BCN-Phyc Herbarium (Documentation Center of Plant Biodiversity, University of Barcelona, Spain). We made molecular analysis of the samples by barcoding following Saunders and Lehmkuhl (2005) and Cremades et al. (2011) procedures. Specific primers for mitochondrial cox1 and the plastid rbcL markers were designed using reference genomic data of *P. cartilagineum* from Genbank (KJ398160; NC031179). The obtained sequence data of *P. cartilagineum* from Antarctic and Mediterranean regions were visualized and processed using Geneious Prime 2019 and aligned to other sequences of P. cartilagineum obtained from Genbank. Evolutionary groups were visualized with the construction of a neighbour-joining tree using PAUP as implemented in Geneious to compare with the phylogenetic representation of the genus obtained by Cremades et al. (2011). Nuclear DNA content estimates based on image analysis of DAPI-stained specimens followed a procedure using a Cooled CCD Miramax RTE 782-Y high performance digital camera placed on a Leica DMRB fluorescence microscope and subsequently analyzed using MetaMorph software (Molecular Devices, Toronto, Canada). Nuclear DNA content was obtained by comparing the fluorescence intensity of sample's nuclei with those of *Gallus gallus* (Linnaeus) erythrocytes, since the later have a stable nuclear DNA content of 2.4 pg. For each species, histograms were constructed with the obtained nuclear DNA values to determine the C-value and assigning ploidy levels. To conduct the morphological study we measured diagnostic parameters based on previous descriptions (Cremades et al., 2011; Saunders and Lehmkuhl, 2005) for the comparison of the samples of the two localities. Our molecular phylogenetic analyses revealed that samples of *P. cartilagineum* from Antarctic region constitute an evolutionary distinct group. These results suggest at least one cryptic entity, not associated with *P. cartilagineum* sensu stricto, exists in Antarctic region. From the morphological point of view, the Antarctic specimens show subtle differences that could represent useful traits for potential taxonomic discrimination. Interestingly, our nuclear DNA content study of samples from the Mediterranean and the Antarctic region support the hypothesis of Antarctic *P. cartilagineum* being a separated evolutionary entity. Even though ploidy levels measured in the samples of both geographical areas are similar (with ploidy ranging from 2C = 0.05 to 32C = 0.8 picograms), the nuclear patterning observed are different between them 1, reinforcing the idea of divergent groups. In the case of Antarctic samples, we have observed a nuclear patterning consisting of cells with a big polyploid nucleus and several smaller nuclei. In the other hand, the nuclear pattern observed in the Mediterranean samples consists only in cells with a big polyploid nuclei, being dissimilar to the previous description of nuclear patterning given for *P. cartilagineum*.

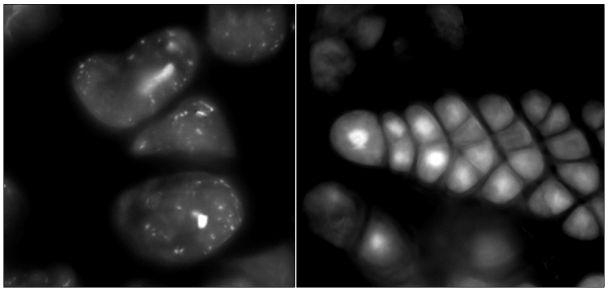

Figure 1: Samples of *P. cartilagineum*.

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Effects of marine heatwaves and acidification on Mediterranean reefs: the response of benthic invertebrates associated to the coralline algae *Ellisolandia elongata*

Mar Santos*[1] , Agnese Marchini[2] , Matteo Nannini[3] , Federica Ragazzola[4] , Chiara Lombardi[3]

*Corresponding author: mar.santos@imbrsea.eu

[1] University of Ghent, Faculty of Sciences, Krijgslaan 281, 9000 Gent (Belgium) and Dipartimento di Scienze della Terra e dell'Ambiente, Università di Pavia, Via S. Epifanio, 14 (Italy).

[2] Dipartimento di Scienze della Terra e dell'Ambiente, Università di Pavia, Via S. Epifanio, 14 (Italy).

[3] Centro Ricerche Ambiente Marino ENEA, Via Santa Teresa - 19032 Pozzuolo di Lerici, La Spezia (Italy).

[4] Institute of Marine Sciences, University of Portsmouth, Ferry road, Easteney Portsmouth PO4 9LY (UK).

Keywords: Bioconstructors, Biodiversity, Peracarid crustaceans, RCP 8.5 scenario

Background

Bioconstructors are known to have a key structural and functional role in marine ecosystems. The construction and provision of complex three-dimensional structures sustains rich assemblages and high biodiversity levels. Among bioconstructional organisms, coralline algae are especially vulnerable to the effects of global change (Williamson et al., 2014), especially ocean acidification due to their mineralogical characteristics (Ragazzola et al., 2016). The coralline algae *Ellisolandia elongata* (J. Ellis and Solander) (Hind and Saunders, 2013) has been selected for the purpose of this study to assess the responses of its associated fauna under the predicted values (marine heat waves (MHWs) and pH levels) of RCP 8.5 for the year 2100 (winter time).

Materials and Methods

The study area is Santa Teresa bay, located in the Gulf of La Spezia (Italy). Two-way crossed experimental design on 36 samples was conducted with two orthogonal fixed factors (MHW and acidification) and two levels each (control and altered). Simulated experimental conditions were created in the lab to test the effects of 4 different treatments (control; CC, MHW, TC; altered pH, CA; and MHW and pH, TA) after 2 weeks on the invertebrate assemblages at 1) a general level (macrogroups) and 2) with a focus on peracarid crustaceans. MHW temperature was calculated according to Hobday et al. (2016) and pH was modified by pumping CO_2 (control: 380 micro atm; altered: 2300 micro atm). Furthermore, an online survey was conducted to explore the level of awareness of the public as regards to the research topic.

Results and discussion

Results show no significant influence of the treatments on the general community structure (Figure 1A) but clear group-specific responses regarding the densities of some molluscs, nematodes and nemertans. Despite no apparent effect on the total peracarid density as a macrogroup, a

deeper analysis shows significant changes concerning their community structure (Figure 1B) and relevant species-specific responses on their densities (Figure 2). As corresponds to the results of the survey on the level of awareness of the general, little concern and poor knowledge on the subject is noticeable, with only 39 percent of the respondents were familiar with the concept of ocean acidification. The study confirms that, in a context of global change, benthic invertebrate assemblages are expected to shift towards an altered community in terms of biodiversity and composition, with particularly remarkable changes at lower taxonomic level of study. In this study, *Ellisolandia elongata* has proven to be an ecologically important species, which hosts a wide variety of species, including some rare Mediterranean natives as well as widespread aliens. Although effects of ocean acidification under current temperature conditions do not impact the associated community structure, marine heat waves seem to exacerbate the effects of acidification, driving shifts on it, and showing that thermal anomalies pose a threat to benthic assemblages, supporting findings of similar studies (Marchini et al., 2019). Although preliminary, the output of this study is remarkable and serves as a baseline for future studies, aiming for efficient conservation and management strategies as regards furure ocean conditions. Besides, the little awareness concerning mainly the concept of ocean acidification highlights the importance of efficient science communication and dissemination in order to bridge the existing gap between the scientific community and general public regarding global environmental issues.

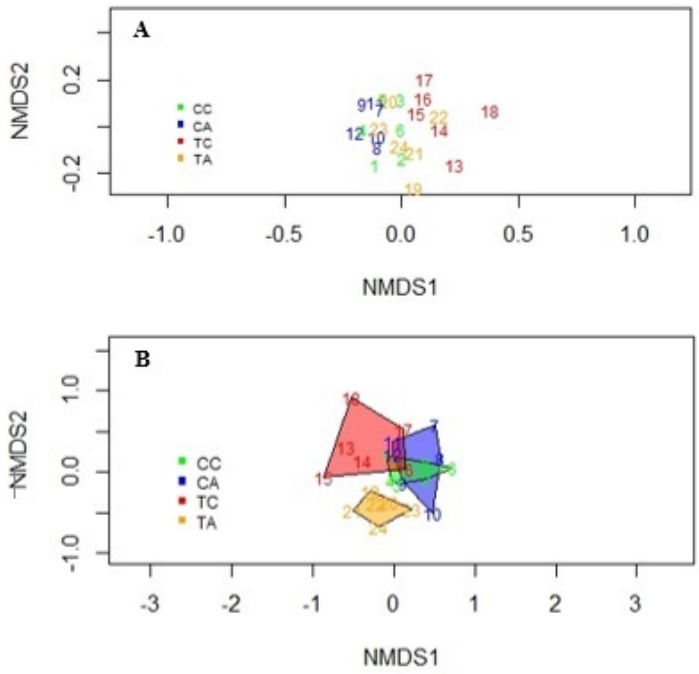

Figure 1: Non metric multidimensional scaling based on dissimilarity index distances of the general community with standardized and scaled results. A) General community analysis and B) peracarid community analysis. Numbers correspond to different samples and polygons are drawn to ease the visualization of the clusters when the PERMANOVA on the dissimilarity matrix resulted significant. Note that the scaling is different.

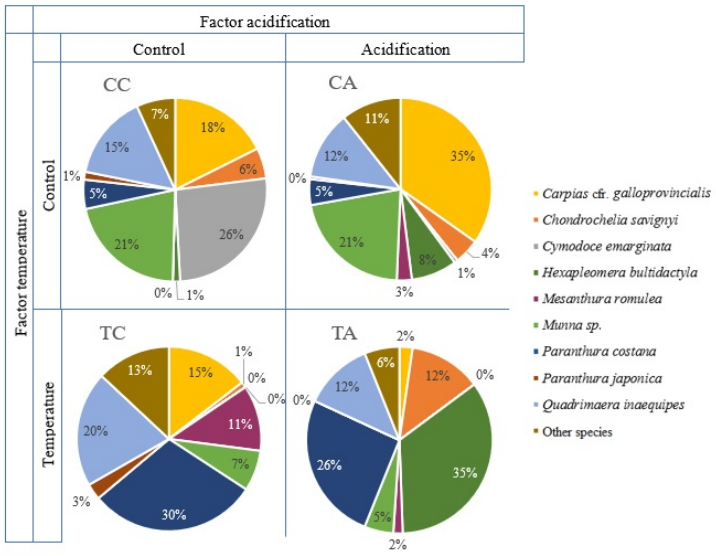

Figure 2: Peracarid community composition under different treatment conditions, shown as a percentage. "Other species" refers to 11 different species with little contribution to the densities and the differences among treatments. Note that the percentual values are rounded to the nearest integer value.

Acknowledgments

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Spatio-temporal distribution and population structure of *Parapenaeus longirostris* and *Nephrops norvegicus* in the northern Alboran Sea

Cristina Ciércoles*[1] , Pere Abelló[2] , Pedro Torres[3] , José Miguel Serna[3] , Jose Luis Rueda[3] , Cristina García-Ruíz[3]

*Corresponding author: criscier@uma.es
[1] Universidad de Málaga. Facultad de Ciéncias. Bulevar Louis Pasteur. Málaga
[2] Institut de Ciències del Mar (CSIC). Passeig Marítim de la Barceloneta 37. Barcelona
[3] Instituto Español de Oceanografía, C.O. Málaga. Muelle pesquero s/n. Fuengirola

Keywords: Alboran Sea, *Nephrops norvegicus*, *Parapenaeus longirostris*, Population structure

Introduction

The deep-water shrimp *Parapenaeus longirostris* (Lucas, 1846) and the Norway lobster *Nephrops norvegicus* (Linnaeus, 1758) are two decapod crustacean species especially targeted by trawl fisheries in the northern Alboran Sea and Gulf of Vera (western Mediterranean). *Parapenaeus longirostris* represents around 30% in weight and 20% in economic value of the total crustacean trawl landings in the area, while *N. norvegicus* represents around 4% in both, weight and economic value (Junta de Andalucía and Gobierno de Murcia, statistics 2017). The aim of this study is to analyze the spatio-temporal distribution and size structure of the *P. longirostris* and *N. norvegicus* populations along the northern Alboran Sea, including the Alboran Island, and the Gulf of Vera. This information is of importance for an appropriate management of fisheries resources in order to achieve a sustainable exploitation.

Material and methods

The study area comprises: (i) the northern Alboran Sea (between Punta Europa and Cape Gata), (ii) the Gulf of Vera (between Cape Gata and Cape Palos) and (iii) the Alboran Island (Figure 1).

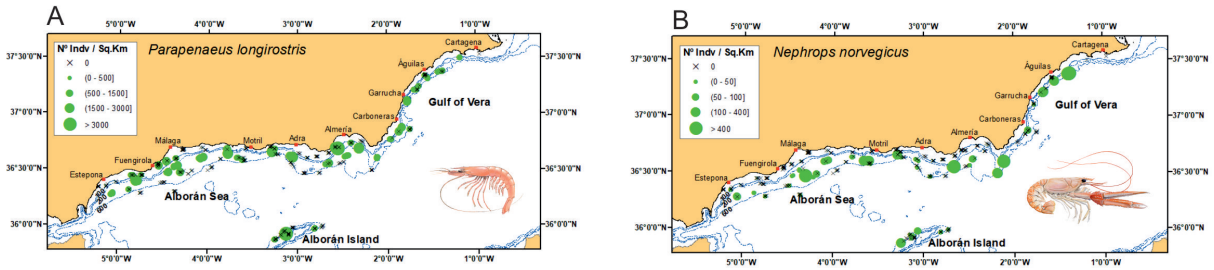

Figure 1: Map of the study area showing the spatial distribution and abundance (ind km^{-2}) of *Parapenaeus longirostris* (A) and *Nephrops norvegicus* (B). Hauls with absence of both decapods are plotted by X.

Data were obtained from 413 hauls performed from 2012 to 2018 under the framework of the European Union research project MEDITS (International Mediterranean bottom trawl survey)

which has been conducted annually (in spring). A stratified random sampling design was used with five bathymetric strata: 10-50 (A), 51-100 (B), 101-200 (C), 201-500 (D) and 501-800 (E). The gear used was a GOC 73 (mean vertical opening: 2.42 m, mean horizontal opening: 18.07 m, cod-end mesh size: 20 mm). The mean towing speed was 3 knots and the trawling time was 30-minute for depths <200 m and 60-minute for depths >200 m. Total catch for both species was separated and quantified by sex and, for each sex, the carapace length (CL) in mm was also recorded. The abundance (expressed as a number of individuals) of *N. norvegicus* and *P. longirostris* were standardized to 1 km^2 (ind km^{-2}) and plotted by year, depth and geographical area. Length-frequency distributions were constructed per 1-mm CL size class for each decapod species and plotted by sex, area, depth and year. The non parametric Kruskal-wallis test was used to test spatial and temporal differences in the mean abundance between depth strata and geographical areas. Parametric tests (Chi-square and Student-t) were used to analyze the spatial and temporal variation of the mean length of each species by depth strata, geographical area, year and sex. The sex-ratio was also calculated and compared by depth strata. Statistic tests were carried out with Statistica software. Statistic comparisons by depth strata were only carried in those strata where the species frequently occur (for *P. longirostris* B,C,D, E depth strata and for *N. norvegicus* D and E).

Results and discussion

Parapenaeus longirostris and *N. norvegicus* were distributed along the study area between 61-711 and 148-793 m depth, respectively. Both species were caught in 30% of hauls and its occurrence was higher within the depth stratum D (200-500 m) (83% of hauls in stratum D for *P. longirostris* and 64% for *N. norvegicus*) (Figure 1). In both decapods, significant differences for the mean abundance were detected in relation to depth (Kruskall-Wallis, p>0.05), with the highest abundance in depth strata D. Differences of the mean abundance between the three areas were not significant. Inter-annual variation of the mean abundance for *P. longirostris* was more acute, than for *N. norvegicus*, and significant fluctuations were detected in both species along the time series (2012-2018) (Figure 2).

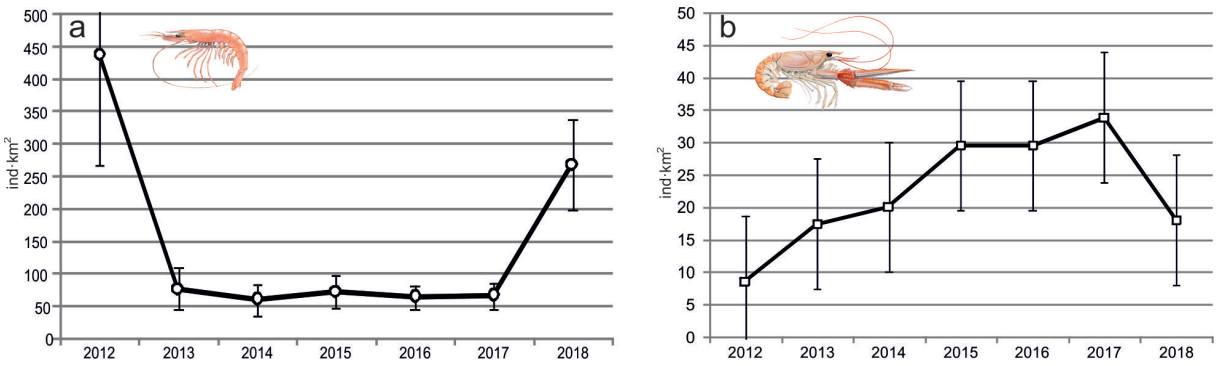

Figure 2: Annual abundance (ind km^{-2}) for *Parapenaeus longirostris* (a) and *Nephrops norvegicus* (b) from annual MEDITS surveys (2012-2018) in the study area.

The overall length-frequency distribution of *P. longirostris* in spring was bimodal while for *N. norvegicus* it was polymodal. Sizes ranged between 10-40 mm CL for *P. longirostris* and 16-69 mm CL for *N. norvegicus* (Figure 3). For *P. longirostris*, there are significant differences in the mean length between depth strata, geographical areas, years and sexes (t-test, p<0.05). Individuals from the deepest strata were significantly larger than those from the shallow strata. Regarding geographic areas, Alboran Island harbored the smallest individuals and the Gulf of

Vera harbored the largest ones. Annually the mean length decreased with mean abundance increases. Regarding sexes, females were significantly larger (mean CL= 25.25 mm) than males (mean CL=22.71 mm) (t-test, $p<0.05$). Both sexes were equally represented but sex-ratio showed significant differences according to depth strata (X^2, $p<0.05$), being females more frequent than males across all strata, except in D stratum. For *N. norvegicus* the mean length was also higher in the deepest stratum (E) (t-test, $p<0.05$). Significant differences of the mean length were found in different geographical areas (t-test, $p<0.05$), with the largest individuals occurring in the Alboran Island and the smallest ones in Gulf of Vera. Regarding sexes, females were significantly smaller (mean CL= 38.64 mm) than males (mean CL= 43.20mm) (t-test, $p<0.05$). The sex ratio was slightly dominated by the females. *Nephrops norvegicus* also showed significant differences according to the depth strata (X^2, $p<0.05$) as found for *P. longirostris*. Females were more abundant in the D stratum while males were more abundant in the deepest stratum (E).

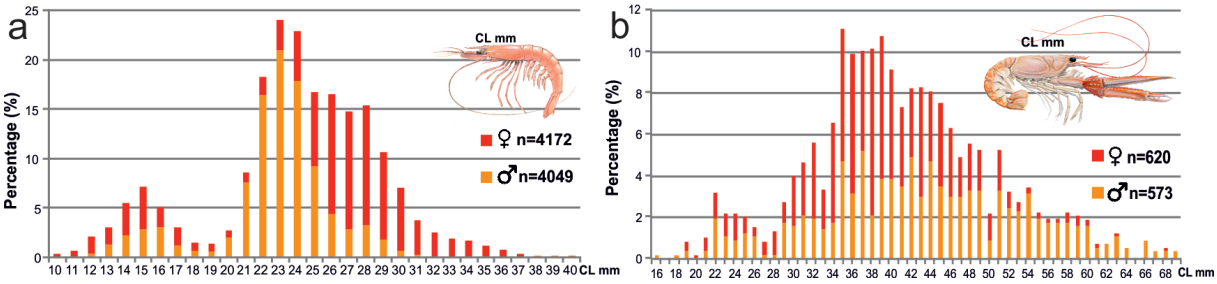

Figure 3: *Parapenaeus longirostris* (a) and *Nephrops norvegicus* (b) length frequency distributions according to sex, obtained from MEDTIS surveys (2012-2018) in the study area. n: number of specimens measured.

As detected in previous studies, *P. longirostris* was more common and abundant in shallower strata (generally between 100-200 m depth) than *N. norvegicus* (generally below 500 m depth)(Abelló et al., 1988; Abelló et al., 2002). Inter-annual variations of the mean abundances for both species were similar to those of others studies in adjacent areas or to commercial landings trends (Sbrana et al., 2019). The differences between species are mainly due to their different life histories and phylogenetic constraints, since they belong to very different branches of decapod crustaceans. Different fishing effort rates and the interaction of environmental conditions with the biological characteristics of the species contribute to reshape their population traits. Both decapods showed sexual dimorphism in size with very similar ranges to those found in other studies: lower size of males for *P. longirostris* and of females for *N. norvegicus* (García-Rodríguez et al., 2009). Additionally, both species also showed a marked, size-dependent distribution with depth, with small individuals generally occurring at shallower waters. The nearly complete absence of *P. longirostris* in shallow waters in the Alboran Island and the geographical differences in mean abundances of both species can be explained by different environmental conditions, bottom characteristics, prey availability or fishing pressure among the areas which may need further studies.

Acknowledgments

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Evidences of sexual segregation of blackmouth catshark *Galeus melastomus* Rafinesque, 1810 (Chondrichthyes: Scyliorhinidae) in the Porcupine Bank, north-east Atlantic

Lola Riesgo*[1] , Francisco Velasco[2] , Francisco Baldó[1]

*Corresponding author: lola.riesgo@hotmail.es
[1] Instituto Español de Oceanografía, C.O. de Cádiz
[2] Instituto Español de Oceanografía, C.O. de Santander

Keywords: Sharks, Elasmobranch, Sexual dimorphism, Sexual segregation, North-east Atlantic

Introduction

Sexual segregation is a general feature of shark populations yet remains to be investigated in the majority of species (Wearmouth and Sims, 2008). Understanding sex-based differences in the spatio-temporal dynamics of animal populations is of fundamental importance for their successful management. This is particularly so for k-selected species, such as deep-water sharks, which typically exhibit slow growth, late maturation and low fecundity.
Galeus melastomus is a small bottom-dwelling shark widely distributed in the north-east Atlantic, being the most abundant shark of the Porcupine Bank (west of Ireland). It is an oviparous species (Costa et al., 2005), considered an opportunistic with a broad generalist niche (Olaso et al., 2005). Its more resilient life history in comparison to many deep-water sharks designates this species of Least Concern on the IUCN Red List (Clarke et al., 2016). However, although the blackmouth catshark is subject to a zero total allowable catch and quota under European Union legislation, in the Porcupine Bank it is caught as by-catch in fisheries targeting hake, anglerfish and megrim because the bathymetric range of the species is within the depth range of these fisheries.

Materials and Methods

The Porcupine Bank lies in the north-east Atlantic, 200 km west of Ireland. The bank forms a structure similar to seamounts with their related anticyclonic structures. The Spanish Bottom Trawl Survey on the Porcupine Bank has been carried out annually each September since 2001 onboard the R/V Vizconde de Eza, a stern trawler of 53 m and 1800 Kw. The area covered extends from longitude 12 W to 15 W and from latitude 51 N to 54 N, following the standard International Bottom Trawl Survey methodology. The sampling design was random stratified to the area with two geographic sectors and three depth strata delimited by the 300, 450, and 800 m isobaths. The blackmouth catshark specimens caught during the survey time series were measured and sex determined.

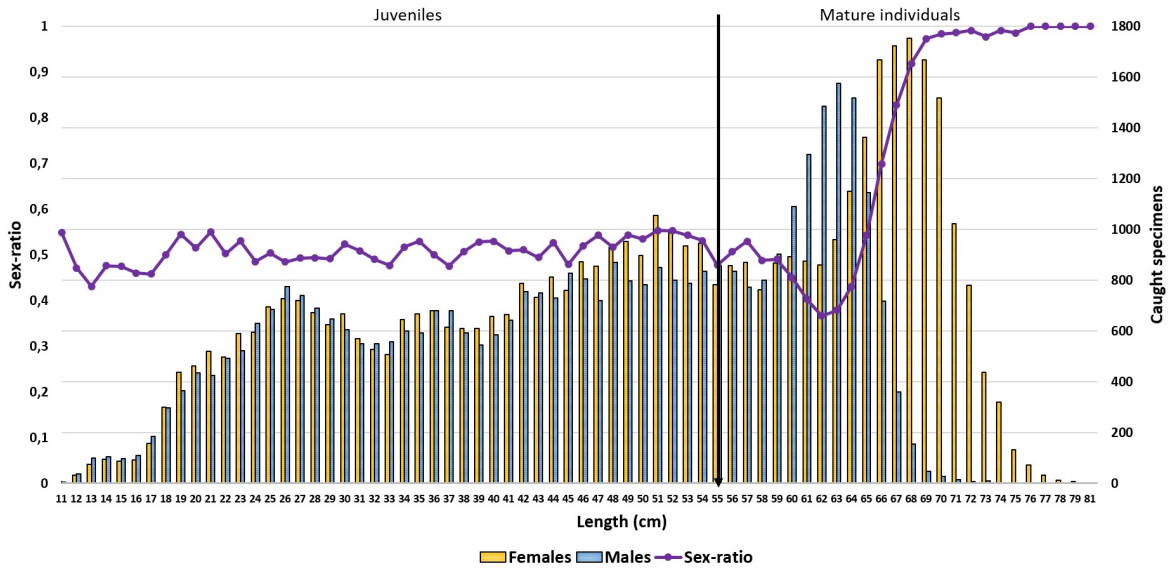

Figure 1: Length distribution and sex-ratio -females/(males + females)- by length.

Results

Elasmobranchs represented 9% of the average stratified fish catch in the Porcupine Survey time series (2001-2018), being *Galeus melastomus* the most abundant species. It represented 65% (74.2 ind./haul) and 42% (31.4 Kg/haul) of the average stratified elasmobranch abundance and biomass, respectively. It has been caught in 1160 hauls of the total of 1509 hauls carried out in the Porcupine Bank since 2001.
During this period, a total of 86,137 individuals (47,724 females and 38,413 males) were caught with females ranging in total length from 11 to 81 cm and males ranging from 11 to 75 cm. The species exhibited a clear sexual dimorphism with respect to body size. The positive Pearson Correlation of the sex-ratio -females/(males + females)- by length ($R = 0.686$, p-value < 0.001) indicates than females attain a greater length than males (Figure 1). In addition, significant differences (one-way ANOVA, p-value < 0.001) in sex-ratio per haul were found between bathymetric strata considered every 100 m (<300 m; 300-400 m, 400-500 m, 500-600 m, 600-700 m and 700-800 m). Pairwise comparisons (Tukey's post hoc test, p value < 0.001) indicated that there were significant differences between the shallowest (<600 m) and deepest strata (600-700 m and 700-800 m), where sex-ratio was higher.

Taking as reference the available published data of length at first maturity at Rockall Through (Moore et al., 2013), adjacent to our study area, we classified the specimens caught into three categories: juveniles (<55 cm), mature males and mature females (>55 cm). The spatial and bathymetric distribution of these categories showed a clear segregation (Figure 2). Juveniles inhabit shallower water (<600 m), where they could minimize competition with adults. The presence of a large number of juveniles, compared to other areas such as Rockall Through, suggests that the Porcupine Bank could be a nursery area. As they increase in size, they move into deeper water where the females occupy preferentially the deepest strata. Sexual segregation between mature males and females could be explained by their reproductive strategy. Gravid females seem to migrate to deeper water where they give birth, maximizing the security of their eggs and reducing predation risk.

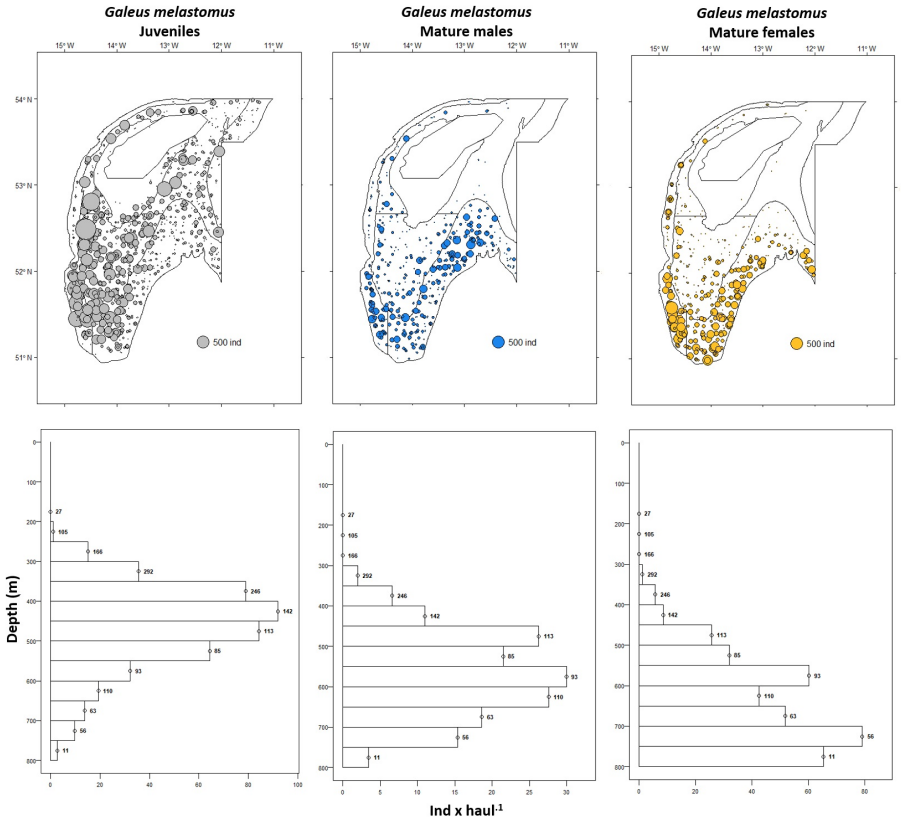

Figure 2: Spatial and bathymetric distribution of juveniles (<55cm), mature males and females (>55 cm). Number of hauls carried out in each bathymetric stratum is shown.

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Motivations and concerns of early career marine scientists

Javier Moreno-Andrés*[1] , Ivana Buselic Garber[2] , AM Wieczorek[3] , H. Abdelouahab[4] , W. Courtene-Jones[5] , F. de Bettignies[6] , M. Fais[7] , M. Custódio[8] , LC Gammage[9] , LG Gillis[10] , E Greene[11] , G Laukert[12] , M López Acosta[13] , M. Lubośny[14] , R McKenna[15] , F Palma Esposito[16] , S Piarulli[17] , K Porter[11] , HK Pradhan[18] , M Protopapa[19] , G Romagnoni[20] , C Scopetani[21] , AC Silva Rocha[22] , G Suaria[23] , T Turk Dermastia[24] , S Våge[25] , RP Vieira[26]

*Corresponding author:javier.moreno@uca.es
[1] University of Cádiz, CEIMAR, INMAR
[2] Institute of Oceanography and Fisheries, Croatia
[3] Ryan Institute - National University of Ireland Galway, Ireland
[4] Institut National de Recherche Halieutique, Morocco
[5] Scottish Association for Marine Science, UK
[6] Sorbonne Université, France
[7] Universidade de Vigo, Spain
[8] University of Aveiro - Centre for Environmental and Marine Studies, Portugal
[9] University of Cape Town - Marine Research Institute, South Africa
[10] Leibniz Centre for Tropical Marine Research, Germany
[11] AquaTT, Ireland
[12] GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany
[13] CSIC - Centre for Advanced Studies of Blanes, Spain
[14] Institute of Oceanology - Polish Academy of Sciences, Poland
[15] Galway Mayo Institute of Technology, Ireland
[16] National Research Council, Italy
[17] Alma Mater Studiorum - University of Bologna, Italy
[18] Alfred-Wegener-Institut - Helmholtz-Zentrum für Polar- und Meeresforschung, Germany
[19] Hellenic Centre for Marine Research, Greece
[20] University of Oslo - Faculty of Mathematics and Natural Sciences, Norway
[21] Consorzio Nazionale Interuniversitario per le Scienze del Mare, Italy
[22] MARE - Marine and Environmental Sciences Centre, Portugal
[23] Institute of Marine Sciences - National Research Council of Italy, Italy
[24] Marine Biology Station - National Institute of Biology, Slovenia
[25] Universitetet i Bergen, Norway
[26] The Centre for Environment - Fisheries and Aquaculture Science, UK

Keywords: Early Career Researcher (ECR)), Capacity building, Marine Sciences Landscape, Cross-sector engagement, Skill sharing

Introduction

EuroMarine is committed to helping early career researchers and promote bottom-up science from the marine science community. The "Orienting Young Scientists of Euromarine (OYSTER)" group was formed early 2018 to engage with young scientists and soon expanded to include a forum of twenty PhDs and post-docs from member institutions located through 13 countries. The members help represent the views of early career scientists in the EuroMarine network and act as a conduit to other young scientists in their own institution. The aims of the OYSTER

group include the contribution to the cohesive outreach and capacity building of young scientists across EuroMarine member organizations and to the development of initiatives to improve the ability of EuroMarine to integrate early career researchers into the European marine landscape. The OYSTER group has recently run a survey spanning institutions across Europe to develop advice for EuroMarine in matters pertaining to the support and development of student and early career researchers (Figure 1). The feedback collected from this survey will help to learn more about current issues concerning young scientists' working environment, financial situation, research interests and future perspectives in science and it will facilitate the development of a supportive network to address some of these issues.

Figure 1: Distribution of respondents by country.

Results

We have gathered responses from over 450 scientists and even though, overall feedback concerning early career scientist work and life was positive some findings from the survey deserve to be given some consideration and require some attention by scientific institutions. Around one in five respondents feels that he/she isn't treated fairly in their workplace. Also, almost 75% of respondents do things that are outside of their responsibilities from which less than half don't mind doing it. Going further, the whole scientific world seems to be based on 1-3 years short term contracts which may have mobilising effects promoting active work of scientists but on the contrary have a huge impact on stability and work-life balance which can lead toward increased work stress, decreasing number of ambitious high-risk high-reward scientific projects and leakage of the best young scientific minds from academia towards stable job in industry. Also going up the career ladder (from MSc to Post-Doc) dreams and hopes about the future career are regularly diminished returning to a satisfactory level only with the stability of e.g. a research fellow position. What is more, young scientist across Europe very often suffer with their earning oscillating around local minimal or low skilled worker wage (tradingeconomics.com data from December 2018): Germany (1414-2050 Euro); Ireland (1614 Euro); Finland (1980 Euro); Poland (480 Euro); Croatia (464-506 Euro); Portugal (659-676 Euro); Spain (858-932 Euro); France (1498-1610 Euro); United Kingdom (1401-1473 Euro); Italy (1130 Euro). This may influence their mental health forcing some of them to drop out or requiring them to maintain second jobs outside academia. These are only a few striking examples from the high amount of data (35 questions) gathered during our 2018 survey. In future OYSTER plan to continue their efforts to raise awareness about problems and concerns of early career marine researchers and support early career researchers through skill-sharing events and an online platform.

Trophic differentation between juvenile bluefin tuna and atlantic bonito in bay of Malaga (Alboran sea - SW Mediterranean)

Ignacio Baro*[1] , Raúl Laiz-Carrión[1] , Antonio Medina[2] , Joan Miquel Sorell[2] , José Luis Varela[2] , Amaya Uriarte[1] , David Macías[1]

*Corresponding author: ignaciobaro_93@hotmail.com
[1] Instituto Español de Oceanografía (IEO)
[2] Universidad de Cádiz (UCA)

Keywords: *Thunnus thynnus*, *Sarda sarda*, SCA, BSIA, AA-CSIA

Introduction

The Alboran Sea holds one of the highest biological productivity rates in the Mediterranean, and thus represents an important nursery habitat in providing food resources for juveniles of many pelagic fishes including tunas like Atlantic bluefin tuna (ABFT, *Thunnus thynnus*) and Atlantic bonito (BON, *Sarda sarda*). Traditional stomach content analysis (SCA) reveals only the composition of recently ingested food; therefore, elucidation of diet composition at broad geographic and temporal scales requires large numbers of samples across space and time. Stable isotope analysis (SIA) has been used as a valuable complement to SCA. Bulk tissue stable isotope analysis (BSIA) of nitrogen (δ^{15}N) and carbon (δ^{13}C) have been used to determine energy sources, flow of carbon to consumers in food webs, and the trophic positions of marine fishes. Values of δ^{15} NBSIA indicate trophic positions of marine animals as the isotopic fractionation leads to enrichment of the isotope values with increasing trophic levels. Values of δ^{13} CBSIA show limited isotopic fractionation with increasing trophic level (Post, 2002). Additionally, compound specific isotope analysis (CSIA) provides measurements of isotope ratios from individual amino acids (AAs). Diet composition and metabolic processes are reflected in the carbon and nitrogen isotopes found in AAs, δ^{13}C AA and δ^{15} NAA. Comparisons of essential and non-essential δ^{13} CAA and trophic and source δ^{15} NAA allow for estimations of primary production sources, which are reflected in variations among basal end members, as well as greater resolution for trophic positions of organisms (Chikaraishi et al., 2009). The aim of this study is to investigate diet composition, trophic level, niche breadth and food overlap of juvenile ABFT and BON in the Alboran Sea. For this purpose, we combine three independent but complementary methodological approaches: stomach content analysis (SC), bulk stable isotope analysis (BSIA) and compound-specific nitrogen isotope analysis of individual amino acids (AA-CSIA).

Material and methods

Trophic interactions between sympatric populations of age-0 bluefin tuna (ABFT) and Atlantic bonito (BON) were investigated in Alboran by integrating stomach content analysis (SCA) and stable isotope analysis of bulk muscle (BSIA) and individual amino acids (AA-CSIA). Young-of-the-year, 3-4 month old Atlantic bluefin tuna (ABFT) were sampled from by-catches of the purse-seine fleet targeting small pelagics in the Bay of Malaga. The stomach contents were removed and blotted dry and prey items were identified to the lowest possible taxa and grouped into taxonomic categories. The relative importance of different prey items in ABFT and BON diets

was estimated using three indices: frequency of occurrence (% O), weight percentage (% W) and alimentary index expressed as percentage (% AI). Additionally, freeze-dried and powdered muscle samples were used for bulk stable isotope analysis (BSIA) and for amino acid compound-specific isotopic analysis (AA-CSIA). δ^{13}C and δ^{15}N values were determined for 12 AAs. The dietary niche width of each group was evaluated using the standardized Levin's index. The isotopic niche width and overlap was calculated from δ^{13}C and δ^{15}N data by Bayesian standard ellipse corrected areas adjusted for small sample size (SEAc) using the SIBER package of SIAR. Estimations of trophic position (TP) for ABFT and BON were derived from data of SCA (TPSCA), BSIA (TPBSIA) and AA-CSIA (TPCSIA).

Results and discussion

In the Strait of Gibraltar and the nearby Alboran basin, BON has been shown to consume only, or almost exclusively teleost fish. Yet, along the western Mediterranean shelf the age-0 ABFT diet is more varied as it also includes a significant amount of small crustaceans (Varela et al., 2019), which are located at lower levels of the food web. Although ABFT and BON have been described as opportunistic and generalist predators, the niche width estimated from the standardised Levin's index indicates that both scombrids show a specialist feeding behavior in the Alboran Sea (B≤0.32). BON only consumed fish, with *Trachurus* spp. being the most important prey overall (% AI = 96.62) (Table 1), whereas ABFT diet also comprised invertebrates and consisted of at least 4 fish categories, 1 cephalopod, 1 tunicate, 1 cnidarian and 2 crustaceans, according with a more euryphagous diet for ABFT (Table 1). Although teleost fish were predominant in the diet of the two tuna groups, significant differences were found in the dietary composition.

Table 1: Results of SCA of the juvenile Atlantic bluefin tuna (ABFT) and bonito (BON) sampled in the Bay of Malaga in 2015. % W, percentage of weight; % O, frequency of occurrence; % AI, percentage of alimentary index.

	%W		%O		%AI	
Prey	BON	ABFT	BON	ABFT	BON	ABFT
Fishes	**100.00**	**99.40**	**100.00**	**87.10**	**100.00**	**99.89**
Trachurus spp.	80.15	6.52	75.00	16.13	96.62	2.81
Sardina pilchardus	11.42	43.70	8.33	22.58	1.53	26.35
Engraulis encrasicolus	5.38	48.00	16.67	54.84	1.44	70.29
Myctophids	2.71		8.33		0.36	
Unidentified fishes	0.34	1.17	8.33	9.68	0.05	0.30
Cephalopods		**0.13**		**3.23**		**<0.01**
Tunicates		**0.06**		**3.23**		**<0.01**
Cnidarians		**0.03**		**6.45**		**<0.01**
Crustaceans		**<0.01**		**3.23**		**<0.01**
Gelatinous zooplankton		**0.39**		**22.58**		**0.10**

Complementary to SCA results, PERMANOVA analysis of stable isotope data and SEAc (Figure 1) indicated that the two tuna species exploited different prey resources. The results show that the both species have differentiated trophic behavior as inferred from the δ^{15}N values. No isotopic niche overlap between species has been observed, which may indicate trophic resource partitioning. Wider SEAc estimated for ABFT suggest more euryphagous diet than BON. The trophic position (TP) of BON and ABFT was estimated using the data obtained from SCA (TPSCA), BSIA (TPBSIA) and AA-CSIA (TPCSIA). The three methods yielded higher TP estimates for BON than for ABFT. The TP values derived from isotopic data of glutamic acid and phenylalanine were lower (3.14 ± 0.23–3.42 ± 0.27) than the estimates based on SCA (4.01 ± 0.15–4.43 ± 0.23) and BSIA (4.28 ± 0.06–5.22 ± 0.13).

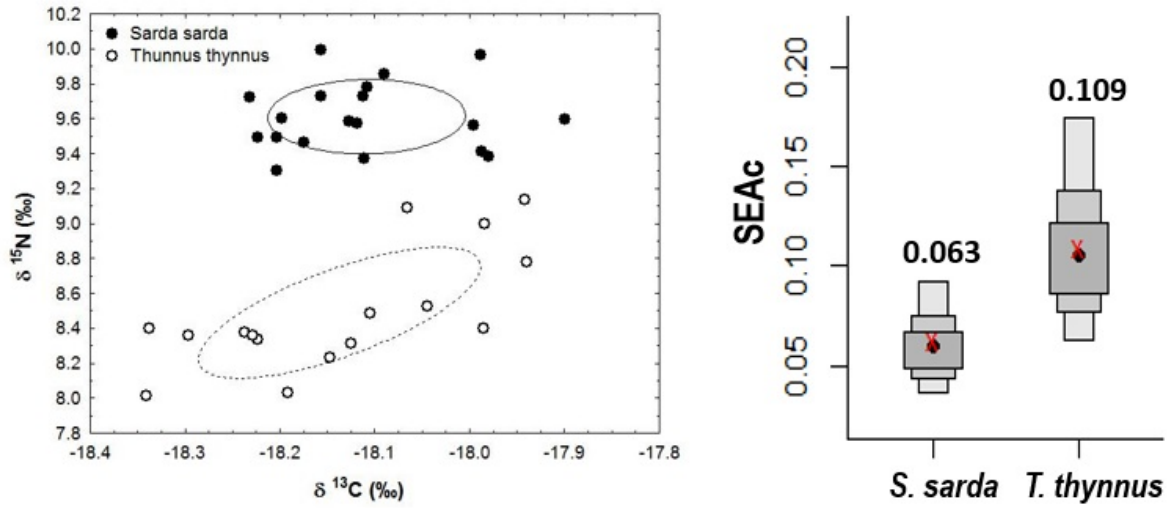

Figure 1: A) δ^{13}C and δ^{15}N bi-plots of muscle tissue and the standard ellipse associated to each specie for the age-0 tuna sampled in the Bay of Malaga (BON, spots and solid line; ABFT, circumferences and dashed line). B) Standard ellipse area (SEAc) estimated as trophic niche width by SIBER (Stable Isotope Bayesian Ellipses in R. The dark, intermediate and light grey boxes are the 50%, 75% and 95% credibility intervals, respectively. X is the standard ellipse area calculated using correction for small sample size (SEAc).

Source AAs had lower δ^{15}N values than bulk-muscle samples, while all trophic AAs showed higher values (Figure 2). This differential δ^{15}N fractionation pattern between the two AA groupings is consistent with the one observed in feeding experiments (Chikaraishi et al., 2009) as well as in muscle tissue of wild yellowfin tuna and age-0 ABFT (Varela et al., 2019).

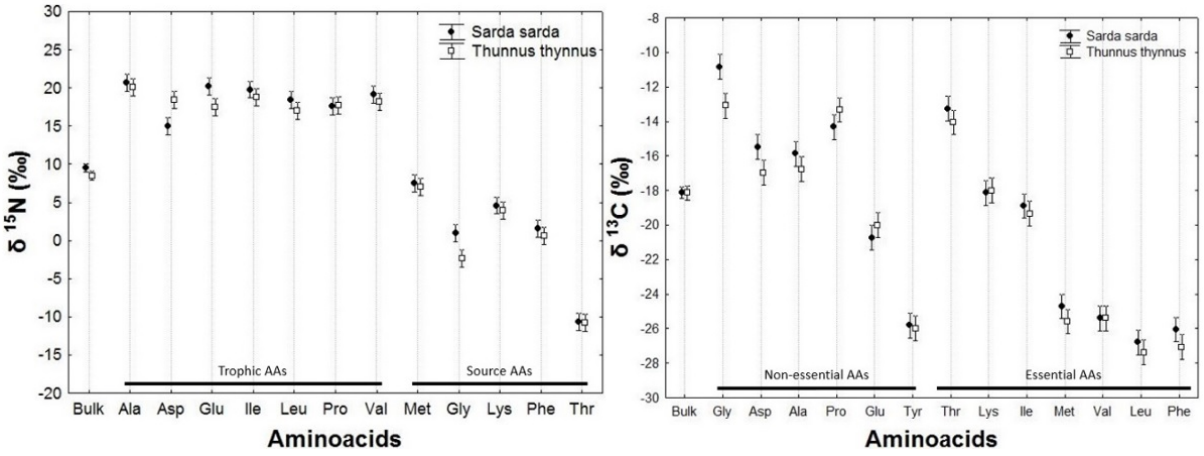

Figure 2: δ^{15}N and B) δ^{13}C values of bulk muscle and amino acids (trophic-sources and essential-non essential AAs respectively) of age-0 tuna samples collected in the Bay of Malaga (BON, circles; ABFT, squares). Ala, alanine; Asp, aspartic acid; Glu, glutamic acid; Gly, glycine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Thr, threonine; Tyr, tyrosine; Val, valine. Bars indicate standard deviation.

BON occupy a higher trophic level than ABFT in the Alboran Sea. The differences in TP obtained from isotopic data might reflect not only foraging on prey located at different trophic

levels, but also the existence of distinct isotopic signatures at the food web base. That $\delta^{13}C$ and $\delta^{15}N$ values of essential and source AAs, respectively, were higher in BON than they were in ABFT may indicate that their trophic baselines were different, and hence these tunas may feed at separate areas before meeting in the Bay of Malaga. Nevertheless, as shown above, the results of SCA actually reveal that the BON diet was richer in teleost fish than the ABFT diet, thereby suggesting occupancy of distinct trophic levels and resource partitioning.

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Bacterial symbiosis in the bivalve *Cardita calyculata* (L., 1758)?

Juan Diego Castro-Claros*[1] , Begoña González-Ruiz[1] , Carmen Salas[1]

*Corresponding author: juandiegocastroc@gmail.com
[1] Departamento de Biología Animal, Universidad de Málaga, Campus de Teatinos s/n, 29071-Málaga (Spain).

Keywords: Bacteria, Biofilm, *Cardita calyculata*, Mollusca, Symbiosis

Introduction

Bacteria are one of the most frequent group in symbiotic relationships with invertebrates, as ectosymbionts (Duperron et al., 2008) or as endosymbionts (Moran and Baumann, 2000). Some symbiotic bacteria seem to be related to biomineralization processes (Braithwaite et al., 2000), but most of them seem to be related to food resources. *Cardita calyculata* (L., 1758) belongs to the ancestral clade Archiheterodonta, in which the presence of bacteria in symbiosis has been observed in other families. So the observation of bacteria on the shell of some specimens of *C. calyculata* raised the question whether this presence is circumstantial or if it is related to its habitat or its phylogenetic position.

Materials and methods

Specimens of different sizes were collected in the intertidal and upper sublittoral zone of the Mijas coast (Málaga), under stones buried in the sediment and between the algae of the upper fissures. These specimens were transported to the laboratory in seawater to be relaxed with magnesium chloride. Later, several of them were fixed in 4% formaldehyde for paraffin inclusion and others in glutaraldehide (2.5% in PBS) for TEM analysis. Some blocks of female specimens with developed gonads were deparaffinized and critical point dried to be seen in the SEM (Scanning Electron Microscopy, JEOL JSM840). The shells were observed in a FESEM (Field Emission Scanning Electron Microscope, Zeis Auriga Cross-Beam Station). The specimens for TEM (Transmission electron microscopy, JEOL JEM-1400) were treated with 1% osmium tetroxide and 2% uranyl acetate to provide contrast to the samples, were included in resin (EMbed 812 of EMS) and ultrathin sections (50-70 nm) were made with an ultramicrotome (Leica Ultracut R). The bacteria from the biofilm present on the shells were cultured in a marine nutrient broth (CULTIMED) at 37°C for 24h. After having made dilutions, was sown on marine nutritive agar (CULTIMED), in order to obtain pure cultures. Later, these bacteria were subjected to Gram staining (observation to optical microcopy) and to Negative staining (observation to TEM).

Results and discussion

A bacterial biofilm was observed on the shell of all specimens analyzed, regardless of the type of habitat (under stone or in algae), bathymetry (intertidal or sublittoral) or size (ultra-juvenile, juvenile and adult). Among the bacterial types we observed different types of bacilli, including coccobacilli, cocci and spirochetes, (Figure 1). However, the shells living under stones in the intertidal showed a greater diversity and density of bacteria than the shells living in the intertidal algae.

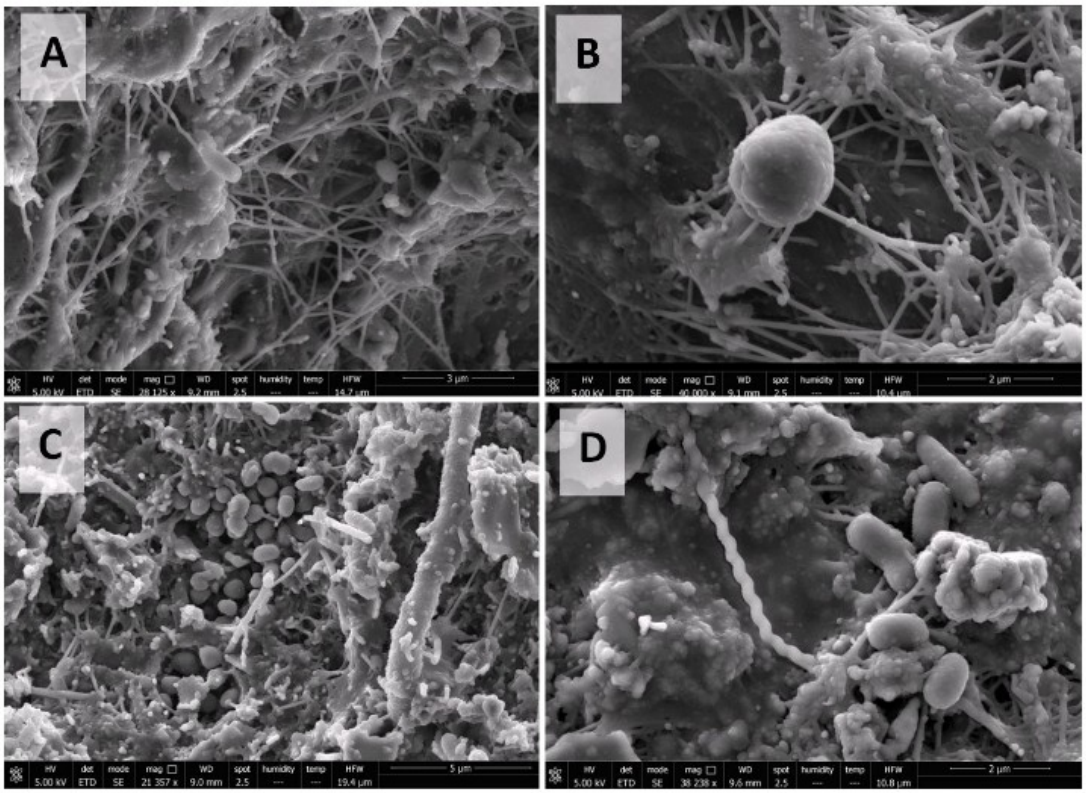

Figure 1: Bacterial biofilm on the shell of the *Cardita calyculata*, observed in FESEM. A. Biofilm produce by bacteria. B. Bacterium type coccus producing biofilm. C. Cocci, coccobacilli and bacilli. D. Spirochete.

From the culture of bacteria we obtained gram-negative bacilli and cocci (Figure 2)

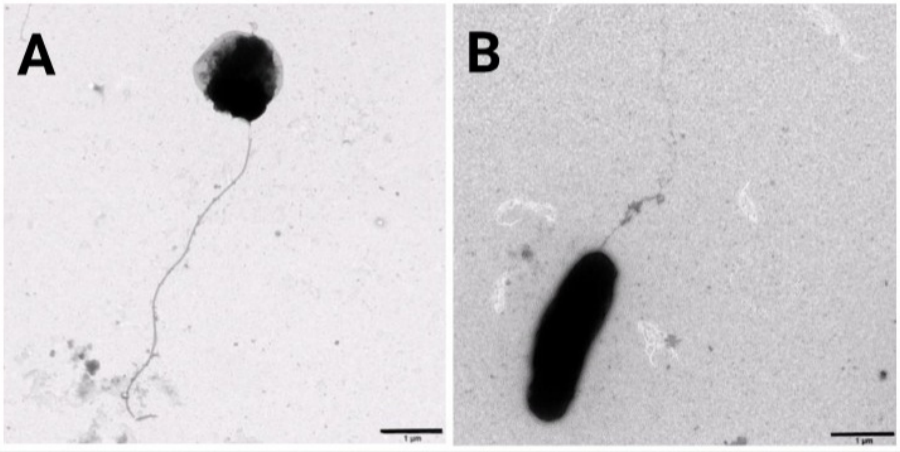

Figure 2: Bacteria from the culture, subjected to Negative staining and observed in TEM. A. Coccus with a flagellum. B. Bacillus with a flagellum.

The analysis of the soft parts of the bivalve allows us to see the presence of bacteria inside the female gonad, on the surface of the oocytes (Figure 3). Also, the presence of bacteria inside bacteriocyte was observed in the mantle edge.

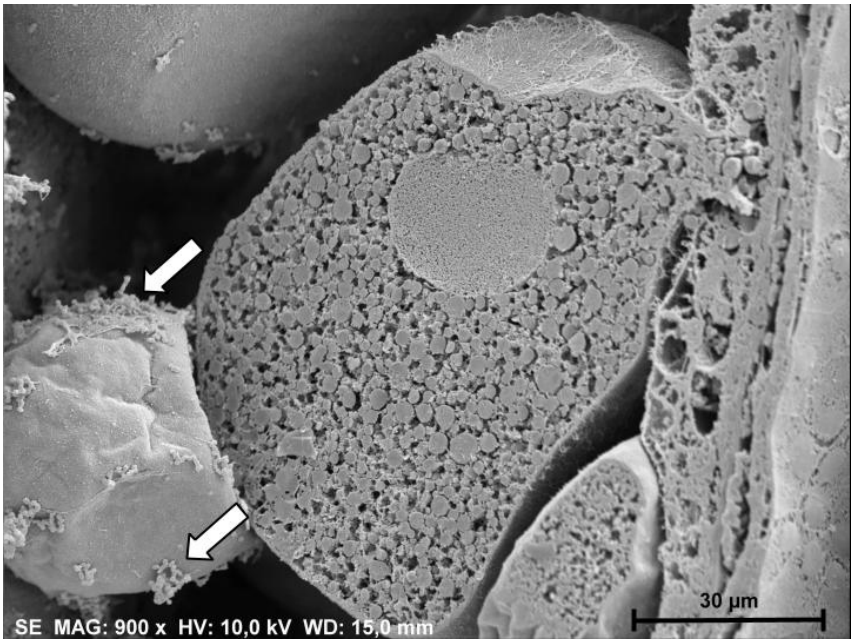

Figure 3: Bacteria inside the female gonad, on the surface of the oocytes, observed in SEM (arrows).

The presence of a bacterial biofilm on the shell surface of all specimens, regardless of habitat or age, seems to indicate that it must be an adaptive selection for the species, since the juveniles settle initially in the algae, but subsequently migrate to cavities of the rocks or under stones. The greater diversity and bacterial density in the specimens living under stones could be related to the decrease of oxygen and the redox potential of these environments, as has been shown in *Montacuta ferruginosa*, species that lives in reducing environments and as well as *C. calyculata* also presents bacterial biofilm (Gillan et al., 1998). On the other hand, the presence of bacteria inside the gonad, on the oocytes, would indicate a vertical transmission of the bacteria from parents to offspring (Bright and Bulgheresi, 2010), independently of that, can be also collected from the environment. The vertical transmission of bacteria in bivalves is considered as an indicator of symbiosis between both organisms.

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Impact of heatwaves in shallow aquatic systems: Changes in O_2, N_2O and CH_4 fluxes in the sediment-water interface

Valle Pérez-Rodríguez*[1] , Emilio García-Robledo[1] , Sokratis Papaspyrou[1] , Ana Sierra[2] , Alfonso Corzo[1]

*Corresponding author: valle.perez@uca.es
[1] Department of Biology, Microbial Ecology and Biogeochemistry laboratory, University of Cadiz, Faculty of Marine and Environmental Sciences, Puerto Real, Spain
[2] Department of Physical Chemistry, INMAR, University of Cadiz, Puerto Real, Spain

Keywords: Climate change, Greenhouse gases, Sediment-water fluxes alteration, Temperature dependence

Introduction

Wetlands and shallow aquatic environments are areas of high ecological value that offer important ecosystem services. Their low thermal inertia, due to their shallowness, makes them highly vulnerable to climatic variations (Cai and Sayles, 1996). The ongoing anthropogenic production and liberation of greenhouse gases (GHG) such as CO_2, CH_4, or N_2O results in climate change and an increase of the average temperature of the planet. In Andalusia, maximum daily temperatures have risen between 0.1 – 0.45 °C every decade for the last half century. More noteworthy is the fact that the frequency of extreme climatic events has increased significantly (Gerald and Tebaldi, 2004), showing a higher occurrence of heat waves (days with higher temperature than 35 °C) in Andalusia. In order to measure the impact of altered temperatures, like the ones observed during heatwaves, a laboratory experiment was performed using sediment cores from the tidal creek Río San Pedro, Bay of Cadiz. The objective of this study was to quantify the consequences of climate change on the net metabolism and fluxes of dissolved inorganic nitrogen (precisely NH_4^+) and greenhouse gases (N_2O and CH_4) in shallow aquatic environments such as the saltmarshes of the Bay of Cadiz.

Material and methods

Intact sediment cores and water were collected in Río San Pedro, a tidal channel in the marshes of Cadiz Bay. After one day of acclimation at 18 °C (annual mean temperature of Cadiz Bay), sediment cores were exposed to a temperature change during 1 day to reach different final temperatures (10, 18, 26, 34 and 42 °C) and the changes in net sediment-water fluxes were measured. Sediment-water fluxes were measured by whole core incubations. The changes in O_2, nutrients and GHG concentrations in the water column (final minus initial) during the incubation (5-7 hours) corresponded to the sediment-water flux. Concentrations of oxygen were measured with a microsensor. Nutrient concentrations were measured as described in García-Robledo et al. (2014) and GHGs concentrations were measured in a gas chromatograph (Bruker® 450). In addition, oxygen microprofiles were measured with an electrochemical microelectrode (Unisense A/S) (Revsbech and Jørgensen, 1986).

Results and conclusions

Oxygen microprofiles were clearly impacted by temperature. Oxygen penetration depth in the sediment decreased with increasing temperatures due to a higher oxygen consumption within the sediment. Therefore, increasing temperature reduced the oxic layer and likely favoured anaerobic processes in the sediment. Sediment was a net sink of NH_4^+ in the range of selected temperatures, suggesting a net consumption by microbial processes such as nitrification. The net consumption of NH_4^+ increased with increasing temperatures, likely reflecting a stimulation of nitrification rates. Sediment was a source of GHGs to the water, releasing CH_4 and N_2O at any temperature. Temperature affected the net release of both gases. In the case of CH_4, its release showed a clear exponential increase with temperature. Flux measured at 42 °C showed a net release of CH_4 twenty times higher than the one measured at 18 °C. The lower solubility of gases and the stimulation of methanogenesis at higher temperatures could be responsible for the increase in the release of CH_4. Although not so clearly, the release of N_2O also increased exponentially with temperature. At high temperatures (34 – 42 °C) the flux was circa 2 times higher than those measured at 18 °C. The net production and release of N_2O could be related to the consumption of NH_4^+ and the first step of nitrification. During the oxidation of NH_4^+ to NO_2^-, a small but significant fraction of nitrogen is released as N_2O. The location of the nitrification process in the oxic layer limits the consumption of N_2O by denitrifiers and enhances its release from the sediment (Figure 1).

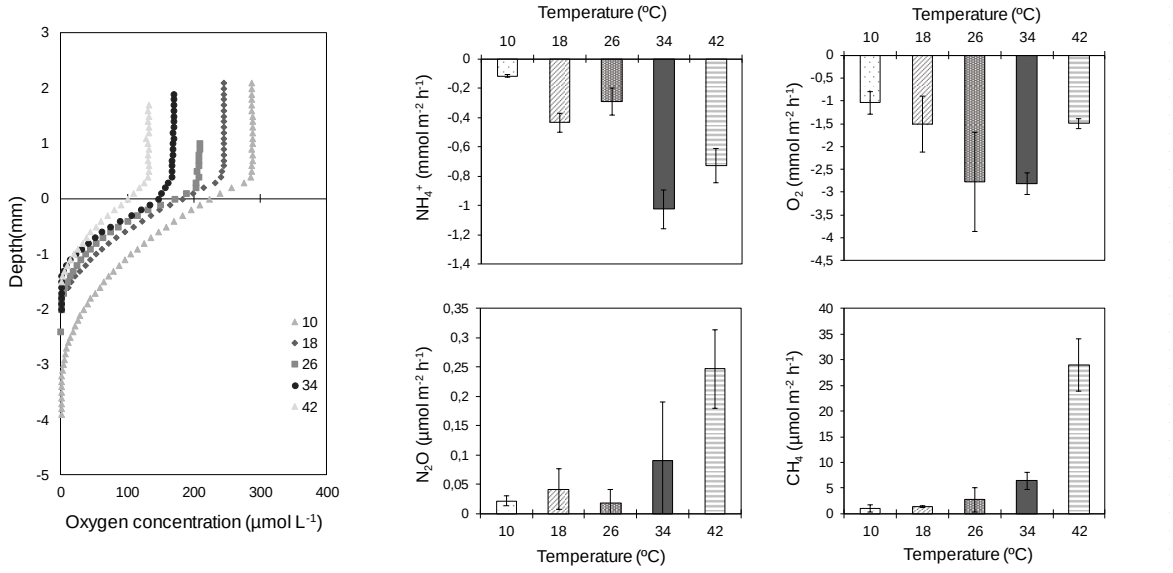

Figure 1: (Left) Oxygen microprofiles measured in sediments from Río San Pedro after being exposed to different temperatures for 24 hours. The oxygen penetration depth decreased with the temperature (p-value < 0.05). The highest penetration depth was observed at the lowest temperature (10 °C) and vice versa. (Right) Average fluxes of O_2, NH_4^+, N_2O and CH_4 across the sediment-water interface in sediment cores from Río San Pedro after being exposed to varying temperatures for 1 day, from an average *in situ* temperature of 18°C. Error bars represent the standard error (n=3).

Therefore, in a scenario of increase in the frequency of heat waves, coastal shallow areas such as the Bay of Cadiz will significantly increase the net release of GHGs such as CH_4 and N_2O. The short but intense temperature changes occurring in heatwaves could produce larger impacts than the steady increase in the average temperatures, with unknown consequences and further feedbacks with global warming.

Acknowledgments

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Marine lobsters off Mozambique (Indian Ocean)

Isabel Muñoz*[1] , Eva García Isarch[1] , Jose A. Cuesta[2] , Enrique Macpherson[3]

*Corresponding author: eli.munoz@ieo.es

[1] Instituto Español de Oceanografía (Cádiz). Puerto pesquero, Muelle de Levante, s/n 11006 Cádiz

[2] Instituto de Ciencias Marinas de Andalucía. Calle República Saharaui, 4, 11519 Puerto Real, Cádiz

[3] Centro de Estudios Avanzados de Blanes. Carrer Accés Cala Sant Francesc, 14, 17300 Blanes, Girona

Keywords: Achelata, Astacidea, Barcoding, Mozambique, Shelf and slope

Introduction

This work presents the preliminary results of two groups of decapods that will be part of the Ph.D Project "*Study of deep-sea decapod crustaceans communities in waters off Mozambique. Comparative with others African communities*". In this study, the results obtained in the revision of the marine lobsters (Astacidea and Achelata infraorders) are presented, as well as the distribution of the main identified species in the study area. This revision has been performed from a morphological and genetic point of view. These two infraorders, known as marine lobsters, are composed of species with important commercial value, some of them being target species of certain national and international crustacean fisheries that develop in waters of Mozambique, Madagascar and/or South Africa, others being bycatch species with high local value for national consumption and others are potentially exploitable (Groeneveld and Everett, 2015). Because traditionally the studies carried out in this area about marine lobsters have focused on the evaluation of fishery resources (Groeneveld and Everett, 2015), there is a significant lack of information about others ecological aspects. Although recently some inventories have been published about decapods from waters of the south-east Africa (Poupin, 2016; Emmerson, 2016 a,b,c), there is still no knowledge about aspects such as the composition, abundance and distribution of the communities that make up the decapods in those waters, and specifically, in relation with the marine lobsters.

Materials and Methods

The specimens studied for this work belong to the Collection of Decapod and Stomatopod Crustaceans of the Oceanographic Centre of Cadiz (CCDE-IEO). Specifically, they come from three oceanographic surveys carried out in waters of Mozambique by the IEO in 2007, 2008 and 2009. The methodology used to capture the specimens was a trawling gear ("*baka*" type). The study area corresponded to the platform and slope off Mozambique, at depths from 100 to 700 meters. Once on board, the specimens were first identified, photographed and preserved in ethanol and later transferred to the IEO in Cadiz, where a more exhaustive identification was carried out. Molecular analyses were performed based on fragments of two mitochondrial genes, the Cytochrome Oxidase subunit I (COI) and the 16S rRNA. The extraction of the total genomic DNA has been carried out from muscle tissue, specifically from the pereiopods (or pleopods in the case of females). Distribution areas and depth ranges of the identified species were analyzed from the survey georeferenced catch data.

Figure 1: Study area

Results

Astacidea is represented in this study by 2 species belonged to the Nephropidae family: the African lobster *Metanephrops mozambicus* and the Indian Ocean lobsterette *Nephropsis stewarti*, both distributed throughout the entire sampling area and at depth beyond the shelf (up to 250 m). About 3000 individuals were captured in the three surveys, and two specimens of each species are conserved at the CCDE-IEOCD. Two families represented the Achelata infraorder, the slipper lobsters Scyllaridae and the spiny lobsters Palinuridae. Around 3000 slipper lobsters belonged to 3 species, *Scammarctus batei batei*, *Scyllarides elisabethae* and *Ibacus novemdentatus* were captured in the three surveys. All of them were captured from 100 to 300 m, only *I. novemdentatus* till 500 m, and they were presented in all the prospected area. One specimen of each species is conserved in the CCDE-IEOCD. Near 2000 individuals of the Palinuridae family were collected, the majority belonging to the species *Palinurus delagoae*. Other two species were identified, *Puerulus angulatus* and *Linuparus somniosus*. Only 2 specimens of *L. somniosus* were captured (in 2007 and 2008). *P. delagoae* had a wide bathymetric and geographical range. *P. angulatus* were present from Mafamede Island to the north of Maputo, near Xai-Xai. The barcoding (16S and COI) of 5 species of these were obtained. In this work, the results obtained for *N. stewarti, S. batei batei* and *P. angulatus* are presented and discussed.

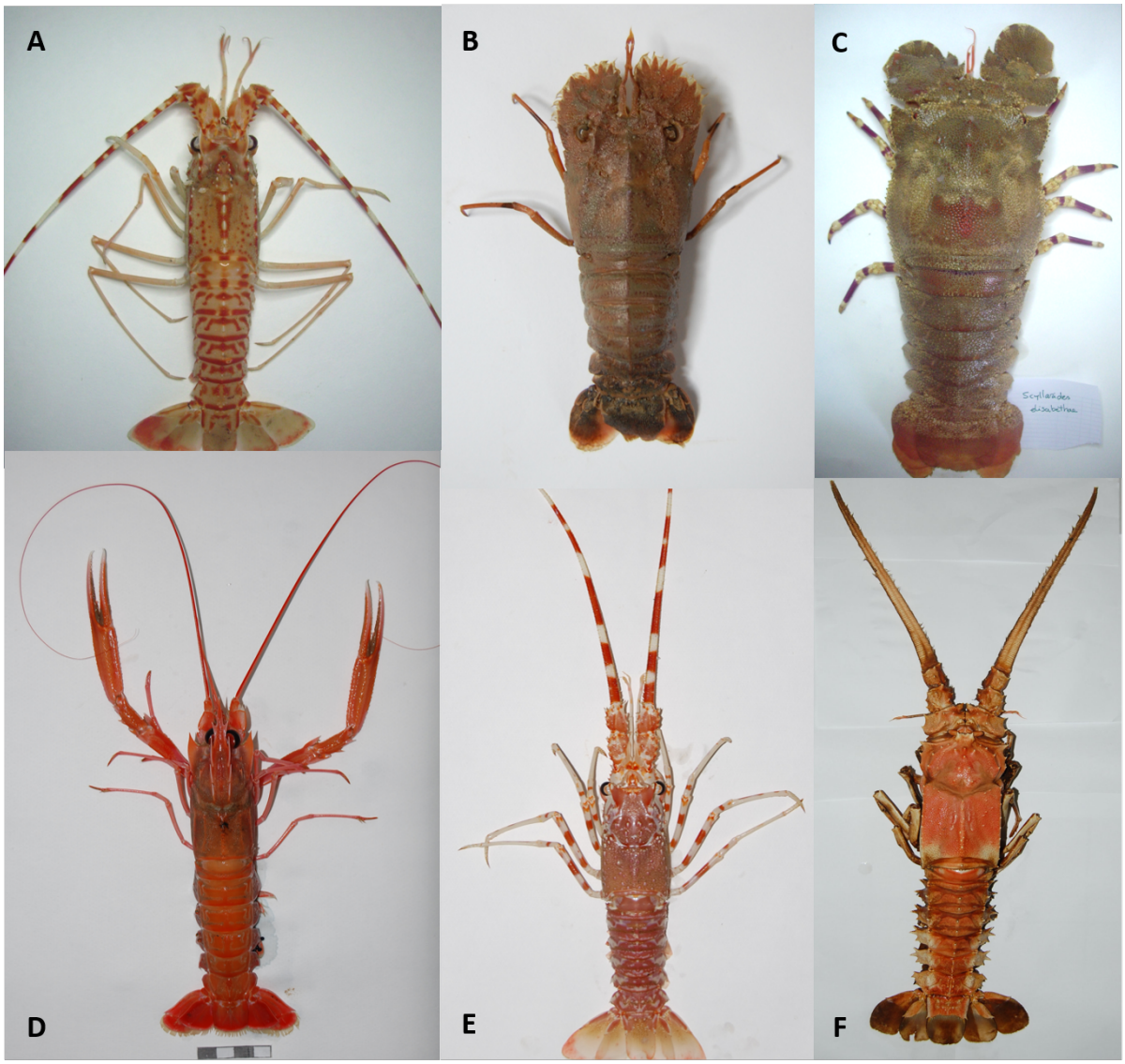

Figure 2: Specimens photographed on board (A: Puerulus angulatus; B: Scammarctus batei batei; C: Scyllarides elisabethae; D: Metanephrops mozambicus; E: Palinurus delagoae and F: Linuparus somniosus)

Acknowledgments

Thanks to all the colleagues and crew that make it possible for this type of study to be carried out.

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Global-change impacts on the metabolism of phytoplankton from coastal environments

Marco J. Cabrerizo*[1] , Juan Manuel González-Olalla[2] , Juan Manuel Medina-Sánchez[2] , Daniel Sánchez-Gómez[3] , Presentación Carrillo[3]

*Corresponding author: marcojc@uvigo.es

[1] Department of Ecology and Animal Biology, Faculty of Marine Sciences, University of Vigo, Campus Lagoas Marcosende, 36310 Vigo (Spain)

[2] Department of Ecology, Faculty of Sciences, University of Granada, Campus Fuentenueva s/n, 18071 Granada (Spain)

[3] Universitary Institute of Water Research, University of Granada, C/ Ramón y Cajal, 4, 18071 Granada (Spain)

Keywords: Global change, Marine ecosystems, Multiple stressors, Phytoplankton

Abstract

Currently, it is poorly known how ecosystems respond to interactions between multiple global change drivers. This is particularly relevant in Southern latitudes of Europe where climatic alterations are far exceeding the natural variability experienced by ecosystems over the last decades (Williamson et al., 2019). It is proposed the use of phytoplankton communities as sensors of the mid/long-term non-additive effects of predicted shifts in sea-surface temperature (cooling and warming), increased dust-aerosol inputs, and high ultraviolet radiation (UVR) levels by the shallowing of upper mixed layers in coastal waters. The main objective of this study is using these organisms as new indicators of environmental sensibility in coastal ecosystems. By combining observational approaches, complex experimental studies, and different temporal response scales it is showed that future global change scenarios can greatly impair photosynthesis, but increase the uptake of organic carbon through bacterivory. Such alterations to a primary producer level could also weaken the strength of the commensalistic phytoplankton-bacteria coupling, and ultimately, trigger alterations in the carbon sink capacity of the ecosystems.

Acknowledgments

This study was supported by the Ministerio de Economía y Competividad (MINECO) and Fondo Europeo de Desarrollo Regional (FEDER) (METAS project, CGL2015-67682-R), and by Campus de Excelencia Internacional del Mar-'Jóvenes Investigadores Ceimar 2018' (MIXICOST, CEIJ-008). MJC was supported by Juan de la Cierva contract (FJCI2017-32318) from the Ministerio de Ciencia, Innovación y Universiades and by a postdoctoral contract 'Contrato Puente' funded by the 7^{th} Plan Propio (FP7/2017) of the University of Granada and METAS project. JMGO was supported by a Spanish Government Fellowship 'Formación de Profesorado Universitario' (FPU14/00977).

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Unraveling the true identity of the hermit crab *Diogenes pugilator* (Crustacea: Decapoda: Diogenidae) and study of the associated species complex

Bruno Almón*[1] , Jacinto Pérez[2] , Eva García-Isarch[3] , J. Enrique García-Raso[4] , Jose A. Cuesta[5]

*Corresponding author: brunoalmon2@yahoo.es

[1] Instituto Español de Oceanografía, Centro Oceanográfico de Vigo. Subida a Radio Faro, 50-52 36390 Vigo, Spain.

[2] Grupo de Estudo do Medio Mariño (GEMM), Puerto Deportivo s/n. 15960 Ribeira. A Coruña. Spain

[3] Instituto Español de Oceanografía, C.O. de Cádiz. Muelle de Levante s/n, 11006, Cádiz, Spain

[4] Universidad de Málaga, Fac. de Ciencias, Dep. Biología Animal, Campus Teatinos, s/n, 29071, Málaga, Spain.

[5] Instituto de Ciencias Marinas de Andalucía, ICMAN-CSIC, Avenida República Saharaui, 2, 11519 Puerto Real, Cádiz, Spain

Keywords: Diogenes, Taxonomy, Iberian Peninsula, DNA, Paguroidea

Abstract

Diogenes pugilator (Roux, 1829) is a widely distributed species which has been recorded profusely since its description based on specimens from the French Mediterranean. Its actual theoretical distribution covers all east-Atlantic shores, the Mediterranean and the Black Seas. It is very common in shallow waters from 0 to 40 m, especially in soft bottoms and sand beaches, where it can be easily spot, with a extremely long left chelae being dragged on the substrate. During the past decades, several morphotypes have been described based on notorious morphologic variations of different body parts, leading in some cases to the description of new species that years later have been largely synonymized to the original *D. pugilator* from Roux (1829). In his work, Roux gives a short description of the species along with a plate that, although it describes well the general form of the body of the species, it is not detailed enough to be able to compare certain important characteristics. Unfortunately, the type specimens of the Roux's species seem to have disappeared after his death, so the clarification of the true identity of *D. pugilator* remains unclear.

Materials and Methods

In the framework of a more global study devoted to the objective of reviewing Iberian species of hermit crabs both from the morphological and molecular point of view, a review of several specimens identified as *D. pugilator* has been conducted, leading to the identification of a certain number of well-defined morphotypes, all previously included in the nominal species. Specimens studied have been collected by a variety of methods and come from different sources, including Biological Reference Colections (Colección de Crustáceos Decápodos y Estomatópodos del Centro Oceanográfico de Cádiz del IEO- CCDE-IEOCD, "Colección Biológica de Referencia del Institut de Ciències del Mar"- ICM-CSIC and the personal collections from José Enrique García-Raso from Málaga University, Jose A. Cuesta from ICMAN-CSIC, and Christoph Schubart from the

University of Regensburg, Germany), from scuba diving, and from scientific cruises devoted to the evaluation of fisheries stocks (ARSA 2017-2018 and MEDITS 2017-2018). Samples were studied under the stereomicroscope and then photographed and sketched to define the main traits defining each of the morphotypes. Drawings were then digitalized in Inkskape, with the aid of a digital tablet. Molecular samples were then taken from at least a pair of individuals from each of the species, one male and one female. Sequences of two mitochondrial genes (16S and COI) were obtained as well as others from GenBank that were also included in the comparative. A complete Maximum Likelihood phylogenetic tree was obtained for the available species of the genus *Diogenes* along with other species of the family Diogenidae in order to contextualize the phylogenetic position of the species of *Diogenes* and validate morphological differentiation.

Results and Discussion

Preliminary phylogenetic analysis agrees with the morphological delimitation of the morphotypes, suggesting that *D. pugilator* is in fact a species complex composed of several well-delimited species, and that all previous records of *D. pugilator* should be revised under the light of the new information. The discussion about which of the identified morphotypes correspond to the original from Roux (1829), is still unresolved due to the absence of type specimens. Nevertheless, the revision of the synonymized species preserved in the Museum Nationale d'Histoire Naturelle in Paris, along with an increasing sampling effort oriented to the area where the type specimens where initially caught, aims to serve as a clue to elucidate the true identity of the Roux species. Unraveling the true identity of *D. pugilator* is one of the most urgent tasks within this project, but it also opens a series of new challenges, since as the result of the designation of neotypes, other morphotypes need to be addressed and described in detail, updating the biological and distributional information associated with each of them. Once this task is finished, a complete revision of previous records and synonymies for this species should be also conducted and old names, perhaps, resurrected. Nevertheless, although this is still a work in progress, preliminary results suggest that specimens from Atlantic coast of the Iberian peninsula are not assignable to Roux species, which is more likely to be restricted to the Mediterranean Sea. Notwithstanding, two different morphotypes are also present in Iberian Atlantic waters being one of them associated with northern latitudes and the second with southern distribution. There are still many questions to solve around this problem, but it is important to reach an consensus about the definition of the species, given that the absence of original material, can lead to the perpetuation of the misidentifications making studies where these species are involved unreliable and the evaluation of the *Diogenes spp.* populations status, consistently overestimated. See Figure 1 and Figure 2.

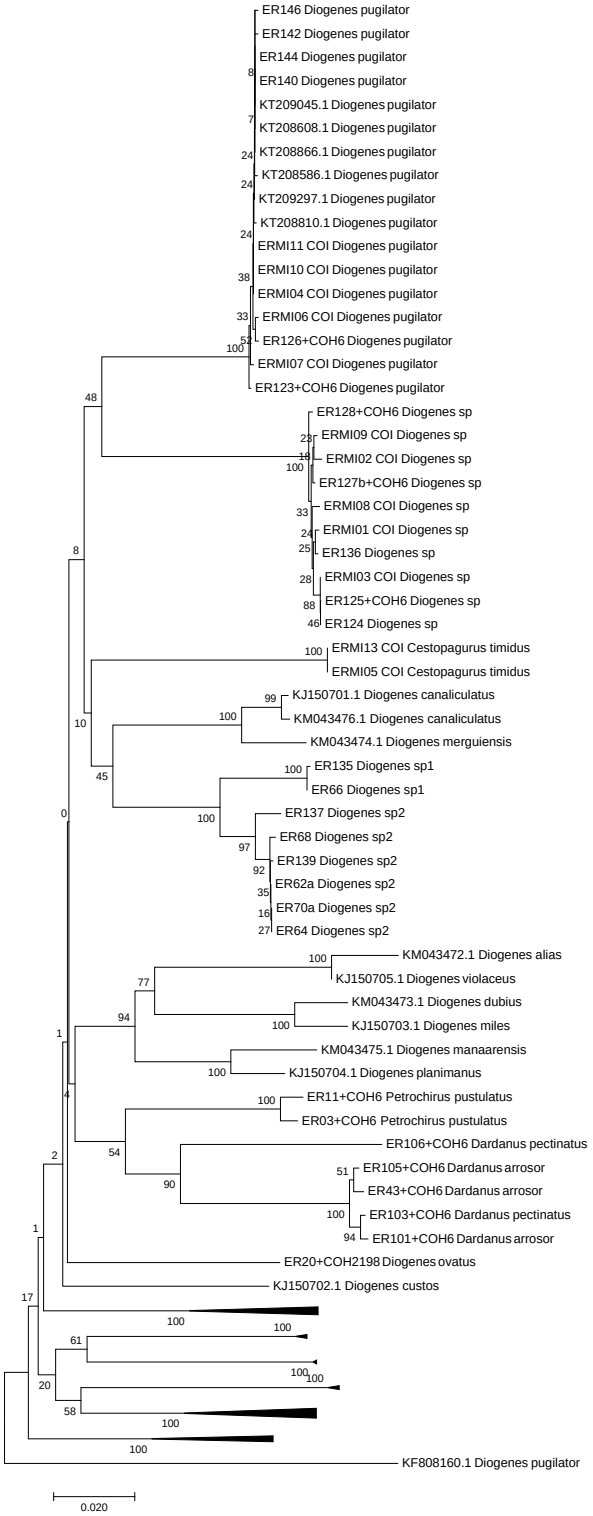

Figure 1: Part of the resulting COI phylogenetic tree showing the different resulting groups involving Diogenes species.

Figure 2: Original illustration from Roux (1829), describing the general morphology of the species.

Acknowledgments

We want to thank Christoph Schubart, Carlos Farias and Pere Abelló, the cession of specimens from their samplings and scientific surveys.

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Trophic plasticity in *Paracentrotus lividus*: herbivory, detritivory and omnivory as a function of resource availability and habitat features

Judith Camps*[1] , Javier Romero[2] , Patricia Prado[1]

*Corresponding author: judithcamps@outlook.es
[1] IRTA-Aquatic Ecosystems. Ctra. Poble Nou Km 5.5, 43540 Sant Carles de la Ràpita, Tarragona, Spain
[2] Department of Ecology, University of Barcelona, Faculty of Biology, Av. Diagonal, 645, 08028 Barcelona, Spain

Keywords: *Amathia verticillata*, *Pinna nobilis*, Pseudo-indigenous species, Seagrass beds, Sea urchins

Abstract

Factors controlling herbivory pressure have a central importance in shaping the seascape. In the Mediterranean, the sea urchin *Paracentrotus lividus* is considered as a keystone herbivore in seagrass meadows and photophilic macroalgal communities on rocky substrates. Here, we explore the trophic ecology of sea urchins in a sheltered, shallow (1 m depth) habitat, constituted by a mosaic of seagrass (*Cymodocea nodosa*) mixed with *Caulerpa prolifera* and sandy patches. We assess the influence of high local availability of the pseudo-indigenous soft body bryozoan *Amathia verticillata* and the abundance of pen shells (*Pinna nobilis*) providing hard substrate and hides on the trophic plasticity of the sea urchin. To this end, an ensemble of food preference (Prado and Heck, 2011) and foraging experiments, stomach contents and stable isotope analyses (δ^{13}C and δ^{15}N) were conducted in the Alfacs Bay (Ebro Delta). Our results show that sea urchins strongly prefer *A. verticillata* over *C. nodosa* and *C. prolifera* (Figure 1, Table 1), confirmed by the high abundance of the bryozoan in stomach contents (ca. 44%) in August, coupled with green and decayed seagrass leaves (29.7 and 26.4% respectively). Stable isotope analyses, which better reflects a long-term feeding behavior, show that ca. 65% of the diet of *P. lividus* seems to be based on decayed seagrass leaves (Figure 2), followed by the bryozoan *A. verticillata* (only available in summer) and green seagrass leaves (21.7 and 13.3% respectively). The local availability of *P. nobilis* also provides a preferred habitat for sea urchins which showed limited foraging movements into the surrounding seagrass beds, particularly when *A. verticillata* was attached to the shells. The apparently high contribution of animal and detrital food to *P. lividus* diet is unprecedented, and suggests an opportunistic feeding behavior in sea urchins in those habitats.

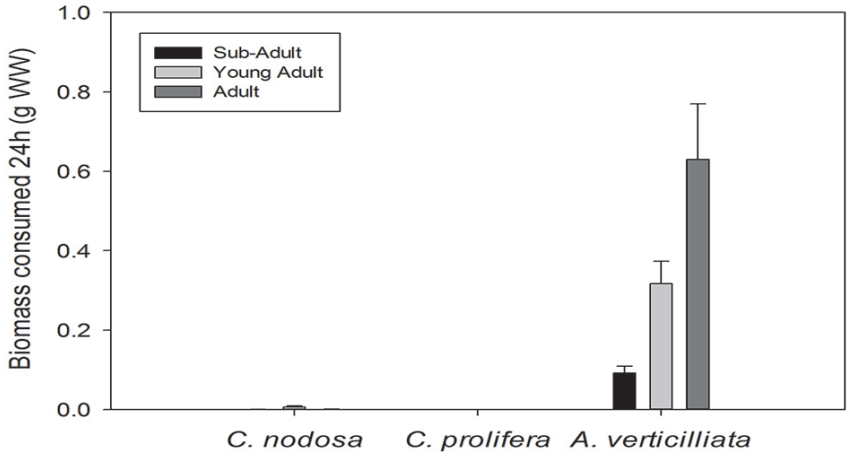

Figure 1: Biomass (g WW) of food items consumed in 24h-lasting experiments of food items *C. nodosa* (CN), *C. prolifera* (CP) and the bryozoan *A. verticillata* (AV)) by sea urchins size classes (Sub-Adults, Young Adults and Adults). Error bars are SE.

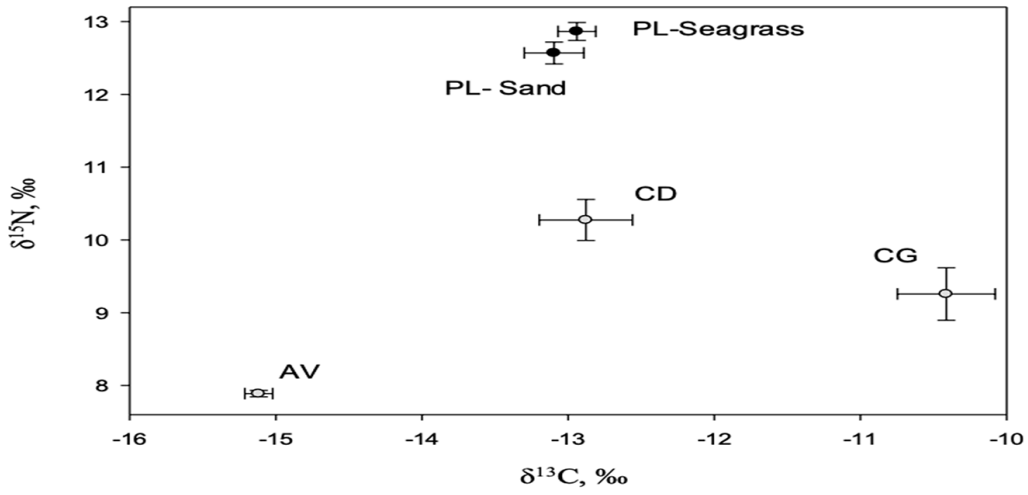

Figure 2: Stable isotope signatures (δ^{15}N and δ^{13}C) of *P. lividus* (PL) from *C. nodosa* (CN) and sand (S) habitats and food items including *A. verticillata* (AV), and green and detrital *C. nodosa* (G-CN and D-CN, respectively).

Table 1: Friedman's ANOVA X2 and Kendall's coefficient of concordance (W) for ranked consumption rates on offered food items including leaves of *C. nodosa* (CN), the bryozoan *A. verticillita* (AV) and the green algae *C. prolifera* (CP). In Wilcoxon matched pairs (WMP) post hoc comparisons, significant differences in consumption rates between pairs of diet items are indicated in bold.

Consumer (n=19, df= 2)	Friedman's ANOVA χ^2	Kendall'sW	*p*
Subadults	25.72	0.714	**0.000**
WMP post hoc:	AV> CN= CP		
Young adults	27.25	0.75	**0.000**
WMP post hoc	AV> CN= CP		
Adults	28.00	0.77	**0.000**
WMP post hoc:	AV> CN= CP		

Acknowledgments

Authors are grateful to Pep Cabanes and to Josep Maria Costas for technical assistance during fieldwork and to David Mateu for this help with sample processing in the laboratory. We are also grateful to Neus Sanmartí for help during stable isotope processing and to Pep Cabanes and Mr. Camps for help during food preference experiments.

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Warmer winters in Kongsfjorden (Svalbard) will compromise the survival of interannual Arctic seaweeds

Manuel Macías Andrade*[1] , Carlos Jiménez[1] , Francisco J L Gordillo[1] , Raquel Carmona[1]

*Corresponding author: manu.maciasandrade@gmail.com
[1] Department of Ecology and Geology, University of Malaga

Keywords: Arctic macrophytes, Polar winter, Warming Arctic, Biochemical compounds, Photosyntesis

Abstract

Four species of representative macrophytes from Kongsfjorden hard bottoms (the Ochrophytes *Saccharina latissima* and *Alaria esculenta*, and the Rhodophytes *Phycodrys rubens* and *Ptilota gunneri*) were incubated in the dark for a period of 16 weeks, simulating Artic winter, at two different temperatures (3.5 and 8^{o}C). Fresh weight, respiration, net photosynthesis, as well as carbohydrate, lipid and protein content were followed. Results indicate that red macrophytes coped with darkness better than the brown ones, as well as with increasing temperature, and nearly no differences in biochemical composition and capacity for O_2 production ocurred during the extended dark cultivation in the former ones, while in kelps a significant loss of biomass was observed accompained by a drop in photosynthetic O_2 production when re-exposed to light after the dark period. In addition, red macrophytes lost just a small proportion of their biomass during darkness. The fate of the thalli as well as respiration and net photosynthesis were affected by temperature, mainly in kelps, and, in general, respiration and thalli degradation was higher at 8^{o}C. Interestingly, *A. esculenta* produced new blades during the last four weeks of the dark incubation, anticipating availability of light. Additionally, the capacity for photosynthetic electron transport was maintained in all thalli, indicating that the light harvesting system was kept fully operational even after 16 weeks in the dark. These results indicate that Arctic macrophytes must be genetically adapted to cope with prolonged dark periods, surviving during the polar winter, and that increasing temperature during winter may significantly affect survival of some kelps (Berge et al., 2015a,b).

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Island shelf and slope geomorphology of La Palma Island (Southern sector)

Ayanta Velazco Martínez*[1] , Juan Tomás Vázquez Garrido[2] , Francisco José Pérez Torrado[3] , Olga Sánchez Guillamón[2] , Desirée Palomino Cantero[2] , Patricia Bárcenas Gascón[2] , Eugenio Fraile Nuez[4]

*Corresponding author: ayanta.velasco101@alu.ulpgc.es

[1] Universidad de Las Palmas de Gran Canaria. Facultad de Ciencias del Mar, 35017 Las Palmas de Gran Canaria

[2] Instituto Español de Oceanografía. Centro Oceanográfico de Málaga, 29640 Fuengirola

[3] Departamento de Física (GEOVOL), Campus de Tafira, Universidad de Las Palmas de Gran Canaria, 35017 Las Palmas de Gran Canaria

[4] Instituto Español de Oceanografía, Centro Oceanográfico de Canarias, 38180 Santa Cruz de Tenerife

Keywords: Geomorphology, Cartography, Multibeam bathymetry, Canary Islands

Abstract

The geomorphology is the study of the Earth's surface landforms, and the analysis of the genetic processes that shaped them in the past and their behaviour in the present. There are different submarine environments characterized by several morphological features that could be generated by different geological processes. In this sense, the study of morphological types allows to infer the dominant geological processes in a region. The studies of submarine geomorphology have had an important development with the upgrade of multibeam bathymetric echosounders and the construction of submarine digital terrain models from these data. Geographic Information Systems are very useful techniques in these studies due to using different spatial analysis tools on bathymetry data allows to recognize different morphological elements and units. The main aim of this work is the geomorphological study of the submarine environments corresponding to the insular shelf and slope of a volcanic island, the southern sector of La Palma at the Canary archipelago (Figure.1).

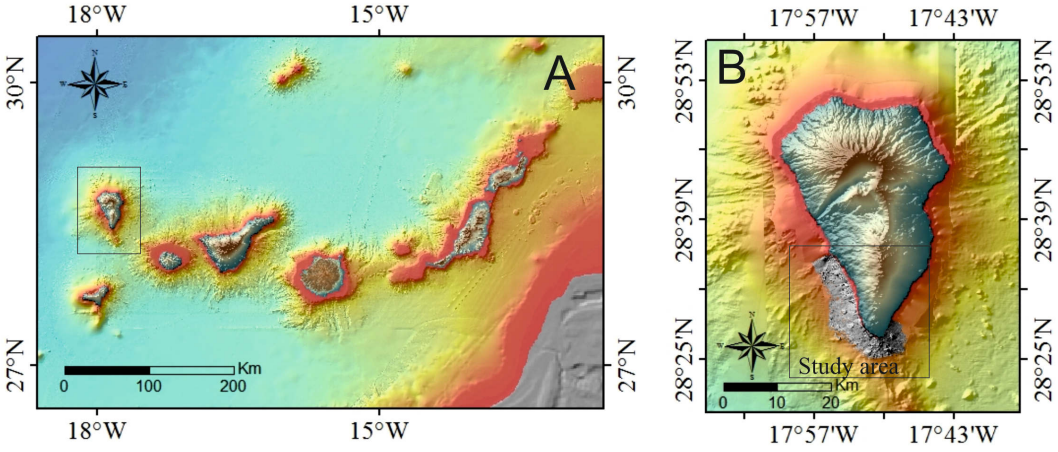

Figure 1: A) The Canary archipelago. B) Map of La Palma and the study area.

This study requires the identification of the geomorphological elements and units present in these submarine environments and their relationship with the dominant geological processes in the area and, by this way, understand the possible evolution and development of the island (Schmincke, 2004). The geomorphology of the study area is explained. To carry out this study a qualitative or handmade mapping (Figure 2) of all the geomorphological elements and units present in the study area has been done. Several morphological types are recognized, such as volcanic cones, flat-topped cones, lava flows, different types of depositional bodies, scarps, landslide scars, valleys and the insular shelf itself, among others. The study of the morphology of these elements have allowed to establish relationships with the processes that have taken place for their formation and their evolution observing, for example, that the scars are related to processes of mass movement by action of gravity, being this a erosive process, while volcanic cones are the result of magmatic activity. These types of relationships are established for all the morphological elements characterized observing that precisely the volcanic activity and the gravitational processes related to the strong gradients of the insular slope and the main morphogenetic processes of the area. In addition, there are two regions with lava flows, which could have come from eruptions that have taken place on land and have spread to the sea.

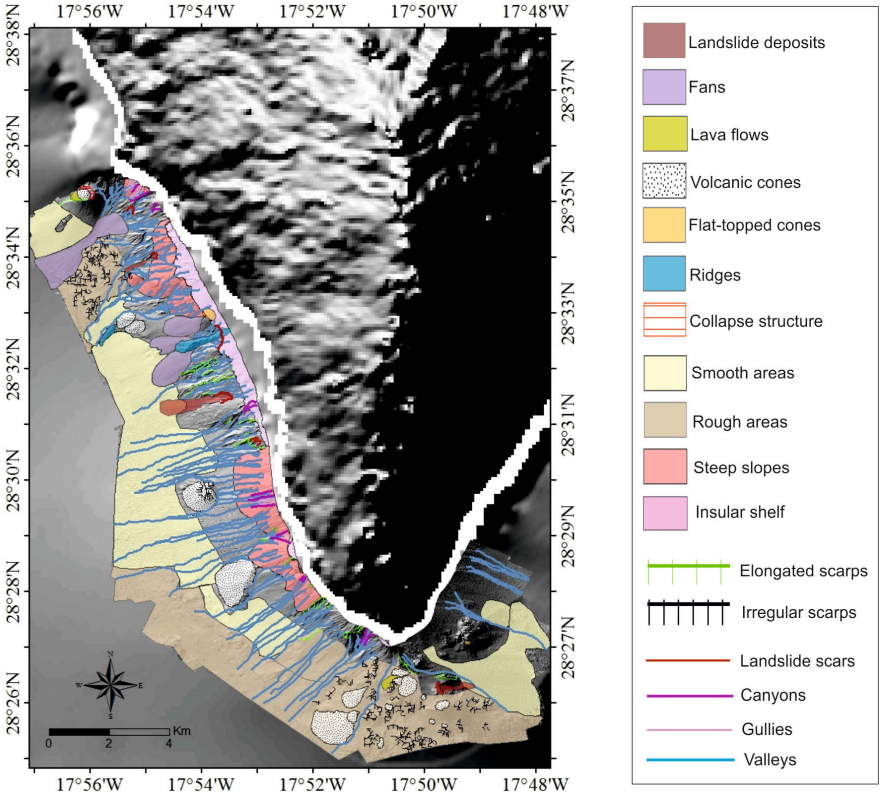

Figure 2: Qualitative or Handmade mapping.

Acknowledgments

Thanks to the “Instituto Español de Oceanografía" and Dr. Juan Tomás Vázquez for allowing access to the data of the VULCANA (IEO 2015-2017) project, without which this work would not have been possible.

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Understanding the biogeographic limits of the intertidal canopy-forming seaweed *Fucus serratus*.

Ana García García*[1] , Sandra Hernández[1] , Brezo Martínez[1]

*Corresponding author: ana.garcia.garcia@urjc.es
[1] University Rey Juan Carlos

Keywords: Climate change, Niche underfilling, Macroalgae, Physiological thresholds, Thermal niche

Abstract

Global warming will probably surpass the physiological tolerance of species, exceeding the lethal thresholds that bound their fundamental thermal niche. Many studies implicitly presume that the wide geographic distribution of species across different thermal regimes can be explained by phenotypic plasticity instead of local thermal adaptation, assuming that conspecific populations fill their potential thermal niche (Breeman, 1988; Sunday et al., 2012). However, others do not and, thus, we do not know to what extent niche underfilling is the exception or the rule. In order to investigate potential population-level responses to temperature, we performed a mesocosm experiment to evaluate lethal temperature thresholds of a dominant canopy-forming marine seaweed *Fucus serratus*. We investigated the differential response of ten different populations distributed in three ecoregions, covering the species' entire latitudinal range from the North Iberian Peninsula to mid Norway. We investigated how this response could affect the filling of its thermal niche by comparing the thresholds of different populations across its native distribution range, and how the species tolerance shapes the filling of the thermal fundamental niche. Further we evaluated the thermal vulnerability of *F. serratus* under the current thermal conditions. Our results suggest low variability in the upper survival threshold (as UST50)(Figure 1), and a realized thermal niche in the field that does not fill the fundamental niche of tolerance. These results suggest that the fundamental niche is not only shaped by ocean temperatures, but also by other climatic and non-climatic factors, such as rising atmospheric temperatures, and harsher conditions during low tide (Martínez et al., 2012). Our results also suggest highest vulnerability of N Spain populations, which experienced the hottest summers in combination with extreme heat wave events (Duarte and Viejo, 2018) Instead, western rias seem to be potential climate refugia for *F. serratus*, which may mitigate the ongoing decline of this species in the N Spain.

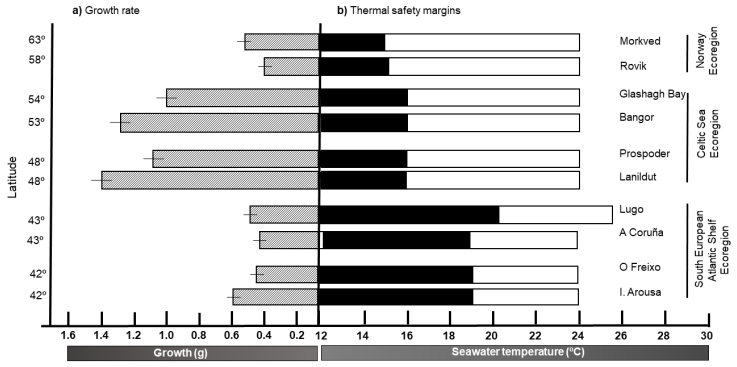

Figure 1: Growth rate and thermal safety margins. a) Growth rate of populations at optimal seawater temperature along the species' distribution range; b) thermal safety margins of studied populations. Distance between solid line and the edge of the box represents the thermal safety margin. White box indicates the range in SSThismax throughout a species' latitudinal range. Black box represents the UST50 evaluated in experimental phase.

Acknowledgments

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PART III

Transversal Sessions

Transversal session "A sea of women"

Olga Sánchez Guillamón[1*] , Mª Carmen García Martinez[1] , Isabel Jiménez Lucena[2] , Maria Macías Gonzalez[3] , Sofía Tristancho Ruiz[4]

[*] Session coordinator
[1] Spanish Institute of Oceanography. Centro Oceanográfico de Málaga (IEO)
[2] University of Málaga, Málaga
[3] Divingmar S.L., Málaga
[4] CEO Futuralga, Cádiz

Keywords: Gender equity, STEM, Women in science.

Abstract

The "A Sea of Women" colloquium opened the cycle of transversal sessions during the II International Congress of Young Marine Researchers. It was dedicated to promoting the diverse professional experiences that a young woman marine student could develop in her future throughout four women´s life experiences coming from the Academia, the public and the private sectors of the marine and research realm.

The session was focused on the main barriers, challenges, opportunities and achievements that our protagonists have dealt with in their internships and jobs, with emphasis to how they have faced inequality and discrimination (lower pay than their male counterparts, job promotions and maternity entitlements), employment and sexual harassment or violence, in a work environment historically reserved for men such as the sea.

This transversal session was held on October 2nd (Lynn Margulis Magna Room), coordinated by Dr. Olga Sánchez Guillamón.

Transversal session "Redes JIs" Young Marine Researchers Networks

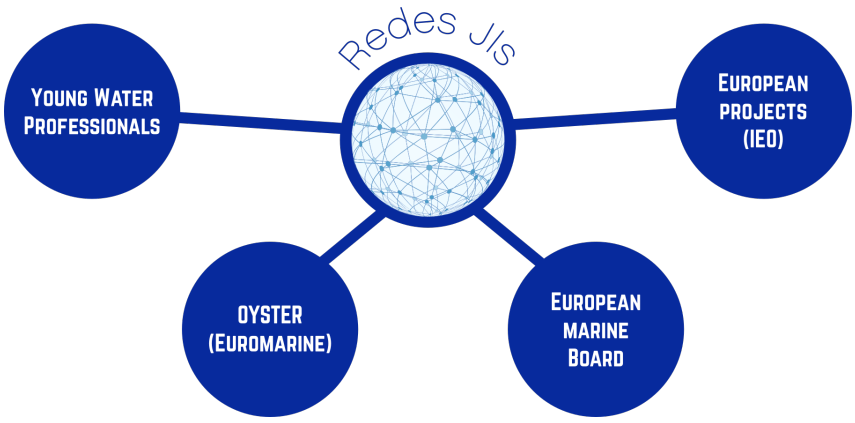
Raquel Sánchez de Pedro[1] , Andrea Phiel Harms[2] , Javier Moreno-Andrés[3] , Ivana Buselic Garber[4] , Alba González Vega[5]

[*] Session coordinator
[1] University of Málaga
[2] Spanish Institute of Oceanography (IEO)
[3] Young Water Professionals (YWP)
[4] Euromarine (OYSTER)
[5] European Marine Board (EMB)

Keywords: Collaborations, Internationalisation, Networking.

Abstract

For many years there was that idea of an isolated scientist at the lab, diving on its own ideas, but today we cannot conceive science without collaborations. Out of the academic world, networking is a common word, which in science we have to start talking about. We live in changing times where collaboration should be first than competition. It is difficult to find a job in academia, which can improve having an effective network in and out of the academic world. In project calls, its a reality that internationalization, and collaborative projects always have more chances to get funding. Networking requires skills, practice but young scientists must start enjoying the benefits of it. This special session aimed to interchange experiences and ideas among professionals and young marine researchers from different marine-related networks. From their experience, we discussed how we can create synergistic collaborations, find opportunities for funding, detect our common needs as young researchers, how to connect science with policymakers and how being involved in active marine networks can strengthen our career paths.



This transversal session was held on October 3rd (Lynn Margulis Magna Room), coordinated by Dr. Raquel Sánchez de Pedro.

Transversal session "JIs Projects"

María López Parages[1*] , Isabel Sáez Casado[2] , Silvana Teresa Tapia Paniagua[1] , Marta Sendra Vega[3] , Laura Cabeza Montilla[4] , Pedro Sánchez Castillo[4]

[*] Session coordinator
[1] University of Málaga
[2] University of Almeria
[3] University of Cádiz
[4] University of Granada

Keywords: CEI·MAR, Early career researchers, Projects.

Abstract

The general objective of the JIs Projects session was the sharing and the exchange of experiences and transfer of knowledge in regards to the first CEI·MAR call of research projects "Young Researchers 2018". Through its day-to-day experience as PIs (Principal Investigators), the speakers shared with the audience their obtained results, the difficulties that could have aroused, how they faced this challenge, whether their experience as PIs has resulted positive from the scientific and professional maturity perspective, as well as, the main aspects to improve with a view to future calls.

This transversal session was held on October 4th (Lynn Margulis Magna Room), coordinated by Dr. María López-Parages.

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