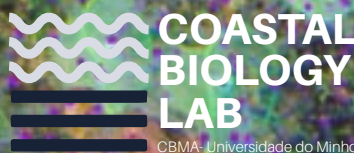


SIEBM

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XX Simpósio Ibérico de Estudos de Biologia Marinha

XX Simposio Iborico de Estudos de Biologia Mariña

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Agenda

September 9	
RECEPTION	08:00
Opening session	09:30
<i>Keynote session: A stroll through the European marine biological data landscape</i>	
Belén Martín (ECIMAT-CIM, U. Vigo, Spain)	10:30
Coffee break	
<i>Special invited speaker: Topics and Trends since 1979 in benthic studies: from sampling techniques to ecogenetic developments</i>	
Javier Niell (Emerit Professor, U. Malaga, Spain)	12:00
Lunch time	
<i>Keynote session: Integrating knowledge among fishermen and scientists for better marine conservation and management: A running example in Southern Portugal (Algarve)</i>	
Ana Marçalo (CCMAR, U. Algarve, Portugal)	14:00
Plenary Session 1	
Adapting fisheries management to the spatial variability in the reproductive cycle: the case of the sword razor clam in Galicia (NW Spain)	
Ana Tubío Gómez	14:45
Assessing the overlap of NAFO fisheries with Vulnerable Marine Ecosystems	
Maria del Mar Sacau Cuadrado	15:00
Space-temporary variation of the population of long-finned pilot whales (<i>Globicephala melas</i>) in the SE of the Iberian peninsula	
Rosa María Canales-Cáceres	15:15
Spatial and temporal characterization (2006-2012) of the trawling activity in the Site of Community Importance “ <i>Volcanes de fango del golfo de Cádiz</i> ” (southwestern Iberian Peninsula)	
Emilio González-García	15:30
North Atlantic Oscillation affects dolphinfish as target and bycatch species in fisheries from the western Mediterranean Sea	
Jose Carlos Báez	15:15
Coffee break	
Atlantic chub mackerel (<i>Scomber colias</i>) growth and reproduction off the Portuguese coast in relation to the population dynamics	
Cristina Nunes	16:30
Univariate approach of blackspot seabream (<i>P. bogaraveo</i>) landings. Linear dependence and volatility	
Víctor Sanz-Fernández	16:45
Reproductive cycle of the sea cucumber <i>Holothuria forskali</i> in the Ria de Vigo: application to fisheries management	
Tania Ballesteros Otero	17:00
Influence of anthropogenic factors in the community of loggerhead sea turtles (<i>Caretta caretta</i>) in Portuguese continental waters	
Lidia Nicolau	17:15
Poster hanging	17:30
Welcome reception at “ <i>Casa Museu Nogueira da Silva</i> ”	
19:30	
Welcome reception at “ <i>Casa Museu Nogueira da Silva</i> ”	
19:30	

September 10

<i>Keynote session: Population structure in marine organisms: from genetics to genomics</i>	
Marta Pascual (U. Barcelona, Spain)	09:00
Plenary Session 3	
Rock-pools as nurseries for co-existing limpets: dynamics of limpet nursery grounds in SW Portugal	

Maria Inês Seabra	09:45
The stalked barnacle <i>Pollicipes pollicipes</i> fishery at Reserva Natural das Berlengas (Portugal): temporal variation of the harvesters perspective	
Alina Sousa	10:00
Quantification of the foraging activity of <i>Patella caerulea</i> and <i>P. rustica</i> under simulated tides using photographic analysis of radula scratches	
Juan Sempere-Valverde	10:15
Drivers of interannual abundance changes in gelatinous carnivore zooplankton in the Iberian Peninsula, (Portugal)	
Sónia Cotrim Marques	10:30
Microplastics: the invisible threat	
Alicia Herrera	10:45
Coffee break	
Plenary Session 4	
Inferred family structure of an endangered species, <i>Pinna nobilis</i> , using molecular analyses: implications of connectivity for conservation	
Elisabet Nebot	11:30
East to West: Population genomics and hierarchical analyses reveal differential adaptation footprints in the edible sea urchin <i>Paracentrotus lividus</i>	
Carlos Carreras	11:45
Environmental and phenotypic drivers affect differential genomic responses in two sympatric congeneric fish	
Héctor Torrado	12:00
Population genomics revealed independent colonisation events of a global ascidian invader	
Xavier Turon	12:15
Disentangling latitudinal diversity gradients in Taxonomic, Phylogenetic, and Functional diversity of Atlantic reef fishes	
Nestor E. Bosch	12:30
LUNCH TIME	
<i>Keynote session: Population structure in marine organisms: from genetics to genomics</i>	
José Guerra-García (U. Sevilla, Spain)	14:00
Plenary Session 5	
Biofilms shaping compositions of macrofouling assemblages: An initial barrier against NIS settlement?	
Eva Cacabelos Reyes	14:45
Do Non-Indigenous Species (NIS) prevailing over native species with climate change effects?	
Nuno Castro	15:00
Sampling Techniques Evaluation For Monitoring And Control Of A Non-Native Crab Population	
Jose Antonio Martin	15:15
Artificial seaweed monitoring structures (ASMS): the NextSea project approach towards a standardized non-destructive epibenthic sampling methodology	
Diego Carreira Flores	15:30
Evolutionary insights derived from comprehensive analyses of DNA barcoding diversity in marine members of the superorder Peracarida (Crustacea: Malacostraca)	
Pedro Vieira	15:45

Coffee break + Poster Session

Plenary Session 6	
Community structure and habitat characterization of <i>Neoschrammeniella</i> cf. <i>bowerbankii</i> (Porifera, Corallistidae) aggregations in the Cantabrian Sea	
Pilar Rios Lopez	16:45
Habitats characterization of circalittoral rocky bottoms of the Avilés Canyon System (Cantabrian Sea)	
Francisco Sánchez Delgado	17:00

High resolution spatial distribution for the hexactinellid sponges <i>Asconema setubalense</i> and <i>Pheronema carpenteri</i> in the Central Cantabrian Sea	
Augusto Rodríguez-Basalo	17:15
In-situ growth rate assessment of hexactinellid <i>Asconema setubalense</i> using 3D photogrammetric reconstruction in El Cachucho Marine Protected Area (Le Danois Bank, Cantabrian Sea)	
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Evolutionary insights derived from comprehensive analyses of DNA barcoding diversity in marine members of the superorder Peracarida (Crustacea: Malacostraca)	
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Coffee break + Poster Session

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High resolution spatial distribution for the hexactinellid sponges <i>Asconema setubalense</i> and <i>Pheronema carpenteri</i> in the Central Cantabrian Sea	
Augusto Rodríguez-Basalo	17:15
In-situ growth rate assessment of hexactinellid <i>Asconema setubalense</i> using 3D photogrammetric reconstruction in El Cachucho Marine Protected Area (Le Danois Bank, Cantabrian Sea)	
Elena Prado	17:30

September 11

<i>Keynote session: Doing things better: marine legislation and recovery of ecosystems</i>	
Angel Borja (AZTI Tecnalia, Spain)	09:00
Plenary Session 7	
Towards a standardized methodology for monitoring and assessing marine mobile epibenthic communities across spatio-temporal scales	
Macarena Ros	09:45

Mapping seaweed beds using multi spectral imagery retrieved by unmanned aerial vehicles	
Débora Borges	10:00

Phase shifts from macroalgae to Zoantharia: effects on subtropical fish community	
Sergio Moreno-Borges	10:15
Predictive model of competition between three species of salt-marsh plants: effects of sedimentary variables and CO2 concentration	
F. Javier Niell	10:30

Comparing toxicity and bioactivity of gold nanoparticles synthesized using two brown algae <i>Cystoseira</i> sp	
Sofia Machado	10:45

Coffee break	
Plenary Session 8	
Collapse of macrophytic communities in a eutrophicated coastal lagoon	
Juan Manuel Ruiz Fernández	11:30

Chemical Characterization of a New Acidified Region to Study Ocean Acidification in Subtropical Ecosystems	
Sara González-Delgado	11:45

History of red coral (<i>Corallium rubrum</i> , L. 1758) change in biometric parameters and carbon retention capacity: a meta-analysis in NW Mediterranean	
Miguel Mallo	12:00

Macroalgal diversity along the North Portuguese shores

Marcos Rubal García	12:15
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Lunch time
Keynote session: Opportunities, threats and challenges of deep-ocean conservation

Marina Cunha (CESAM, U. Aveiro, Portugal)	14:00
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Plenary Session 9	
Deep water fish ontogenic changes in habitat preferences in several NE Atlantic ecosystems	
Alberto Serrano López	14:45
New data of Tetractinellida (Porifera) in Aviles Canyon system and Le Danois Bank (Cantabrian Sea). Results of the SponGES Project	
Javier Cristobo	15:00

Distribution patterns of intertidal zoantharian species in the Canary Islands and their relation with ocean temperature	
Cataixa Lopez Batista	15:15

Using Biological Traits To Assess Fishing Gear Impacts In The Seabed. The INTERMARES Experience	
José Manuel González-Irusta	15:30

Simulating trophic impacts of recreational fishing scenarios on two oceanic islands using Ecopath with Ecosim	
Lorena Couce	15:45

Coffee break + Poster Session

Plenary Session 10	
Investigation of juvenile Angelshark (<i>Squatina squatina</i>) habitat in the Canary Islands to inform protection of this Critically Endangered species	
David Jiménez Alvarado	16:45

Stock structure of the common mora in the mid-North Atlantic (Azores archipelago)	
Régis V. S. Santos	17:00

Long term evaluation of spearfishers tournaments in the Canary Islands	
Airam Guerra-Marrero	17:15

Effect of diet on growth and development of the jellyfish <i>Phyllorhiza punctata</i> (Cnidaria, Scyphozoa) in laboratory conditions	
Inês Matos Duarte	17:30

September 12

Keynote session: Physical-biological interactions in the ocean: case studies from the northern Canary Current Upwelling Ecosystem

A. Miguel Santos P. (IPMA, Portugal)	09:00
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Plenary Session 11	
Environmental Education, Citizen Science and Dissemination of Science in a coastal Atlantic community: the role of the CMIA of Vila do Conde	
Silvia Morim	09:45
Funchal Marine Biology Station: 20 years of research and promotion of Madeiran and Macaronesian marine biodiversity (NE Atlantic Ocean)	
Mafalda Freitas	10:00

Improving perception and acceptability of aquaculture through hands-on experimental activities for schools	
Marta Filipa Vitorino Correia	10:15

The Importance of Environmental Education Activities Assessment: the “School Of Nature” Project Case Study	
Leonor Cruz	10:30

Acquired knowledge about Ciguatera Fish Poisoning in the Canary Islands population	
Alberto Bilbao-Sieyro	10:45

Plenary Session 12

Combining artificial substrates, morphology and DNA metabarcoding for investigating macrozoobenthic communities in NW Iberia

Barbara Rocha Leite 11:30

Metabarcoding the benthic boundary layer: the role of sampling method and marker characteristics in the DNA signatures obtained at the interface between benthos and plankton

Adrià Antich 11:45

Molecular and morphometric combo reveals extraordinary hidden diversity in European polychaetes from the Phyllodoceidae family

Marcos A. Teixeira 12:00

Patterns of spatial and temporal variation in estuarine meiofaunal communities assessed through DNA metabarcoding: a case study in the Lima estuary (NW Portugal)

Maria Fais 12:15

LUNCH TIME

Keynote session: *From microbes to mammals: comprehensive analysis of biodiversity using DNA metabarcoding*

Mehrdad Hajibabaei (University of Guelph, Canada) 14:00

Plenary Session 13

Phylogeography of the deep-sea morid codling *Lepidion lepidion* reveals the presence of two ancient atlantic/mediterranean lineages

David Barros-García 14:45

Lipidomics of solar-power animals: a tool to unravel the process of kleptoplasty

Felisa Rey 15:00

Health status-associated microbiota fingerprints within the oral cavity of cetaceans

Pedro Soares-Castro 15:15

Occurrence of the toxic dinoflagellate *Alexandrium ostenfeldii* in the coastal waters of the southeastern Bay of Biscay

Luis Ferrer 15:30

Presence of *Vibrio mediterranei* associated to major mortality in stabled individuals of *Pinna nobilis* L

Patricia Prado Villegas 15:45

Cephalopods from the Canary Current upwelling ecosystem (Northwest Africa)

Amanda Luna 16:00

A glance into the global phylogeographic history of hydroids of the superfamily Plumularioidea (Cnidaria, Hydrozoa)

Carlos Moura 16:15

Coffee break + Poster Session

Closing session 17:30



Keynote and Invited Speakers

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This documents is intended solely to facilitate the participants follow-up during the XX SIEMB and it isn't, by no means, the final eBook that will be published in Front. Mar. Sci. Conference Abstract: XX Iberian Symposium on Marine Biology Studies (SIEBM XX). Due to limitations on the Frontiers platform, from which the text in this document was extracted, italics and superscript text are not supported. Although we tried to minimize the problem in the title of the communications, it was not possible to correct all instances in the main text. Please accept our apologies and keep the document as a provisional publication

A Stroll Through the Marine Biological Data Landscape (If You Love Your Data, Set Them Free)

Belén Martín Míguez

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Scientists are trained to zoom in at issues, problems, features... Part of our work as researchers consists of coming closer to the object of our research, and acquiring as much evidence as possible around it in order to understand it better. It is like we were equipped with a magnifying glass, that enabled us to see the smallest details. That brings about an inevitable feeling of ownership, of possession. The more familiar we are with a subject, the more comfortable we feel remaining in our zone of comfort and the more convinced we are that we have rights on the knowledge we are handling. Psychologists warn us about the risk of confusing love with possession. Sting sang "If you love somebody, set them free". Obviously, loving a person is not the same than loving your data... but the mechanism behind may not be that different. This talk intends to contribute to a change of model, from the "Data belongs to scientists, to "Scientists only hold data in usufruct". We will explain first the reasons behind this paradigm in the case of marine data. This will require looking at the marine data landscape and the myriad of institutions that have a say on the way research is eventually developed, i.e., the ocean governance framework. We will pay particular attention to those projects, organizations, policies and funding mechanisms related to marine biology, and how they can appear at different levels: international, European, regional... We will explain how free exchange of data is increasingly encouraged in this ocean governance framework. We will also shed some light on concepts such as "Open Data" "Democratization of information" and "FAIR principles". Finally, we will tackle the issue of the proliferation of data portals, where data are made available for free but in such a way that the user is often confused. Once we have found our way in that soup of acronyms, we will describe some of the advantages of setting free our data (beyond complying with the funding programmes requirements) by publishing them, including keeping them for the long-term, more possibilities of collaborations with other scientists and additional citation. We will then present two examples of initiatives related to making marine biological data more accessible. In the first one, we will focus on biodiversity data, from an international and European perspective touching upon initiatives such as OBIS and EMODnet. Secondly, we will provide an example of a European Research Infrastructure built around Marine Biological Stations (EMBRIC, European Marine Biological Resource Centre) and we will show how this distributed infrastructure can foster free exchange of knowledge through its different services, including a Transnational Access Programme, and the compliance with Nagoya protocol.

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Wilkinson et al. (2016). The FAIR Guiding Principles for scientific data management and stewardship. *Sci Data*. 2016 Mar 15;3:160018. doi: 10.1038/sdata.2016.18

Keywords: Marine Biology, Marine data exchange, EMBRC, Open Data, FAIR

Topics and Trends Since 1979 in Benthic Studies: From Sampling Techniques to Ecogenetic Approaches

F. Xavier Niell (Emeritus Professor of Ecology)

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Forty years ago, Miguel Ibañez from San Sebastian gathered several Portuguese (L. Saldanha, from Lisboa) and Spanish (V Ramos from Alicante; F.X Niell and J.D Ros, Barcelona) biologists to held the first Symposium on Marine Benthos. Forty years later, the world has changed a lot and today we are at the 20th edition of what is now called the Simposio Iberico en Estudios en Biología Mariña.

In this presentation I will provide some brief comments comparing the research carried out since then. The 1st edition of the Symposia took place when Climate Change have been "detected" in the local studies in Southern Europe. I'd like to remind the name of Edouard Fischer Piette, that at 1945, started a yearly pilgrimage from Brittany to Gibraltar. He reported a continuous changing in the distribution frontiers of the big European seaweeds and intertidal mollusks; the biologists of that time called it "mediterraneization". Nowadays, we know that there was a marked rise in temperature from 1966 to 1969. At the end of the sixties, under the initiative of IGBP, it was suggested to encourage associative research mainly focused in the standardization of methods and procedures to study the benthos, inspired in terrestrial ecology studies.

The logistics problems related to sampling in environmental studies were of more quantitative nature in pelagic that in benthic systems. The aims of the IGBP was to replace the current collections by strict and reproducible sampling procedures and the adoption of standardized routines in data analysis. A topic of paramount interest was, following IGBP recommendation, the measurement and the comparison of ocean production, in order to evaluate the sustainability of oceans in face of a progressive general water eutrophication. The comparison between a "message" and a list of species leads to the heavy controversial concept of diversity until 1975, when this concept dyes after a Hulburt's study in which the diversity was qualified as non-concept. The meaning of diversity is no more than a specific quantitative composition index, with no strict relationships between structure and function of ecosystems and values of diversity. The refoundation of the concept of biodiversity leads to R. May, who wants to force the interest of politicians for species extinction, but without adding something new to the previously established species richness concept.

Modeling was scarce and it was still limited because of the lack of adequate computers resources. Clustering tried to replace the primitive sociobiology of communities, based upon the Zurich-Montpellier school developed in the South of France by Peres and Picard and was used in the description of Mediterranean communities; the Atlantic phycologist usually preferred the concept of zonation to classify the assemblages of plants and animals in the benthos.

Biogeochemistry and marine Microbiology as well as Ecophysiology had scarce number of studies, with dynamical focusing. These two disciplines made still a description too much static clearly lacking the basic measurements, among others, of the turnover of processes. The experimental studies with factorial design has been few frequent, as well as the use of appropriate statistics to the test of new and "ad hoc" hypothesis. Manipulation experiments *in situ* and in combination with laboratory and field experiments were imposed since the 90's. The most amazing addition to the studies in marine biology has been the hybridization of genetics and ecology. The genetic identity of organisms represented until now by the different enzymatic composition of allosteric enzymes was replaced by nucleic acid sequencing. All these technics gave fruitful results since 2012 as applied to Marine Biology.

Keywords: SIEBM, Ecology, marine Biology, benthic studies

Integrating knowledge among fishermen and scientists for better marine conservation and management: A running example in Southern Portugal (Algarve)

Ana Marçalo

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The participation and dynamic engagement of different stakeholders (co-management) at all stages of a management processes is an essential prerequisite for satisfactory results in the implementation of conservation actions (1). In fisheries and for marine resource management, decades of top-down expert based approaches have failed to achieve urgently needed conservation objectives. Works worldwide have shown that fishers are usually not happy with existing structures, but willing to cooperate towards elaborating better sustainable solutions if they are approached in a respectful manner and language is not too technical. In this manner, trust bonds created between scientists and fishers to share their knowledge have created a vital engagement for effectiveness. Thus, the promotion of more inclusive decision-making processes allowing several stakeholders, especially the primary (fishers) to participate in a down-top approach has been an important tool to generate “win-win” situations, in which social, economic and environmental needs will be simultaneously satisfied. Appropriate tools should be used to attain the best results and are area, fisheries and species specific. This presentation will overview projects coordinated by the University of Algarve and its Centre for Marine Studies (CCMAR) that used a participatory multi-actor approach using various tools in order to achieve the best results in specific subjects for better coastal fisheries and resource management. Focus will be given to an ongoing project that works towards solving competition between cetaceans and coastal fisheries. Increasing interactions between fisheries and marine protected species (e.g. cetaceans) are a reality and occur worldwide in many forms (2). Working towards minimizing cetacean–fishery interactions requires the implementation of case-specific localized management strategies with the active participation of fishers. The negative aspects of these interactions for both fishermen (e.g. loss of catch through depredation or gear damage) and for the animals (e.g. incidental capture leading in most cases to death) are of most concern. In the Portuguese southern coast (Algarve), little is known and documented about these interactions. The only works available, indicate that several cetacean species interact with many coastal fisheries in the area, with the purse seine fishery best documented (3,4). Furthermore, work is still needed to evaluate other fisheries and work toward mitigation strategies. To better understand the level of interactions of the coastal fleet operating in the area, we performed harbor interviews ($n = 209$) in the most relevant ports in the Algarve coast ($n=11$). Analysis indicated that the gears of most concern were gill and trammel nets operated in the eastern side of the Algarve. Mitigation strategies are in progress for the detected areas and fisheries of most concern and fishing harbours have been selected accordingly. This work is made under the scope of the project iNOVPESCA that uses frequent exchange of knowledge and ideas between stakeholders involved (fishermen, scientists and governmental entities), through participatory meetings, workshops and harbour approaches.

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al. 2015. Cetacean occurrence, habitat preferences and potential for cetacean-fishery interactions in Iberian Atlantic waters: results from cooperative research involving local stakeholders. Aquatic Conservation: Marine and Freshwater Ecosystems, 25: 138–154.

Keywords: Participatory Management, Co-management and sustainability, Fisheries Management, mitigation, Cetacean-fisheries interactions

Population structure in marine organisms: from genetics to genomics

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Ecosystems survival relies on the capacity of species to cope with environmental change. Connectivity among populations is a key factor to ensure long-term survival of these ecosystems and the species within them. The establishment of networks of marine protected areas can allow preserving biodiversity at large scale. The evaluation of population genetic structure across taxa is fundamental for the effective design of these networks. Genetic differentiation differs among species according to their dispersal capabilities, reproductive season or behaviour traits, among others. Moreover, oceanographic discontinuities reducing gene flow in the study area, as well as environmental characteristics determining local adaptation, are drivers of population differentiation sometimes undetected. In marine species, several molecular markers have been successfully applied to identify patterns of genetic differentiation across geographic gradients and through time. However, the ability to identify population structure may depend on the marker diversity, the genome region being analysed, and the species population history. For the last decades, our group has analyzed invertebrate and vertebrate marine species, from habitat formers to highly migratory species, mostly with mitochondrial and microsatellite DNA markers. Overall, our research is showing the importance of historical and current processes on the spatial distribution of genetic diversity. In recent years, the application of high-throughput sequencing technologies in species without reference genome is allowing us to tackle new challenges in the assessment of marine biodiversity, conservation, adaptation, connectivity, and human impact, in both native and introduced marine species across different taxa. Genome-wide data at the individual level is offering us the opportunity to assess the effect of dispersal, selection and genetic drift on population structure. Understanding these evolutionary processes is fundamental to provide scientifically sound information for managing marine biodiversity, at large as well as at fine scales. Examples in native and introduced marine species will be discussed.

Acknowledgements

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Keywords: Population genetics, adaptation, connectivity, Genomics, Marine species

The importance of amphipods: from taxonomy to applications

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During the last years the annual rate of newly described amphipod species has significantly increased, including not only taxonomic information (both morphological and molecular) but also ecological and behavioural data. The active participation of amphipodologists in WoRMS is also remarkable. Important advances using amphipods as models in proteogenomic, transcriptomic and mitogenomic studies have been achieved throughout the last decade, and detailed studies dealing with invasive species, life histories, behaviour and trophic ecology have also been conducted. The progress in the use of amphipods as bioindicators and as resource in aquaculture is especially interesting. They have been used successfully in environmental programs for monitoring TBTs, heavy metals and organic pollution. Their high nutritional content (fatty acids omega 3 and proteins) makes them suitable as food for several species of commercial interest. Taking into account that they are mainly detritivorous, they have been proposed as elements of Integrate Multitrophic Aquaculture systems. Amphipods have also attracted the attention of different media during the last few years, and two amphipods, *Liropus minusculus* and *Epimeria quasimodo* have been among the top ten new species, in 2014 and 2018 respectively, selected from a total of ca. 15,000 species described annually. Despite the increasing interest in amphipod research, there is still a long way to go to understand and convey the importance of this group.

Keywords: Amphipoda, Crustacea, Taxonomy, ecology & behavior, Bioindicator, Aquaculture

Doing things better: marine legislation and recovery of ecosystems

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In recent years seas are facing really important challenges, such as climate change, biodiversity loss, increasing pressures, etc., and media are highlighting some problems which certainly require urgent solutions (plastics and microplastics, emerging pollutants, etc.). However, are they the most harmful problems at sea? We know that one of the most important and pervasive problems that our seas are facing is overfishing, which extends the impacts to many ecosystem components (foodwebs, benthic communities, fish, seabirds, mammals, etc.). Despite this, in the last decades new legislation in Europe (Water Framework Directive, Marine Strategy Framework Directive), as well as new policies (Common Fisheries Policy, Biodiversity Strategy, etc.) seem that have provided the necessary management resources to revert the situation, at some extent. Hence, trends in different seas and for different physico-chemical and biological elements are improving and the global status of European seas, although still requiring management actions, has started to be close of the good status. Here, I will present some examples of improvement of the environmental status in Europe, as well as the evaluation at the different European seas, using the Nested Environmental status Assessment Tool (NEAT). Also, I will highlight how the recovery of marine systems is able to provide new and more ecosystem services for human wellbeing.

Keywords: MSFD, assessment, recovery of ecosystems, Monitoring, Europe

Opportunities, threats and challenges of deep-ocean conservation

Marina R. Cunha

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The deep ocean is currently one of the most exciting frontiers for science, technology and economic activities. Deep-ocean ecosystems are likely to be biologically fragile, relevant to carbon cycling and susceptible to catastrophic events and global change. Over the past century advances in technology have met some of the challenges of the deep allowing exciting new discoveries and prospects to pursue novel research themes (Rogers et al., 2015). Such advances also permitted unique opportunities for economic activities and industrial exploitation of the deep ocean biological and abiotic Resources (Wenhai et al, 2019). Alongside, increasing human population in tandem with climate change became the drivers of unprecedented levels of anthropogenic and natural pressures that call for urgent governance and management actions (e.g. Armstrong et al., 2019; Ramalho et al., 2018; Stratmann et al., 2018; Almeida et al., 2017). Nonetheless, a huge gap in the fundamental knowledge on the biodiversity and functioning of deep ocean ecosystems still persists, hindering not only an effective assessment of their economic value but also the implementation of science-based policy and governance models (Folkersen et al., 2019). Which is then the way forward? A crucial scientific aspect is a better knowledge of ecological processes, such as connectivity, paramount for the renewal of biological populations and their resilience to disturbance (Balba and Metaxas, 2019). Also critical, is a wider coverage and higher resolution of the ocean observations, both at spatial and temporal scales (Levin et al., 2019). We advocate that advanced technology, collaborative networks of infrastructures for deep ocean observations and cross-disciplinary research are needed to address emergent societal issues and scientific challenges, and harmonise a responsible Blue Economy with the conservation of the deep ocean.

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Physical-biological interactions in the ocean: case studies from the northern Canary Current Upwelling Ecosystem

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The Canary Current Upwelling Ecosystem is one of the four major upwelling regions of the Eastern Boundary Currents (EBC) of the global ocean (Fig. 1). These EBC systems are highly productivity and host large population of small pelagic fish (e.g., sardines and anchovies; SPF), which sustain important fisheries (e.g., the “anchoveta” fishery off Peru is the world’s largest). The collapses of these SPF fisheries have enormous negative economic and social effects on the fishing nations that border these EBC (Aristegui et al., 2009; Checkley et al., 2009). In order to avoid the negative effects (e.g., food availability and settlement conditions) of Ekman offshore transport due to coastal upwelling the marine organisms that inhabit these regions developed mechanisms to assure inshore transport and/or larval retention. In this review several examples of these mechanisms will be presented from phytoplankton to fish. Relatively high offshore Chlorophyll-a concentrations were detected during a winter (February 2000) upwelling event off NW Iberia (Fig. 2). The response of the surface waters to this event was strongly affected by two distinct local features observed during wintertime in the region: the Western Iberia Buoyant Plume (WIBP) and the Iberian Poleward Current (IPC). The WIBP provided conditions for the development of a shallow Ekman layer nearly coincident with the stratified upper meters (Ribeiro et al., 2005). This surface stratification was an important factor in assuring the stability needed for phytoplankton growth, as well as in establishing a vertical retention mechanism for fish larvae and a suitable environment (food availability) for their survival. The observed transport, induced by the joint effect of wind-driven dynamics and the IPC, comprised a westward advection and stretching of the plume, with little entrainment with the offshore deep mixed layer waters, and a northward displacement of sardine larvae (Fig. 3) (Santos et al., 2004). In conclusion, the IPC and the WIBP introduce important fluctuations in the transport patterns of the region, these conditions provided retention and convergence mechanisms that influenced the distribution of phyto- (Ribeiro et al., 2005) and ichthyoplankton (Garrido et al., 2009) in the area and modulate the impact of winter upwelling events in the survival of larval fish (Fig. 4) (Chicharo et al., 2003; Santos et al., 2004; 2006). In the Portuguese continental shelf decapod larvae are retained on the continental shelf along 3 meridional bands parallel to the coast (Fig. 5), independent of larval phase duration or taxonomic group but closely related to their parental populations: inner shelf species larvae are distributed close to the shore (maximum of larval abundances along the 30 m isobath), shelf species are distributed along the continental shelf (maximum of larval abundances in middle shelf) and finally slope species are distributed close to the shelf break (along the 200 m isobath). This distribution pattern (i.e. in bands) results from the relationship between larval behaviour (e.g., vertical migration) and local oceanographic processes (dos Santos et al., 2008; Peliz et al., 2007). Ontogenetic vertical migration behaviour is evident for almost all the taxa, in which the older stages (last zoeal stages, decapodids and megalopae) were usually found in deeper strata of the water column (Fig. 6) (Bartilotti et al., 2014). *Upogebia pusilla* and *U. deltaura* are two common congeners ghost shrimps species occurring in European estuarine and shelf areas, respectively. Their planktonic larval phase lasts around 3 weeks and consists of 4 zoeal stages and a decapodid that must settle in the benthos before recruiting to adult populations. Inhabiting different habitats of the same geographic area and exposed to similar oceanographic conditions these species developed different dispersal/retention mechanisms (e.g., emission points, vertical distribution and ocean circulation) to return to the estuaries or for the suitable settlement substrates where adult populations occur (Fig. 7). These species are good models for other coastal invertebrates that reproduce in summer and have short larval development (Pires et al., 2013). Cephalopod planktonic paralarvae of the neritic species occur during a considerably extended period of the year with two or three abundance peaks within the highly productive upwelling system of the western Portuguese coast and contrasting with the Gulf of Cadiz area. There is an apparent relationship of loliginid and *Octopus vulgaris* paralarvae with upwelling dynamics. The eventual advection from the shelf during upwelling events is probably prevented by their diel migration behaviour (Fig. 8). The summer inshore counter-current is suggested to advect *O. vulgaris* paralarvae from

the warm summer spawning grounds on the northern Gulf of Cadiz to the western upwelled waters around Cape S. Vicente. *O. vulgaris* paralarvae are located on the outer-shelf during the upwelling season and near the shore during autumn, possibly following the Ekman dynamics of cross-shelf transport (Moreno et al., 2009). Satellite-derived sea-surface temperature (SST) data revealed a sudden change in the intensity of the coastal outcrop regime in the Canary Current Upwelling System between the 1980s and 1990s, contrasting with the quasi-decadal oscillations of the SST anomaly in the open ocean over the same period. The outcrop indices and the SST gradient showed that this sudden change occurred earlier (~ 1992) in the northern part of the EACC (off the Iberian Peninsula) and a few years later (~ 1995) off the coast of NW Africa. Changes in the productivity of several small pelagic fish species observed for the same period suggest that there was a response of the ecosystem to these changes (Fig. 9) (Santos et al., 2005). The European sardine (*Sardina pilchardus*) is the most important small pelagic fishery of the Western Iberia Upwelling Ecosystem (WIUE). Recently, recruitment of this species has declined and this can be, at least partially, explained by changing environmental conditions. Simulation studies using a Regional Ocean Modeling System climatology (1989–2008) coupled to the Lagrangian transport model, Ichthyop, showed that there is a weak, continuous alongshore transport between release areas, though a large proportion of simulated ichthyoplankton transport north to the Cantabrian coast (up to 27%). We also show low level transport into Morocco (up to 1%) and the Mediterranean (up to 8%) (Fig. 10). The high proportion of local retention and low but consistent alongshore transport supports the idea of a series of metapopulations along this coast and to explain how these small pelagic fish have adapted their reproductive strategies in a coastal upwelling system to ensure coastal retention and recruitment success (Santos et al., 2018). The optimal temperature for larval sardine development varies between 13 and 17 °C and survival outside these boundaries is reduced, particularly during the first weeks of life (Garrido et al., 2016). At the same time, larvae depend of large food concentrations to be able to survive (Caldeira et al., 2014). Thus, low larval survival linked with sub-optimal temperature and low food availability may result in low recruitment strength. In general, high recruitment years are associated with high food availability (Chla) and low temperature (SST), but this is recruitment area-specific. High recruitment years are mostly related to high food availability, particularly during the last quarter of the previous year. In Western Iberia and in the Gulf of Cadiz, high recruitment years were also associated to lower SST, whereas in the Bay of Biscay, where SST during the winter was generally below the optimal range ≈11–12 °C for sardine larval development, higher recruitment is associated with high SST (Garrido et al., 2017).

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Keywords: Coastal upwelling, Phyto- and zooplankton, Cephalopod paralarvae, ichthyoplankton, Sardine (*Sardina pilchardus*), small pelagic fish, transport, retention, Dispersal, Connectivity (B)

From microbes to mammals: comprehensive analysis of biodiversity using DNA metabarcoding

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Genomic technologies—especially high throughput sequencing (HTS) platforms—provide the capacity to generate vast amounts of genetic data from all types of specimens. DNA metabarcoding utilizes this capacity to derive biodiversity data from the marker genes (e.g. DNA barcodes) embedded in the organisms residing in a bulk environmental sample—from water to soil to sediments. The ability to identify all organisms, from microbes to mammals, from a simple water sample is transformative for scientific and socioeconomic applications. This is reflected in the rapid rise of publications using metabarcoding for biodiversity analysis. These investigations have mainly focused on: 1) a small subset of biodiversity (e.g. invasive species, endangered species); 2) specific assemblages (e.g. ecological bioindicator species); or 3) whole communities (e.g. habitat assessment, beta diversity analysis). These objectives are generally achievable by utilizing small/medium DNA sequencing platforms. However, recently introduced ultra-high-throughput sequencers are capable of further advancing this field by providing comprehensive coverage of all species present in a given sample from a single test. In the face of a global biodiversity crisis, the next phase of ecosystem investigations will need to involve the standardized large-scale application of DNA metabarcoding technologies to generate comprehensive, near real-time biodiversity information.

Keywords: Genomics, Biodiversity, metabarcoding, environmental DNA (eDNA), Biomonitoring, Environmental assessment



Oral presentations

Adapting fisheries management to the spatial variability in the reproductive cycle: the case of the sword razor clam in Galicia (NW Spain)

Ana Tubío Gómez, Tania Ballesteros Otero, Alba Hernández, Rosana Rodríguez, Mariana Herrera, Roberto Gómez, Enrique Poza, Damián Costas, Gonzalo Macho and Elsa Vazquez

The knowledge of the spatial variability of the population dynamics of benthic organisms is essential for sustainable fisheries management, not only from a biological point of view, but also with a socioeconomic focus. More and more fisheries are intended to be managed with a social-ecological systems approach, but this approach requires a large amount of information. Fishers in Galicia point out two problems regarding sword razor clam fisheries management; 1) the two-month reproductive closure causes local market shortages and the importation of foreign razor clams, 2) excessive discards of broken razor clam during some months (foot breakage when harvesting fully mature individuals). Both problems are related and remain unsolved. Razor clams' stocks, like many other sedentary organisms, are highly spatially structured, forming meta-populations. By inhabiting spatially heterogeneous environments razor clams are patchily distributed within populations, which generates a spatial variability in their population dynamics (e.g. reproduction), even between very near areas (e.g. Robinson and Richardson, 1998; Rabaoui et al., 2007). Thus, studies of the reproductive cycle need to take into account the spatial variability of this resource in order to adapt the fisheries management, with the intention to reduce the problems highlighted before. The sword razor clam *Ensis magnus* is the most important commercial species of the razor clams in Spain and one of the most important shellfisheries in Galicia (NW Spain), being the Ría de Vigo the second most productive area of Galicia (landings accounted for 130t and ~1.5 million €; 23% of the landings in 2018). The reproductive cycle of *E. magnus* in the Ría de Pontevedra (Galicia) revealed spatial differences in the reproductive pattern caused by variations in environmental conditions (Hernández-Otero et al., 2014). And, although this species has already been studied in the Ría de Vigo (Darriba, 2001), the variability in reproduction at a mesoscale level in the ria is still unknown. Consequently, the aim of the study is to describe the reproductive cycle of *E. magnus* in several beds of the Ría de Vigo and its relation with environmental factors, in order to adapt the fisheries management to the biological spatial variability. From January to December 2018, reproductive cycle of *E. magnus* was studied in five exploited beds of the Ría de Vigo using histological methods (following the scale proposed by Hernández-Otero et al., 2014). These five beds were chosen taking into consideration their location in the ria: two in the inner part, two in the middle part, and one in the outermost part of the ria. Samples were taken monthly, during the sexual rest (July-September) and fortnightly, during the sexual period. Environmental variables (temperature, salinity and chlorophyll a concentration) were downloaded from the INTECMAR (Technological Institute for the Control of the Marine Environment of Galicia, Xunta de Galicia; <http://www.intecmar.gal>). Results show a reproductive cycle of *E. magnus* in the Ría de Vigo characterized by a sexual rest stage during summer and early autumn, a start of gametogenesis in autumn and a period of successive spawns interspersed with gonadal restoration during winter and spring, as it was found before in this ria (Darriba, 2001) and in the Ría de Pontevedra (Hernández-Otero et al., 2014). This reproductive cycle is similar to the one previously described in the intertidal in the Ría de Vigo (Darriba, 2001), and in the subtidal beds of the Ría de Pontevedra (Hernández-Otero et al., 2014). The relation with environmental conditions was found to be similar to the results reported by Darriba (2005) and Hernández-Otero et al. (2014). During summer, when temperature is higher and the water is rich in nutrients, *E. magnus* accumulates reserves and stay at sexual rest. In autumn, when temperature and nutrients decrease, gametogenesis started at the expense of the reserves stored. These results may be used for managing the fishery, adapting the rotational harvesting plan and the closure period to the spatial variability found in the gametogenic stage of the razor clams within the ria. This approach would avoid harvesting in fully mature beds, reducing therefore the discards due to the break of razor clams when its gonad is fully mature, and reducing as well the closure period, without reducing the reproductive output of the population. Nevertheless, reproductive timing varies from year to year due to environmental conditions, and moreover, monitoring the reproductive status of each fishing bed is very time (and money) consuming. To tackle this, easy and cheap data collection programs should be implemented in the fishery under an adaptive system that allows quick decisions to be collectively taken between stakeholders.

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Keywords: sword razor clam, *Ensis magnus*, reproductive cycle, Fisheries Management, Galicia

Assessing the overlap of NAFO fisheries with Vulnerable Marine Ecosystems

Maria del Mar Sacau Cuadrado, Pablo Durán Muñoz, Francisco Baldó Martínez and Corinna Favaro

In 2006, the United Nations General Assembly (UNGA) adopted the Sustainable Fisheries Resolution 61/105, calling on states and Regional Fisheries Management Organizations (RFMOs) to take action to protect Vulnerable Marine Ecosystems (VMEs) in the high seas. Over the last decade and in response to UNGA Resolutions, the Northwest Atlantic Fisheries Organization (NAFO) has spent a significant international effort to delineate and protect cold water corals and sponges. Fourteen areas around the high-seas portion of Grand Bank and Flemish Cap were implemented in order to protect deep-sea coral and sponge habitats from the impacts of bottom contacting gears. The protection provided by these closures has been underpinned by the identification and delineation of VMEs, and the mapping of fishing effort in order to assess the potential impacts of fishing on those vulnerable habitats. To understand the extent of fishing activities overlapping with VMEs taxa polygons we have carried out a characterization of distribution and intensity of fishing effort during a three-year period: 2016-2018. This characterization was done on the basis of two data sources: Haul by haul logbook information and Vessel Monitoring System (VMS) data. Through this analysis, fishing footprint layers were created for fisheries-specific and cumulative fishing effort using VMS data and haul-by-haul catch data (logbook). Afterwards, we conducted an overlay analysis to estimate the area of VME polygons that were overlapped by the cumulative fishing footprint and fisheries-specific footprints. The results indicate that logbook data and VMS are complementary and the coupling of both datasets is a powerful methodology for describing the spatial distribution of impact on the VMEs at a much finer resolution.

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Keywords: Vessel Monitoring System (VMS), Vulnerable Marine Ecosystems (VMEs), logbook, NAFO, Fishing effort

Space-temporary variation of the population of long-finned pilot whales (*Globicephala melas*) in the SE of the Iberian peninsula

Rosa M. Canales-Cáceres, Francisco Gomariz-Castillo, Francisco Alonso, Isabel Abel and Francisca Giménez-Casalduero

The long-finned pilot whale (*Globicephala melas*) is a delphinid that frequently inhabits temperate and cold waters. It has a preference for deep and sloped areas. In the Mediterranean the population is catalogued as “insufficiently known” by the Spanish Red Book of the Vertebrates and as “Data deficient” by the IUCN. In the SE of the Iberian Peninsula, there is hardly any published work on the evolution of the populations of these cetaceans. The coastline of the Gulf of Mazarrón is a privileged area for whale watching. Specifically, Mazarrón Bay, having a very narrow continental platform and submarine canyons that reach depths of approximately 2500 metres, very close to the coast, favours the appearance of processes and upwelling and therefore the proliferation of plankton, fish, cephalopods, marine birds, turtles and cetaceans. In these waters 7 species of cetaceans can be sighted in a more or less habitual way, being the pilot whale one of them. This work aims to provide data on changes that have been observed in the sightings of these animals by means of opportunistic data taken on tourism boats for watching cetaceans in the area around the Gulf of Mazarrón during the period 2004-2014. Between the years 2004 and 2014 a total of 1042 trips have been made in a whale watching boat in different months of the year. On each day, an experienced watcher remains at the boat’s surveillance post, keeping a 180° horizon watch with 7x50 mm binoculars. After locating the animals, data were taken on the position of the groups sighted, data on group structure, behaviour, number of individuals, interactions with other species and meteorological information, as well as photographs of the individuals. These sighting records are transferred to an spreadsheet for further processing. The sightings are standardized with the number of trips in order to make comparisons in different seasons and years. The data of presence in the different years, the seasonality and the composition of the groups are analysed. A distribution model has also been carried out for this species. To enlarge the data set of the model, information from online databases has also been added: The Global Biodiversity Information Facility (GBIF), The European Marine Observation and Data Network (EMODnet) and The Ocean Biogeographic Information System (OBIS). The random forest algorithm has been used using 20 predictors that include variables of distance to the coast, geomorphometric variables of the seabed estimated from bathymetries and variables of the marine surface obtained from satellite information. The spatial information was stored in GIS format and is processed with GRASS. Most of the derived layers used as predictors were obtained with GRASS or SAGA. The R randomForest library was used to obtain the interpolation and presence-absence models. A total of 1836 sightings were recorded, of which 437 corresponded to pilot whale, making this species the second most sighted (the first is the striped dolphin, *Stenella coeruleoalba*, with 872 sightings). They make up almost 24% of the total number of encounters. The results show that the pilot whale is a cetacean that can be observed in the Gulf of Mazarrón with relative frequency, since it seems to show a certain fidelity to the appropriate area for its feeding. After studying the presence data, it seems that in the zone they have a marked seasonal tendency, preferring the months of warm waters, where they form more numerous groups. In the presence model the distance to the coast was the most relevant variable, showing a peak of presence around 10 km of coast. The flatness variable seems to indicate a preference for abrupt bottom zones.

Keywords: Long-finned pilot whales, distribution model, gulf of Mazarrón, Whale watching, Temporary changes

Spatial and temporal characterization (2006-2012) of the trawling activity in the Site of Community Importance “Volcanes de fango del golfo de Cádiz” (southwestern Iberian Peninsula).

Emilio González-García, Ángel Mateo-Ramírez, Javier Urra Recuero, Gerardo Bruque and José Rueda

Human activity is causing different environmental impacts that accelerate changes in the marine environment both in the species of commercial interest and in the habitats where they occur, promoting a loss of biological diversity (Rice et al., 2016). Fishing, mainly bottom trawling, is one of the activities with the highest negative impact on marine ecosystems. Currently, the use of combined information from logbooks and Vessel Monitoring Systems (VMS) is a booming technique to study the spatial distribution of the fishing activity. This technique allows detailed spatial and temporal analyses of the fishing activity in specific areas (Hintzen et al., 2012). In the present study we analyze the spatio-temporal evolution of the fishing effort of the bottom trawling fleet as well as catches of the main species, both within the Site of Community Importance (SCI) of the “Volcanes de Fango del golfo de Cádiz” and in the external zone of the SCI (adjacent). The resulting data will be taken into account for the development of future SCI management plans in order to promote sustainable fisheries and habitat protection. The SCI is located in Spanish waters of the Gulf of Cadiz between 350 m depth and the upper slope 700 m, and the study focuss mainly in the Shallow Field of Fluid Expulsion (SFFE) of the SCI. Numerous trawlable grounds of important species occur in the SCI such as the Norway lobster (*Nephrops norvegicus*), the deep-water rose shrimp (*Parapenaeus longirostris*), European hake (*Merluccius merluccius*) and blue whiting (*Micromesistius poutassou*), among others (Ramos et al., 1996). Data from the logbooks and the VMS provided by “Secretaría General de Pesca” have been used for the period 2006-2012 for analyzing the evolution of the spatial distribution of the fishing effort and catches of the main species by the bottom trawling fleet. Regarding the VMS, this localization device sends a signal every 2 hours (ping) and provides data from the vessel itself, fishing status, date, time, position (latitude and longitude), course and speed. This information is previously processed in R software and analyzed using the VMStools package. This package calculates the time and average speed between successive signals and identifies the beginning and end of each fishing trip. The distribution of average speed frequencies was used to delimitate the speed range when vessels were trawling. Subsequently, depending on vessel and date, the data was crossed with the logbooks for analyzing catches of commercial species. The spatial distribution of the fishing effort as well as of the different catches made for the period 2006-2012 was estimated using a grid size of 1 km² in which the number of hours of trawling and the catch assigned to each ping was calculated. The data obtained were integrated into the ArcGIS desktop software for representing both the fishing effort and the average catches (kilograms) of important commercial species in each 1 km² cell. The spatial distribution of the trawling activity, both in hours and vessels, extends throughout sedimentary bottoms of the shelf and slope of the northwestern Gulf of Cádiz, with a greater pressure in the shelf throughout the years. High trawling activity areas are mostly located outside the SCI, however specific areas with high trawling were also detected in the SCI, mainly in the northern (around Anastasya mud volcano) and the central parts. The main targeting species in the SCI areas is the Norway lobster. In the deep zones of the SCI (> 700 m depth) as well as in hard bottoms and/or with strong near-bottom current speeds, trawling activity decreases considerably (Figure 1). An overall increase of the trawling activity was detected from 2009 to 2012, possibly due to the closure of the Moroccan fishing grounds and the reorganization of the trawling fleet. The fleet belonging to the Huelva province presented the greatest activity within the SCI, probably related with the technical characteristics of its fleet (e.g. large dimensions, powerful engines). Catches of the commercial species are generally higher outside the SCI. The main target species in the SCI is the Norway lobster with an upward trend throughout the years (3.7t in 2006 to 28t in 2011). The deep-water rose shrimp catches fluctuated between 9t in 2007 and 155t in 2009 and the European hake and monkfishes (*Lophius spp*) catches were stable during the period with average values of 13.5t and 3.8t respectively. The information on the spatial distribution patterns of the trawling activity and the catches of the main target species throughout the study period represent an important tool in the management measures of SCI. This is of importance for minimizing the impact of bottom trawling fleet and estimating the socio-economic impact of the closure of some areas to trawling. Figure 1. Spatial distribution, with a mesh size of 1 km², of the trawling activity effort (average annual fishing hours) for 2012 in the SCI and outside the SCI. No color cell indicates no trawling detected.

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Keywords: Fisheries, Gulf of Cádiz, mud volcanoes, SCI ESZZ12002, trawling activity

North Atlantic Oscillation affects dolphinfish as target and bycatch species in fisheries from the western Mediterranean Sea

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Dolphinfish (*Coryphaena hippurus*) in the western Mediterranean Sea support both commercial small-scale fishing and recreational fisheries. This species is also caught as a bycatch of drifting longline fisheries. The Western Mediterranean Sea is an important fishing ground where a Spanish drifting longline fleet operates targeting mainly swordfish *Xiphias gladius*, bluefin tuna *Thunnus thynnus* and albacore *T. alalunga*.

The North Atlantic Oscillation (NAO) reflects fluctuations in atmospheric pressure at sea-level between the Icelandic Low and the Azores High. The NAO is associated with many meteorological variations in the North Atlantic region, affecting wind speed and direction and differences in temperature and rainfall. The NAO is a dominant pattern of coupled ocean-climate variability in the North Atlantic and Mediterranean basin. Many authors have observed a relationship between the NAO and changes in fishery target species abundance and recruitment.

The main aim of the present study is to understand the effect of NAO on the capture per unit effort of dolphinfish (CPUE) in Spanish drifting longline from Western Mediterranean Sea. This is basic to improve the assessment and management of the Mediterranean dolphinfish stocks.

Catch and effort data for longline fisheries were collected in the framework of the Spanish Oceanographic Institute (IEO) on-board observer training Program, planned according to ICCAT recommendations. The IEO on-board observer Program provided commercial fish catch and bycatch data collected on-board longline vessels from 2000 to 2014. During the selected period, six metiers have been described: LLALB, Drifting surface longliners targeting albacore; LLHB, Traditional longliners targeting swordfish; LLAM, American longliners targeting swordfish; LLJAP, Drifting surface longliners targeting bluefin tuna; LLSP, Drifting semi-pelagic longliners targeting swordfish; and LLPB, Demersal longliners targeting swordfish.

Of the 6 longline gears (metiers) pointed above, the LLHB and LLALB are the longlines gears that captured more dolphinfishes. Thus, for the current series we observed a correlation in the CPUE of the dolphinfish ($r=0.88$; $P=0.049$). Thus due to the gaps per months observed in the series both LLALB and LLHB, and the high correlation between LLHB and LLALB CPUE of dolphinfish bycatch, we analyzed together both LLHB and LLALB gears.

Therefore we estimated the CPUE of the dolphinfish between 20 and 80 cm in length, for the period between September and December of the years 2000 to 2014 pooling together the LLHB and LLALB gears.

We use the NAO as a global climate index to describe the climatic conditions of the fishing area, theoretically affecting dolphinfish. Monthly values used of the NAO index were taken from the website of the USA National Oceanic and Atmospheric Administration:

http://www.cpc.noaa.gov/products/precip/CWlink/pna/nao_index.html, and <http://www.esrl.noaa.gov/psd/data/correlation/ao.data>.

The atmospheric oscillations present strong inter-annual and intra-annual variability. However, several studies have shown that changes in NAO trends have a delayed effect on aquatic ecosystems due to ecosystem inertia. For this reason, we used monthly gap, between one to six months.

To find the relationship between NAO index and the number of dolphinfish per each 1000 hooks (i.e. CPUE of dolphinfish), we performed a logistic model, a binary logistic stepwise forward/backward regression to test whether the probability of obtain a CPUE of dolphinfish in the longline was to higher than mean or lower, in function to the NAO as an independent variable.

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). 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 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 Figure 1 shows the spatial distribution of observed fishing effort of dolphinfish by-caught in drifting longline.

For the monthly longline CPUE, we obtained a statistically significant logistic model, with the variables NAO1 previous month (NAO1) and NAO6 previous months (NAO6). The model's goodness-of fit was significant according to the Omnibus test (Omnibus test= 10,532, $df=2$, $P<0.01$; Hosmer and Lemeshow test= 3.094, $df=7$, $P=0.876$), and its discrimination capacity was good (AUC = 0.8779).

The logit function from logistic regression was:

$$\text{logit} = -1.68 - 1.053 * \text{NAO1} + 1.019 * \text{NAO6}$$

Studies on the impact of climate change on exploited marine fishes are numerous. However, the impact of these effects is hard to predict due to the inherent uncertainty in these models, and the lack of knowledge on the biological response of the target species in short time periods. Thus, some authors have modelled the response of marine species to large-scale climate phenomena, such as the NAO or El Niño South Oscillation (ENSO). Thus, through the response to climatic oscillations, the resilience capacity of marine resources can be understood. In the case of the dolphinfish from the Mediterranean Sea, our results indicate a strong linkage between NAO and CPUE of dolphinfish in the Spanish longline fleet in the Western Mediterranean, which could relate to processes affecting dolphinfish abundance, availability and catchability to the fleet. The stronger relationship with the NAO index with 6 month gap could indicate a possible effect of the NAO on the recruitment of dolphinfish to the Spanish Mediterranean fishery. Dolphinfish are fast growing species, reaching the sizes ranges analyzed in this paper (20 to 80 cm) in last than 6 months. A positive NAO phase results in dry and stable periods on Mediterranean areas. These prevailing conditions could favour dolphin fish larval survival.

Keywords: Longline, Mediterranean, nao, bycatch, *Coryphaena hippurus*

Atlantic chub mackerel (*Scomber colias*) growth and reproduction off the Portuguese coast in relation to the population dynamics

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Atlantic chub mackerel (*Scomber colias*) is a middle-size pelagic species which mostly inhabits warm waters, distributed along the Eastern Atlantic coasts from South Africa to the Bay of Biscay, and occupying mainly the continental shelf and the upper slope depths. Population abundance and distribution in Portuguese waters significantly fluctuate over time, with maximum values observed in the middle of the last 3 decades. In this area, it is fished mainly by the purse seine and the artisanal multi-gear fleets. Landings have increased since the mid-2000's in Iberian waters, which represented in 2012 to 2017 1/4 to 1/3 of the landings in the Portuguese mainland (from 19000 ton. in 2017 to 45000 ton. in 2015). This rise is possibly correlated with the significantly decrease of sardine availability, the increase in abundance of chub mackerel, and a growing economical (canning industry, feeding of aquaculture species) and gastronomical interest on the species. However, landings are not regulated, the species being solely subjected to a minimum landing size and general technical restrictions, and despite the importance of this resource, no formal scientific assessment and advice are undertaken at present, with no guarantee that the exploitation rate is sustainable with the current catches estimates. Additionally, the dynamics of this species within the pelagic ecosystem in Atlantic Iberian waters are poorly known. Most chub mackerel caught in Iberia are young individuals (up to 2-3 years old), older fish being rarely observed in surveys and from the commercial fleet. Spawning, recruitment and nursery areas are not clearly identified, and the connectivity of individuals between adjacent Iberian areas and with the Moroccan waters is not known. The understanding of the reproductive dynamics and growth pattern of chub mackerel is relatively limited; there are no estimations of the species spawning biomass and/or reproductive potential, and age determination is uncertain due to the presence of false rings. In view of the above, the objective of this work was to contribute to building knowledge on the growth and on the reproductive biology of chub mackerel off Portuguese waters. During this study, chub mackerel samples were collected bi-monthly during 2012 in Peniche, and in 2014-2015 in Matosinhos harbours. Sagittal otoliths edge analysis (interpretation of whether the edge zone under formation is opaque or translucent) was performed in order to verify the existence of an annual growth pattern by examining the growing edge type of otoliths along time. The progression of diameter frequency was analysed in a subset of otoliths to identify different age groups. From the results obtained, the seasonal pattern of the otolith increments was described, growth taking place mainly during spring and summer months. Age was determined from counts of opaque (transmitted light) annual growth zones, the growth pattern was analysed, and the growth parameters were estimated for the chub mackerel off the West coast. Samples collected regularly in 2014-2016 from the commercial fleet in both Matosinhos and Sesimbra harbours and during the winter and spring acoustic surveys in 2014-2018, were used to investigate chub mackerel reproductive biology, by means of the analysis of individual macroscopical and microscopical information (from histological preparations of preserved gonads). The results obtained allowed to microscopically determine the size at first maturity of chub mackerel in the Western Atlantic Portuguese waters ($L_{50} = 20.57 \pm 0.52$ cm) and to describe the seasonal dynamics of the reproductive activity, key input to evaluate the spawning population (the fraction which is to contribute to the reproductive output), chub mackerel reproducing mostly during winter and spring months, though a very high proportion of inactive females were observed even at peak spawning off the West coast. Additionally, chub mackerel individual biological data collected during the Portuguese Winter and Spring Groundfish and Acoustic surveys for the period 2000-2018 was analysed to investigate possible geographical differences in reproductive activity and growth pattern. The above results were compared to and discussed in relation to the available similar information in adjacent geographical areas, with the view of attempting to understand the species dynamics in Atlantic Iberia, the final aim being to improve the scientific information for an accurate data-based assessment and management for this marine exploited resource.

Acknowledgements

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Keywords: Atlantic chub mackerel, reproductive activity, Growth pattern, Histology, otoliths analysis, Geographical differences

Univariate approach of blackspot seabream (*P. bogaraveo*) landings. Linear dependence and volatility.

Víctor S. Fernández and Juan Carlos G. Estrada

In the present study, the characterisation and modelling of the historical series of recorded blackspot seabream landings in the Strait of Gibraltar, carried out by the Spanish and Moroccan fleets from 1983 to 2016, has been carried out. With this objective, 2 different modelling methodologies were applied: ARIMA (integrated autoregressive moving average) and GARCH family (generalized autoregressive conditional heteroskedasticity). The ARIMA models (Box and Jenkins, 1976) explain the behavior of a temporal variable from their past observations, generating a linear dependence between the different observations of the variable. On the other hand, the GARCH model family (Bollerslev, 1986) models the changing variance or volatility of time series, indicating the absence of autocorrelation between their observations and at the same time the dependence of the variance in the past. The main difference between the different GARCH models lies in their definition of variance as an equation. The GARCH models selected for this study were: APARCH ("Asymmetric Power Arch Model") and FIGARCH ("Fractionally Integrated GARCH Model"). Different probability distributions were tested in the realization of the models, so that the one that presented the best values in the validation phase was chosen: normal distribution, skew normal distribution, generalized error distribution, skew generalized error distribution, student-t distribution, skew student-t distribution, normal inverse gaussian distribution, generalized hyperbolic distribution y Johnson's reparametrized SU distribution. For the implementation of the GARCH models, the variance of the series was first individually modelled, varying the parameters of the models (p,q) within the range [1-9]. Once all the combinations were made, the model chosen was the one that presented the least Akaike information criteria (AIC). Once the variance modelling was finished, an ARMA (1,1) was integrated to the best GARCH model in order to consider the effects of the mean, obtaining a resulting ARMA (1,1) + GARCH (p,q) model. The range of variation of the parameters (p q d, P Q D)s of the ARIMA models was [0-6]. All models were developed with rstudio. The best model will be one that presents a high level of variance (R^2 , ARV, E^2) and persistence (PI) and a low absolute error value (RMSE, MAE), relative (%SEP), Akaike (AIC and a modified of AIC, AICc) and Bayesian (BIC) in the validation phase. From 1983 to 2016, the blackspot seabream catches showed a negative trend and a marked seasonality, which were characterized by two cycles with a maximum in June 1997 (144.6 t, historical maximum) and in June 1999 (138.9 t) and a minimum in March 1999 (8.2 t) and February 2004 (5.1 t). The trend in catches indicated a progressive decrease at the rate of -0.0011 t/year, reaching the historic low of 1.2 t in March 2003 (Figure 1). In terms of seasonality, the months of April, May, June, July and August accounted for 53.80% of catches followed by 25.06% provided by October, November and December, being the most important months for the blackspot seabream landings. The ARMA (1,1) + APARCH (1,6) models with skew student-t distribution and ARMA (1,1) + FIGARCH (2,3) with normal distribution were the ones that presented the best values of the error sources in the validation phase (Figure 1). Explaining 41% of the variance, with a loss of information of 26%. The best ARIMA model was (103) (011)12 with 61 % variance explained and 24 % loss of information. Comparing both methodologies, we can observe that the ARIMA model presented better values of the validation measures than the ARMA models (1,1) combined with the GARCH family. The ARIMA models had greater variance explained, less loss of information and less time lag and therefore greater persistence (Table 1). The results show that volatility is not a determining factor in the evolution of the series. The average together with the seasonal factor explains 60 % of the behaviour of the series, whereas the ARMA+GARCH volatility would only explain 40 %. On the other hand, the good results obtained after the application of these univariate techniques could suggest a good characterization or almost complete approximation of blackspot seabream landings in the Strait of Gibraltar. ARIMA models have been able to detect linear and seasonal effects and ARMA+GARCH models have indicated that the blackspot seabream fishery shows associated uncertainty. However, a univariate modelling is not able to incorporate the effect of other variables involved in the fishery (fishing power, climate), fact could introduce significant changes in established relationships and the accuracy of the model will be reduced. Therefore, in future studies, a multivariate approach (hybrid models: ARIMA + computational neural networks, GARCH + computational neural networks) could optimize the characterization of the series and the intensity of the linear and non-linear effects, making it possible to study the relationship between different species and variables (climatic, fleet and economic) of the same region. Figure 1: Monthly series of blackspot seabream landings

(t) during 1983-2016 with the fit provided by the best models. A) ARMA (1,1) + APARCH (1,6). B) ARMA (1,1) + FIGARCH (2,3) C). ARIMA (103) (011)12 Figure 2: Parameters and error measures of the best ARIMA (p,d,q) (P,D,Q)s and ARMA (1,1) + GARCH (p,q) models.

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Keywords: ARIMA - GARCH models, landings, Blackspot seabream fishery, Univariate approximation, Strait of Gibraltar region

Reproductive cycle of the sea cucumber *Holothuria forskali* in the Ría de Vigo: application to fisheries management

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The knowledge of the life cycle of marine resources is essential for a responsible fisheries management. However, the exploitation of the sea cucumber (*Holothuria* (*Panninogothuria*) *forskali* Delle Chiaje, 1823) in the Ría de Vigo (NW Spain) is recent (2015) and it has been done until now with scarce or no information about some key biological aspects as reproduction, recruitment and growth. To overcome these gaps, and to move towards a sustainable resource management, the study of the reproductive cycle and growth of *Holothuria forskali* is being studied in a bed of the Ría de Vigo. To describe the reproductive cycle, samples were taken fortnightly during 2018. Gonadal condition index (GCI, calculated as wet gutted weight/wet weight) and the gametogenic stages obtained by the classic histological methods were applied. A gonadal status was assigned to each individual according to a scale modified from TUWO & CONAND, 1992; RAMOFAFIA et al. 2000; NAVARRO et al. 2012; and SANTOS et al. 2016, which describes a new state of gonadal restoration, not described until now for this species to our knowledge. The reproductive cycle of *Holothuria forskali* in the Ría de Vigo is characterized by a sexual resting stage during spring, when temperature and daylight hours are lower; beginning of gametogenesis during summer, when temperature and daylight hours are higher and sea bottom is rich in nutrients; and a period of spawns interspersed with a possible gonadal restoration during autumn and winter, when temperature is lower and food is scarce. Sex ratio is 1:1 and the population studied is not synchronized, since females initiate maturation earlier than males. Comparison of the histological results with GCI indexes suggests that GCI is a good indicator for gonadal stage. The fisheries management strategies, as rotational scheme and/or the closure period must be adapted to the reproductive stage. Thus, we recommend to avoid harvesting between November and January in order to increase the spawning potential ratio and consequently the recruitment.

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Keywords: sea cucumber, *Holothuria forskali*, Fisheries, reproductive cycle, Management, Ría de Vigo

Influence of anthropogenic factors in the community of loggerhead sea turtles (*Caretta caretta*) in Portuguese continental waters

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Among marine megafauna, sea turtles were highly impacted by anthropogenic activities. The most significant threats include direct take, coastal development, pollution and pathogens, global warming and the impact of fisheries. Sea turtles are protected by various international protocols, including CITES, the Bonn Convention, The Bern Convention and the Habitats Directive. This work shed light onto various aspects of the ecology of loggerhead sea turtles (*Caretta caretta*) along the Portuguese coast, where information was previously very scarce. For this species, some aspects related to its distribution and cause of strandings, diet and major threats were evaluated in order to promote and contribute to their conservation in Portuguese continental waters.

With respect to the analysis of marine turtle strandings in continental Portugal, in the period 1978-2013, 57.1% of the recorded strandings corresponded to individuals of loggerhead turtle. Also, higher relative density values of loggerhead sea turtle strandings occur in the south coast of Portugal during spring and summer. The analysis of the causes of stranding and mortality revealed that interaction with fisheries was the main cause of stranding for this species. Regarding the feeding ecology of loggerhead sea turtles, we analyzed 95 digestive tracts of loggerhead turtles, stranded along the Portuguese continental coast, between 2010 and 2013. In the Portuguese continental coast, crustaceans were the main group of prey in the diet of loggerhead sea turtles, with the Henslow's swimming crab (*Polybius henslowii*) and hermit crabs (*Pagurus* spp.) being the most important species. Sea turtles have recently been proposed at European level as good environmental status indicators (GES) under the MSFD (2008/56/EC), particularly with respect to monitoring descriptor D10 relating to changes in abundance of marine debris. In this context, out of the 95 loggerhead sea turtles whose digestive tracts were analyzed, 56 (59.0%) had marine litter in their tracts. Of these, more than half (56.8%) had ingested plastic items. It was also possible to deepen other aspects related with ocean pollution, such as the levels of inorganic elements in various tissues of loggerhead turtles. This study analyzed 38 loggerhead sea turtles and the results showed high concentrations of cadmium in kidney tissue ($34.67 \pm 3.21 \mu\text{g/g}$), which may contribute to the deterioration of turtle health.

The present study, involving various aspects of the ecology of loggerhead sea turtles, is a pioneer in Portugal and provides an important basis for the development of future conservation efforts for this species in Portuguese continental waters. Overall, monitoring marine turtle strandings, the evolution of their diet, the presence of marine litter in their digestive tracts and their contamination levels may help to outline measures for their conservation within several European Directives frameworks, particularly in the context of Directive 2008/56/EC which establishes guidelines for marine environment policies in the European Union.

Keywords: *Caretta caretta*, strandings, fisheries interactions, Gut content analyses, Anthropogenic threats, marine litter, Inorganic Elements

Rock-pools as nurseries for co-existing limpets: dynamics of limpet nursery grounds in SW Portugal

Maria Inês Seabra, Teresa Cruz, Cristina I. Espírito Santo, João J. Castro and Stephen J. Hawkins

Limpets are key-organisms structuring rocky intertidal assemblages. Here we studied recruitment patterns of the patellids *Patella depressa* and *Patella ulyssiponensis*, and of the pulmonate striped-limpet *Siphonaria pectinata*, which are common inhabitants on Iberian rocky shores.

Rock-pools are presumably important nursery grounds for limpets, due to high sensitivity of their early-benthic life-stages to environmental variation present in open-rock habitats at emersed conditions. However, the relative importance of rock-pools as nurseries relatively to other habitats (namely other wet and patchy microhabitats) has been rarely assessed.

In Sines region (SW coast of Portugal), the shoreline has experienced major anthropogenic changes since the '70s due to the setting of the Port of Sines, a deep oceanic port with worldwide maritime importance. Inside the limits of the Port, man-made breakwaters mainly compose the rocky-intertidal environment, where rock-pools are mostly absent. A lower density of limpets inside this Port, compared with outside adjacent areas, was previously reported.

In this study, the following questions are addressed, separately for each of the three species: i) what is the relative importance of rock-pools as habitats for juvenile limpets comparatively to other intertidal habitats?; ii) what is the temporal (seasonal and inter-annual) variation of limpet recruitment?; iii) how does limpet recruitment vary spatially among one shore located within an oceanic port and two nearby shores located at increasing distances relatively to the port? Moreover, inter-specific correlative hypotheses on the patterns of limpet recruitment into rock-pools were tested, namely if recruitment of co-existing limpet species is correlated in time and in space.

Two field-surveys (August / Early September 2007 and February 2009) focused on the search for limpet juveniles (maximum shell length – MSL – of less than 10 mm for *Patella* spp. and less than 5 mm for *S. pectinata*) were carried out at three natural shores in the region of Sines, covering a wide range of intertidal habitats (rock-pools, open-rock and crevices at different tidal levels; North and South-oriented vertical walls; patches of mussels, *Lichina pygmaea* and *Fucus*).

Temporal variation in recruitment was examined in a series of mid-shore rock-pools present in the same three shores, which were limpet-cleared at three-monthly intervals from May 2005 to July 2008. Recruitment to three months was defined as the density of individuals measuring less than 5 mm in MSL (recruits) recorded at each sampling time in each limpet-cleared rock-pool. This size limit (MSL < 5mm) was established in common for all the three species, based on a preliminary analysis of size-frequency distribution of each species densities during the total period of study. Among-shore variation of recruitment to three-months into rock-pools was also assessed for each species (one natural shore located inside the Port, and the other two shores were outside the Port, 4 and 9 km away).

In general, juvenile densities of all species were significantly highest in rock-pool habitats, although the spatial pattern of distribution and abundance of juveniles across intertidal habitats was unique for each species.

Recruitment to three months of *Patella depressa* and *Patella ulyssiponensis* into rock-pools was consistently low during all summer periods of the whole study period. The density of recruits of these species was higher during other seasonal periods but there was inter-annual variability of the highest recruit density peaks. The maximum density of *Siphonaria pectinata* recruits (average of 102 individuals in 15 x15 cm²) was recorded for the summer of 2005. During the following years recruitment of *S. pectinata* was comparatively low, but consistently higher during summer or autumn comparatively with the other seasons.

Recruitment of *Patella depressa* varied significantly among all the three shores, being lowest in the shore inside the Port and highest in the farthest shore from the Port. Recruitment of *Patella ulyssiponensis* and *Siphonaria pectinata* was significantly higher in the farthest shore from the Port than in the other two shores.

Overall, Pearson correlation analyses showed a significant positive relationship between recruitment of *Patella depressa* and *Patella ulyssiponensis* along space and time, and that recruitment of *Siphonaria pectinata* was not correlated with any of the other two species.

Keywords: Rocky-shore ecology, recruitment, *Patella*, *Siphonaria*, Sympatry, Intertidal habitats

The stalked barnacle *Pollicipes pollicipes* fishery at Reserva Natural das Berlengas (Portugal): temporal variation of the harvesters perspective

Alina Sousa, David Jacinto, Nélia Penteado, Diana Pereira, Teresa Silva, João J. Castro, Sergio Leandro and Teresa Cruz

The stalked barnacle *Pollicipes pollicipes* is a cirriped crustacean that inhabits very exposed rocky shores. It can be considered the most important ecological resource on rocky shores of North Spain and continental Portugal and it is highly prized and heavily exploited. Berlengas archipelago is situated ~10Km from Cape Carvoeiro (Peniche, Portugal) and it is composed by three main groups of islands: Berlengas, Estelas and Farilhões. In 1981, Berlengas and Estelas were declared a Natural Reserve (RNB), that was reclassified in 1998 to include Farilhões. From 1989 to 2000, *P. pollicipes* harvesting was forbidden in RNB, but barnacles were exploited and its surveillance was considered to be ineffective [1]. In 2000, RNB was the first area in Portugal to be managed for this fishery by implementing a management plan that allowed professional fishers to harvest at RNB and that included spatial and temporal closures, a limited number of harvester's licenses, size and bag limits, and catch reports in logbooks (specific rules have changed in 2011). In 2011, RNB was declared by the Man and Biosphere Program (MAB) of UNESCO as a biosphere reserve. The main objective of the present study was to evaluate the perception of the licenced barnacle harvesters at RNB about: the state of *P. pollicipes*; the agreement and sense of fulfilment of the management plan in practice; and the harvester's concordance about the implementation of a co-management system to manage this resource at RNB. The temporal variation of the harvester's perception was evaluated by individual and direct interviews to professional barnacle harvesters that have licence to harvest at RNB. These interviews were performed in 2005 (n=38), 2013 (n=32) and 2018 (n=39). The state of *P. pollicipes* was evaluated by inquiring the fishers on their perception of change in the amount, size, quality and overexploitation in the 5 years prior to each interview. The percentage of harvesters that considered that there was a decrease in the amount of barnacles was 50% (2005), 66% (2013) and 59% (2018). The percentage of harvesters that considered that there was a decrease in the barnacle's size was 34% (2005), 63% (2013) and 56% (2018). Regarding the quality of the barnacles, the majority of the harvesters considered that there has not been a change in the quality of the barnacles in the 5 years prior to the interview (59% in 2019, and 69% in 2013 and 2018). The interviews also revealed an increasing percentage of harvesters that considered that *P. pollicipes* is overexploited at RNB (66% in 2013 and 78% in 2018; no data from 2005). Overall and considering the amount, size and overexploitation perception of the harvesters, these data revealed a negative tendency of the state of this resource. About the agreement with the management plan, several questions revealed an increasing concordance of the harvesters over the years about the regulation in practice, namely about the temporal closures, the bag and size limits, the catch reports in logbooks and the maximum limit of 40 licenses. However, the harvesters revealed an increasing discordance, overtime about the existence of no-take zones (44% in 2013, 72% in 2018). Regarding the sense of nonfulfilment of each management rule, a negative tendency for the temporal and spatial closures rules was observed (weekly closure- 51% (2005), 40% (2013), 79% (2018); monthly closures- 50% (2013), 91 % (2018), no data for 2005; no-take-zones-59% (2013), 92% (2018), no data for 2005). Finally, most of the harvesters agreed with the implementation of a co-management system to manage barnacle harvesting at RNB (81% in 2013 and 97% in 2018) and consider that this change could bring advantages to their activity.

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Keywords: Stalked barnacles, *Pollicipes pollicipes*, Reserva Natural das Berlengas, Management, fishery

Quantification of the foraging activity of *Patella caerulea* and *P. rustica* under simulated tides using photographic analysis of radula scratches

Juan Sempere-Valverde, Francisco Sedano, César Megina, José C. García-Gómez and Free Espinosa

Limpets are one of the most important herbivorous grazers of temperate rocky shores. Their feeding activity promotes diversity and influences the structure and functioning of intertidal communities. In the current context of global change, harvesting pressures on accessible intertidal and habitat modifications caused by coastal urbanization are threatening these organisms in many coastal areas. In this regard, the improvement of ex situ maintenance techniques, combined with the understanding of limpets' behavioural traits, would help to improve the effectiveness of conservation strategies and mitigation efforts. The behaviour ecology of limpets in the natural environment is well understood (i.e. *Patella caerulea* activity is higher during spring tides and when splashed by moderate waves while *P. rustica* is more active when splashed during high-wave episodes) and the laboratory microcosm should reflect what we know about limpets in their natural environment. However, there are many issues in limpet aquaculture (either for commercial or conservational purposes), due to their sensitivity and ecological requirements, that remain unknown in laboratory conditions. The aims of this study are to study the feeding activity of *P. rustica* and *P. caerulea* under simulated tides (spring and neap) and to describe a quick photographic method to quantify radulae marks, which would expedite samples treatment. Methodology *Patella caerulea* and *P. rustica* individuals of 2-3 cm length were obtained within a rip-rap structure in Algeciras, Spain (36°07'02.2"N, 5°26'07.6"W). After 10 days acclimation, limpets were placed in groups of five over 40 × 35 cm limestone plates (replicates) and introduced into the tanks with a 45° inclination for a period of 10 days (Fig. 1-A). For each species, four replicates were carried out under spring tides (24 cm tidal oscillations) and four under neap tides (8 cm tidal oscillations – Fig. 1-B). To record grazing activity, each replicate had 16 wax discs of 3 cm diameter (Fig. 1-C). After the experiment, grazed wax discs were primed with black water-based paint (Fig. 1-D). After it dried, discs were re-painted using the dry brush technique with a second white primer to enhance the contrast of radula marks (Fig. 1-E). Then, discs were photographed and the images' RGB layers, brightness and contrast were modified using Adobe® Photoshop to obtain monochromatic images representing radulae marks (Fig. 1-F). Marks that were not made by limpets were manually deleted from images. To obtain radula marks coverage, black area on images was measured using the "Analyze particles" tool of ImageJ® software. To erase small particles and pixels errors, a minimal particle size was set up and optimized for each image. A two-way ANOVA was carried out using radula marks coverage data to test orthogonal factors Species (fixed, two levels: *P. caerulea* and *P. rustica*) and Tide (fixed, two levels: Spring and Neap). IBM® SPSS® Statistics 22 software was used to perform these analyses. Results During the experimental phase, three home-scar marks were found and two different radula scratch patterns observed over wax surfaces (Fig. 1-G). The first pattern was thick and highly covered with marks and the second was scattered and sinuous. Overall, the grazed area as coverage mean was 2.24% (SD: ± 1.41) of wax discs surface, being higher for *P. caerulea* (3.41%; ± 1.65) than for *P. rustica* (1.07%; ± 0.87 - Table 1). Intra-specific differences in feeding behaviour were detected only for *P. caerulea*, which grazed more actively during Spring than Neap tides. On the contrary, *P. rustica* grazing behaviour was not influenced by tide amplitude. Discussion The radula patterns found over wax surfaces match those described for the displacement and intensive grazing phases of limpets' feeding excursions. In previous studies, *P. caerulea* showed circa-tidal rhythms under a scenario of simulated tides, which may reduce stress when compared with aquariums without tidal simulation. In this study, grazing activity of *P. caerulea* was also affected by tidal amplitude in a similar way as it occurs in natural areas. Therefore, tidal range should be taken into consideration when specimens of limpets are maintained in aquarium tanks. Regarding the methodology used, wax discs were originally processed by identifying the mark patterns and estimating their coverage using a binocular microscope. In this regard, the proposed digitalization of the processing method would simplify the obtention of mark coverage, a heavy task avoided in subsequent studies after the original method proposed in 1997.

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Keywords: *Patella*, Aquariums, artificial tides, photographic method, Grazing activity

Drivers of interannual abundance changes in gelatinous carnivore zooplankton in the Iberian Peninsula, (Portugal)

Sónia C. Marques, Maria Elena D'Ambrosio, Ana L. Primo, Juan-Carlos Molinero and Miguel A. Pardal

The persistent massive blooms of gelatinous zooplankton recorded during recent decades may be indicative of marine ecosystem changes. In this study, we investigated the potential influence of the North Atlantic climate (NAO) variability on decadal abundance changes of gelatinous carnivore zooplankton in the Mondego estuary, Portugal, over the period 2003-2013. During study period, the community of gelatinous carnivores comprise a larger diversity of hydromedusae than siphonophores; the former dominated by *Obelia* spp., *Lizizia blondina*, *Clythia hemisphaerica*, *Liriope tetraphylla* and *Solmaris corona*, while the latter dominated by *Muggiaea atlantica*. Gelatinous carnivore zooplankton displayed marked interannual variability and mounting species richness over the period examined. Their pattern of abundance shifted towards larger abundances ca. 2007 and significant phenological changes. The latter included a shift in the mean annual pattern (from unimodal to bimodal peak, prior and after 2007 respectively) and an earlier timing of the first annual peak concurrent with enhanced temperatures. These changes were concurrent with the climate-driven environmental variability mainly controlled by the NAO, which displayed larger variance after 2007 along with an enhanced upwelling activity. Structural equation modelling allowed depicting cascading effects derived from the NAO influence on regional climate and upwelling variability further shaping water temperature. Such cascading effect percolated the structure and dynamics of the community of gelatinous carnivore zooplankton in the estuarine system (Fig. 1).

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Keywords: jellyfish, nao, North Atlantic climate, Mondego Estuary, upwelling

Microplastics: the invisible threat

Alicia Herrera, Ico Martínez, Jorge Rapp, Eugenio Raymond and May Gómez

Plastic pollution is affecting marine ecosystems on a global scale. Microplastic particles are of particular concern as, due to their size, they can enter marine food webs. The damage that microplastics can cause, not only physical, due to ingestion, but also the effects of the associated chemical pollutants in marine life, is still unknown. Within the MICROTROPIC project we evaluated the levels of microplastics and associated chemical pollutants on beaches, sea surface and fish in the Canary Islands. In the north and northeast oriented beaches, maximum abundances of up to 244 gr/m² were found in the tidal line. On the sea surface, the maximum values were found at Las Canteras Beach, a semi-enclosed bay, with concentrations of more than one million particles/Km². On the other hand, in 78% of the fish studied, microplastics were found in the gastrointestinal content, mainly fibres. Finally, chemical contaminants associated with microplastics were analyzed on 4 beaches with different levels of anthropogenic pressure. High levels of persistent organic pollutants (POPs) were found, among them DDT, Polycyclic Aromatic Hydrocarbons (PAH), and emerging contaminants such as those derived from UV filters. The sum of DDT and its metabolites was significantly higher in Gran Canaria, the most inhabited and industrialized island, with maximum values of 13.488 ng/g. On the other hand, UV filter levels were higher in Las Canteras and Famara, the beaches with higher touristic pressure, with maximum values of 3.740 ng/g. The values, both the concentration of microplastics and POPs, are similar to those found in the most polluted sites on the planet. The next step in our research is to determine, in controlled laboratory

experiments, the possible effects of these contaminant levels on the physiology of fish, jellyfish and crustaceans.

Keywords: Microplastic (MP), POPs (Persistent Organic Pollutants), Plastic pollution, marine pollution, Marine life, Plastic wastes

Inferred family structure of an endangered species, *Pinna nobilis*, using molecular analyses: implications of connectivity for conservation

Elisabet Nebot, Maite Vázquez, Emilie Boissin, Claire Peyran, Salud Deudero and Serge Planes

PROBLEM STATEMENT Coastal Mediterranean marine biodiversity is under threat due to several anthropogenic impacts. Marine protected areas (MPAs) are an essential tool to counteract these impacts by preserving biodiversity, natural habitats and maintaining genetic diversity. Knowledge of the genetic structure and connectivity patterns of keystone and vulnerable species is essential, since it helps on the preservation of the structure and function of ecosystems (Daily et al., 2000; Libralato et al., 2010). Here we focus on the genetic structure and connectivity patterns of the emblematic and endemic bivalve *Pinna nobilis* (Linnaeus, 1758) in a MPA from the Western Mediterranean. Recently, in autumn of 2016, the occurrence of a new parasite species (*Haplosporidium pinnae*, Catanese et al., 2018), is producing a mass mortality event (MME) in the whole Mediterranean Sea. As a result, *Pinna nobilis* has been declared as critically endangered species in Spain. Given its current conservation status, and the low knowledge of their genetic structure and connectivity at a local level, there is an urgent need to know more about these topics, in order to be able to proposed proper conservation measures for its recovery. **METHODOLOGY** The study was carried out in the Marine National Park of Cabrera, situated in the south of Mallorca. A total of 771 *Pinna nobilis* individuals were sampled from nine different sites between 2011 and 2014 (Vázquez-Luis et al., 2014), representing a low percentage of the population inhabiting the MPA before the MME (Vázquez-Luis et al., 2017). Total genomic DNA was extracted for each individual and genotyped at 23 polymorphic microsatellite loci. Population genetic analysis and parentage analysis were performed. **RESULTS** *Pinna nobilis* individuals from the National Park of Cabrera were grouped into one single panmictic population. Of the 771 sampled individuals, almost half (45.01%) of them were related with other individuals. Estells de Dins (EDD) had the highest number of related individuals (63.16%), while Dimoni (DIM) showed the lowest number of related individuals (36.84%) (Fig. 1). In total, 562 relationships were detected, mostly occurring in Santa Maria (STM, 60.14%) and Puerto (PORT, 14.95%). Specifically, most of the relationships in Santa Maria were between individuals from Santa Maria (57.99%), Santa Maria - Puerto (16.57%) and Santa Maria - Olla (7.69%). While most of the relationships in Puerto were between individuals from Santa-Maria (66.67%) and between individuals from Puerto itself (9.52%). In total 126 families were found, composed from 2 to 8 individuals, 66.67% of the families were composed with 2 individuals, 12.70% were composed with 3 individuals and only 1 family was composed with 8 individuals (Fig. 2). The maximum number of different sampled sites per family were four. In addition, no parents-offspring relationships were detected. **MAJOR CONCLUSIONS** A high number of individuals related have been found despite the low percentage of sampled individuals, concurring with the lack of genetic structure at the scale of Cabrera and pointing to high levels of connectivity of all sampled sites in the National Park. The high percentage of families with 2 members could be due to the reproductive characteristics of the species (external fecundation), to the difficulty of sampling a high percentage of the population (due to the high densities existing previous to the MME), the existence of pelagic larvae (dispersal process); and to the high mortality rates of the first years. However, the existence of families with a total of 8 members living in up to four different sites points out the high connectivity across the National Park. Moreover, based on results obtained, Santa Maria seems to be a source site with high number of connections to the rest of the sites within the National Park, making it a key site for future restocking programmes. **FIGURE LEGENDS** Figure. 1. Study area and percentage of related individuals per site Figure. 2. Number of individuals per family

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Keywords: Pinnidae, Bivalve, parentage analysis, Kinship analysis, Endemic species

East to West: Population genomics and hierarchical analyses reveal differential adaptation footprints in the edible sea urchin *Paracentrotus lividus*

Carlos Carreras, Alex Garcia-Cisneros, Owen S. Wangensteen, Víctor Ordóñez, Creu Palacín, Marta Pascual¹ and Xavier Turon

The population structuring of living organisms is shaped by the combination of different evolutionary forces such as migration, genetic drift or selection. The rise of genomic studies during the last decade has opened new research opportunities including the analysis of outlier loci to look for genomic signals of local adaptation potentially associated to environmental cues. Species with wide distribution ranges may encounter diverse environmental conditions conferring different population selective pressures. The Atlanto-Mediterranean edible purple sea urchin, *Paracentrotus lividus*, is a commercially exploited keystone species in littoral benthic communities. To assess the current global status of the species under a genomic perspective, we used Genotyping by Sequencing (GBS) to characterize 241 individuals sampled in 11 localities along its distribution range. The 3,348 loci retained after filtering identified significant structuring and a gradient matching the longitudinal position of the localities. Two main genetic clusters (Atlantic and Mediterranean) were detected, linked by a transition zone across the Gibraltar Strait and the Alboran Sea. Adaptation to salinity appeared as an important driver of this axis of structuring as revealed by the molecular function of outlier loci and their significant correlation to mean and maximum salinity. However, a hierarchical analysis revealed subtler patterns of differentiation within these two main clusters. Outlier candidate markers identified between and within these two main clusters were mostly different, likely indicating different selective pressures on different regions. Other stressors, such as minimum temperature, seem to define the structuring within the Mediterranean. Furthermore, regionally missing loci were found, showing a geographic pattern of frequency consistent with the Atlanto-Mediterranean axis of genetic differentiation and reflecting a regional distribution of null alleles. Our study shows the potential of population genomics to analyse the fine scale genetic structure and adaptation signatures of marine species. Hierarchical analyses (i.e., defining groups of genetic clusters and analysing them separately) are needed when different levels of structuring are present to detect large and fine scale adaptation processes.

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Keywords: Genotyping by sequencing (GBS), Population Genomics, Oceanographic discontinuities, Benthic communities, Engineer species

Environmental and phenotypic drivers affect differential genomic responses in two sympatric congeneric fish

Héctor Torrado, Carlos Carreras, Núria Raventós, Marta Pascual and Enrique Macpherson

Connectivity and local adaptation are two contrasting evolutionary forces highly influencing population structure. To evaluate the impact of early life traits and environmental conditions on genetic structuring and adaptation we studied two sympatric species, *Symphodus tinca* and *S. ocellatus* reproducing in spring and summer respectively, caught along a temperature/productivity gradient in the Western Mediterranean Sea. For both species, we individually measured early life history traits from otolith readings and obtained genome-wide markers by Genotyping-By-Sequencing (GBS). As suggested from our results, the reproductive period seems to influence population connectivity and how they adapt to local environmental conditions. Species are more prone to show signals of selection to local conditions when they reproduce during environmental stable periods, e.g. summer for *S. ocellatus* that shows genomic signals related to productivity and temperature. Contrarily, species reproducing in environmental variable conditions, such as *S. tinca* reproducing in spring, a period with higher turbulence, may show lower genetic structuring. Interestingly, mostly heterozygous individuals at loci associated to environmental conditions were detected in the same three localities at both *Symphodus* species. Furthermore, we observed that for both species some homozygous genotypes associated to hatching date seem to reproduce earlier. Overall, our results suggest that unstable environments may favour connectivity and reduce local adaptation. Nonetheless, standing genetic variation within localities can be under selection suggesting the potential of local adaptation in a few loci regardless of environmental stability.

Keywords: Western Mediterranean Sea, GBS (genotyping-by-sequencing), *Symphodus*, GWAS - genome-wide association study, Early life traits

Population genomics revealed independent colonisation events of a global ascidian invader

Xavier Turon, Maria Casso and Marta Pascual

Invasive species constitute a major threat to biodiversity and can cause high economic and ecological negative impacts. To assess genomic diversity and population structure of non-indigenous species it is crucial to develop adequate management strategies. We developed a protocol combining whole genome amplification (WGA) and genotyping-by-sequencing (GBS). WGA is suitable for small sized organisms, colonial forms with small zooids, or studies where only trace material (scales, hairs...) is available, as is often the case for elusive and/or endangered species. This protocol was applied to the worldwide invasive colonial ascidian *Didemnum vexillum* using a single zooid per colony to avoid any genetic heterogeneity (chimeras). The obtained reads were filtered and analysed using the GIBPS toolkit to get the loci dataset. WGA-GBS performance was first tested using half zooids, providing empirical demonstration for genotyping reliability. The Percentage of Shared Genotypes (PSG) was calculated from the table of haplotypic genotypes to determine the reliability of the WGA-GBS protocol. Then, we analysed 296 individuals from 12 localities worldwide including native and main invaded areas. PSG identified some clones, always within the same locality, and only one sample per clone pair was kept for further analyses. Polymorphic loci datasets generated by locality, area and globally, identified genetic differentiation at all levels. The two groups found in Japan, the native area, matched Cytochrome Oxidase I (COI) clades and were strongly differentiated at the genomic level suggesting reproductive isolation and a speciation process. Our genomic analyses confirmed that only one COI clade spread worldwide. Genetic diversity and differentiation was high in the introduced localities as well as in the native area. MDS and STRUCTURE analyses showed that three independent colonisation events determined the distribution of

the species, although all population pairwise comparisons were significant. Human-mediated transportation seems to drive the distribution pattern of this species worldwide and regionally, as there is a lack of isolation by distance within introduced areas. Diverse and well differentiated populations point to a high expansion potential of this worrisome worldwide invader.

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Keywords: invasive species, Population Genomics, genotyping-by-sequencing, whole genome amplification, *Didemnum vexillum*

Disentangling latitudinal diversity gradients in Taxonomic, Phylogenetic and Functional diversity of Atlantic reef fishes

Nestor E. Bosch, Thomas Wernberg, Timothy J. Langlois, Dan A. Smale, Pippa J. Moore, João N. Franco, Pierre Thiriet, Eric Feunteun, Claudia Ribeiro, Pedro Neves, Rui Freitas, Karen Filbee-Dexter, Kjell Magnus Norderhaug, Alvaro Garcia Lazzati, Francisco Otero-Ferrer, Fernando Espino, Ricardo Haroun and Fernando Tuya

The rate of biodiversity loss in the Anthropocene have triggered a research agenda that move beyond the description of global patterns and drivers of species diversity (i.e. taxonomic diversity - TD) to the distribution of phylogenies (i.e. phylogenetic diversity - PD) and functional traits (i.e. functional diversity - FD). Understanding the complementarity between these three biodiversity facets have provided important insights in biodiversity-ecosystem functioning relationships and community assembly mechanisms, and provide a useful framework for conservation planning of both terrestrial and marine ecosystems. Yet, empirical studies for marine fishes covering broad latitudinal gradients remain scarce. Importantly, understanding regional differences in the macroevolutionary context over which these diversity patterns have arisen can provide important insights into the balance between scale-dependent community assembly processes - i.e. competition vs. environmental filtering. Here, using standardized underwater visual census techniques, we compiled an extensive quantitative dataset on eastern Atlantic shallow-water reef-dwelling fishes (148 species within 48 families, including bony and cartilaginous fishes) covering a latitudinal extent of 0° to 60° (including 66 sites within 6 marine ecoregions). We used the unique historical and biogeographic context of the eastern Atlantic basin to: i). test for congruency in latitudinal diversity gradients between TD, PD and FD, ii). investigate the level of phylogenetic overdispersion vs. phylogenetic clustering across different ecoregions. We hypothesized that environmental filtering play a major role in shaping the structure of fish assemblages at cool-temperate ecoregions (North Sea and Celtic Seas), generating assemblages in which the constituent species are more related than chance (i.e. phylogenetic clustering). Whilst, in warm-temperate (South European Atlantic Shelf, Macaronesia) and tropical ecoregions (Gulf of Guinea Islands, Cape Verde) competition have been the major structuring force, generating assemblages in which the constituent species are less related than chance (i.e. phylogenetic overdispersion or evenness). In summary, this study represents a novel approach to understand mechanisms of generation of diversity, in a relatively understudied ocean basin.

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We acknowledge the extensive network of collaborators involved in gathering this dataset

Keywords: Biodiversity, Reef fishes, community assembly, Eastern Atlantic, Trait conservatism

Biofilms shaping compositions of macrofouling assemblages: An initial barrier against NIS settlement?

Eva Cacabelos, Patrício Ramalhosa, João Canning-Clode, Jesus S. Troncoso, Celia Olabarria, Cristina Delgado, Sergey Dobretsov and Ignacio Gestoso

Maritime traffic facilitates the transport and arrival of non-indigenous species (NIS) into new regions through ballast water and/or encrusting organisms attached to hulls of ships. The introduction of NIS into the environment represent a great risk due to their potential impacts on structure and functioning of receiving ecosystems, that result in loss of ecosystem services and huge economic costs. Monitoring their presence and impacts is therefore critical for marine environmental management. Microfouling or biofilms, mainly composed of bacteria and diatoms, can have an important role in larval and spore settlement of macrofouling organisms, such as barnacles, polychaetes, ascidians and mussels. Biofilms can induce, repel or have no effect on larval and spore settlement of macrofouling species. Within this context, we proposed that settlement of native and NIS macrofouling species is facilitated by the presence of particular microbial biofilms. In order to test this hypothesis, in January 2019 we deployed a set of PVC settlement plates in several ports subjected to different maritime pressures, i.e. port located in marine protected areas ("pristine" biofilms), ports with almost no maritime traffic and without protection, i.e. controls, ports experiencing high maritime traffic (fisheries, recreational vessels and commercial traffic) and ports affected by secondary traffic (recreational boats and short ferry crossing). Using both modern genetic and classical taxonomic analyses, we will describe and compare the composition of "pristine" and "local" biofilms. After one month, PVC plates colonized by "pristine" biofilms were transplanted to the impacted habitats. We will determine how biofilms developed in pristine vs. impacted habitats affect the settlement of invertebrate larvae, determining both biodiversity and functioning of macrofouling communities. Preliminary analysis indicates greater recruitment and biomass of macrofouling species in secondary ports, especially on those plates colonized with "local" biofilms. Early detection monitoring of NIS is critical for marine environmental management. With the deployment of the PVC plates in January 2019 in the several ports subjected to different maritime pressures within the Ría de Vigo, we established a monitoring network of sessile organisms. These plates were and will be periodically (3-monthly) sampled to determine biodiversity and detecting both the presence of NIS already established and the new NIS arrivals in the different ports of the estuary, as well as their possible expansion along the coast. The information obtained within this project will be useful for the future optimization of standardized survey, impact assessment protocols, and prevention and control mechanisms to mitigate threats posed by NIS.

Keywords: non-indigenous species, Biofilms, macrobenthic assemblages, Maritime traffic, Monitoring

Do Non-Indigenous Species (NIS) prevailing over native species with climate change effects?

Nuno Castro, João Canning-Clode, Patrício Ramalhosa, Eva Cacabelos, José Lino V. Costa and Ignacio Gestoso

Climate change is increasing the frequency, duration and intensity of marine heat waves (MHWs) events [1]. Documented effects of MHW episodes all over the world include a variety of negative environmental impacts. In addition, extreme heat events can interact with another main component of global change in coastal ecosystems, non-indigenous species (NIS) invasions [2]. In this context, we designed a study to first compare the responses of invaded versus native marine fouling communities to a simulated gradient of short- and long-

term MHWs conditions, and second to evaluate the effects of MHW on the invasibility of marine communities. For that, two sets of experimental assemblages were previously collected by deploying a total of 60 artificial plates in the subtidal coastal habitat of Madeira Island: 30 plates at Quinta do Lorde Marina (Madeira) (i.e. high NIS propagule pressure environment) and 30 outside this marina area (i.e. low NIS pressure) in order to recruit assemblages with dominance of NIS and dominance of native species, respectively. After the 4-months period all units were sampled, through a binocular microscope and photographs taken for processing through image software analysis. Species identity, abundance and biogeographical status (i.e. native/cryptogenic versus NIS) were assigned. The different replicates will be placed in the mesocosm facility, randomly, to be subjected to heat waves simulated conditions. The units will be held independently at ambient seawater temperature (20 °C, control), or +4 °C (24°C, moderate intensity) or +8°C (28°C, extreme intensity) for a period of 5 (short duration) or 10 days (long duration). After that time, the communities will be sampled again to examine the influence of the heatwaves and then deployed back in the marina (i.e. high NIS pressure) for an additional 3 month period for testing the resistance/resilience of heat stress communities to NIS recruitment.- Univariate and multivariate statistics were applied to verify the hypothesis that (1) NIS dominated communities would be more resistant to MHW effects than native species dominated communities, (2) the combination of extreme intensity and long duration (i.e. maximum cumulative intensity 28°C during 10 days) event will have the stronger impacts on both NIS and native marine species, and (3) the biotic resistance of native communities will be lower after a MHW event. Preliminary results showed high variability in the responses of species to heat stress and an overall negative impact under extreme intensity (i.e. +8°C) independently of the MHW duration. Recruitment of NIS in those stressed marine communities was greater particularly in those native species dominated communities from the maximum cumulative intensity treatment. Evidences found here remark the relevant consequences that MHW can have for the conservation of coastal communities and how negative impacts on native species can promote the NIS invasion success. Management actions should give attention to the potential interactive impacts that the simultaneity of global change stressors can imply for the conservation of marine coastal communities, especially in the context of an island ecosystem.

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Keywords: Heat wave and severe heat wave, Climate Change, Mesocosmos, fouling communities, invasibility

Sampling Techniques Evaluation for Monitoring and Control of a Non-Native Crab Population

Jose A. Martín, Raül Triay-Portella, Jose M. Lorenzo, Israel Boderó-Izquierdo, José A. González, Angel Luque and Jose Gonzalez-Pajuelo

Cronius ruber is a non-native crab species who has now largely spread and adapted throughout the Canary Islands (north-eastern Atlantic) in few years. The aim of this work is to determine the effectiveness of different sampling techniques for the control and monitoring of this species. The study was carried out in six different locations, between 2 and 6 m depth, around Gran Canaria Island. In each location two scientific divers installed 2 fixed transects on a rocky-bottom and 2 on sandy-bottom with seagrasses (*Cymodosea nodosa*). The techniques that we have tested were visual census, visual census with attractors and baited traps. The transects were 75 m² and the effective fishing area (EAF) of each group of baited traps was 1,650 m². In each transect, all the different techniques mentioned were carried out. Due to the nocturnal activity of the species, all the techniques were developed during the night time. The crab densities were compared according to the type of

the bottom, and for each sampling technique. The visual censuses with attractors were the most appropriate technique for determinate the crab abundances in high densities areas. However, baited traps were more effective sampling technique for determinate a low crab density location. The use of one technique versus another will be determined by the phase of evaluation and control of the invasive species, being more appropriate the use of baited traps to determine the presence or absence of the species, and on the other hand the census with attractors allows a more precise evaluation of the density evolution of *Cronius ruber* population.

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Keywords: Alien invaders, non-native, Monitoring and control, crab, Sampling technics

Artificial seaweed monitoring structures (ASMS): the Next-Sea project approach towards a standardized non-destructive sampling methodology

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Bottom physical heterogeneity, associated or not to the structural complexity of the habitat-forming organisms, are the main factors that contribute to the difficulty in assuring replicable benthic marine macrofauna quantitative sampling. This is particularly true in very heterogeneous nearshore areas and is one of the methodological challenges in the study of marine benthos. Although quantitative data may not be relevant to inventory (presence/absence) or taxonomic studies, quantitative sampling of benthic macrofauna is needed to understand process changes and trends over time. The need of quantitative replicable sampling is fundamental in order to obtain statistical valid data. This kind of sampling is always destructive and not always desirable in certain sensitive habitats, as it can compromise the long-term survival of the study target.

Quantitative and standard sampling of benthic macrofauna is mandatory to understand how the abundance and diversity of species are controlled (Hauser et al. 2006), especially those associated with habitat-forming organisms, such as macroalgae (Hansen et al. 2011; Veiga et al. 2014) and gorgonians. In the context of global change, pollution monitoring and environmental management, the development of a standardized methodology that does not imply the destruction of the natural occurring elements of the system is required. Therefore, artificial substrates (AS) appear as a simple solution that may overcome the main environmental and anthropogenic problems mention, since they are easy to implement and not too expensive. With a known structural complexity and volume, AS are adequate to remove the variability of the sampling devices from the equation in quantitative sampling (García-Sanz et al. 2014; Ransome et al. 2017).

In temperate regions, seaweeds are naturally abundant habitat builders (depending on their structural complexity, size and antifouling properties) for many organisms of ecological and economic importance. Hence algae are good targets for monitoring studies based on colonization processes, mainly for the benthic and hyperbenthic macrofauna that is elusive most of the times (Davenport et al, 1999). Nevertheless, their collection may not be sustainable and will compromise the algae settlements in the long-term. In order to enable these kind of studies, AS may be viable non-destructive alternative, provided that the algae structural complexity is mimicked by the artificial substrates (Cacabelos et al. 2010).

Sampling through the use of AS is not instantaneous and rely on the availability of organisms to colonize them. It means that a longer period of time is going to be needed, in order to allow the process of colonization by benthic organisms. It is also well known that different kinds of substrates are going to be colonized differently (Schneider et al. 2003; Vazquez-Luis et al 2008). The same kind of substrate may also give different results, depending of the time of the year of deployment (García-Sanz et al. 2014) .

In NextSea project we aim to find an adequate procedure that can overcome the problems related with quan-

titative sampling of the macrofauna associated with habitat-forming algae. Therefore, the sampling of different macroalgae and the deployment of distinctive types of AS have been tested, both in intertidal and subtidal environments. *Saccorhiza polyschides*, *Cystoseira tamariscifolia*, *Codium tomentosum* and *Chondrus crispus* were sampled both in the Northern Portuguese and Galician (Spain) coast. Four types of AS were also tested, in an attempt to mimic the structure of different habitat builder seaweeds in these ecosystems (Artificial seaweed monitoring system, "ASMS").

The ASMS were made using polyethylene plants (ASMS-1: potted arborescent plants; ASMS-4: artificial plant mat), and sisal rope (ASMS-2 and ASMS-3), made of natural vegetal fibers. The sisal rope was frayed and coated with an innocuous resin to give it rigidity (ASMS-2) or arranged in order to mimic the structure of the rhizoid and frond of a kelp (ASMS-3). ASMS-1 is the most structurally complex followed by ASMS-4, ASMS-2 and, finally ASMS-3. All ASMS were deployed, attached to concrete plates and retrieved after about 6 months of exposition. All algae were collected between June and August and all organisms sorted and counted per unit of sampling (algae or ASMS). Although quantitative sampling has been made, for this analysis only presence/absence data will be presented and discussed.

A total of 305 taxonomic entities (species or families) were collected, from which 92 were only found on AS and 17 were exclusive from algae. A dissimilarity analysis using a presence/absence matrix measured by the Bray-Curtis distance shows the net distinction between the macrofauna associated with macroalgae and AS, on both geographic locations (Fig. 1). Concerning macroalgae, the more structural complex *C. tamariscifolia* and *S. polyschides* supported a greater richness than the simpler ones. The same effect can be seen with AS: the more complex (ASMS-1 and ASMS-4) were also the ones that supported the greatest richness. The influence of the latitudinal gradient is also evident and the results showed that the latitudinal difference between locations is always stronger than the difference between algae and AS in the macrofauna sampled.

With the results obtained we can conclude that ASMS-3, with the minimal structural complexity, was not adequate for sampling. From the remaining ASMS, the ones with more structural complexity (ASMS-1 and ASMS-4) were more successful in the collection of organisms than ASMS-2. When comparing ASMS-1 and ASMS-4 to the macroalgae, a greater richness and abundance was always achieved by the ASMS. Nevertheless, the macrofauna assemblages collected with ASMS-1 and ASMS-4 differ markedly from the ones colonizing the algae. Different algae are colonized by different macrofauna assemblages, suggesting a close relation with the biological nature of the substrate and implying that each assemblage is not a true representation of the potential colonizing organisms present in the water mass.

Our results suggest that i) a strategy based in more than one kind of ASMS is needed to adequately sample the benthic and epibenthic marine communities and ii) structural complex substrates are more adequate than simpler ones. In spite of the more efficient sampling results achieved by AS, some species were only present in algae, reinforcing the need to search for complementary AS. The complementary use of AS mimicking structurally complex algae and bottom structures such as *S. polyschides* seems to be a better solution than the use of a single type of AS..

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Keywords: Artificial substrates, benthic sampling,

Evolutionary insights derived from comprehensive analyses of DNA barcoding diversity in marine members of the superorder Peracarida (Crustacea: Malacostraca)

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Problem statement Large-scale global initiatives aiming to acquire a comprehensive catalogue of all living species and tackle the different biodiversity knowledge shortfalls (Hortal et al., 2012), have promoted the generation of vast amounts of DNA sequence data. The use of DNA barcodes as a universal system for DNA-based species identification, namely the mitochondrial cytochrome c oxidase gene (COI), has been shown to be successful in species classification and delimitation in several marine groups (Weigand et al., 2019). Furthermore, it has been contributing to improve the detection of potential new species, particularly cryptic species, and it has been used in numerous evolutionary studies (e.g., Vieira et al., 2019). This vast amount of COI data creates an unprecedented opportunity for extensive investigation of core aspects of molecular evolution in whole taxonomic assemblages, such as the Peracarida. Peracarida is a Superorder of the subphylum Crustacea and one of the most diverse and widely distributed groups of crustaceans. In marine coasts, peracaridean species are among the most ecologically important invertebrates, with high relevance in trophic interactions and constituting one of the dominant groups and key components of marine benthic communities (Ruffo, 1998). According to the World Register of Marine Species (WoRMS), it is composed by 12 orders and more than 20 000 described species, a third of the total number of crustacean species. However, they are also among the least known crustaceans, with an estimated two thirds of undiscovered species. Moreover, detailed analyses of evolutionary insights and patterns of molecular variation in marine members of this group are nearly inexistent. Here we conduct a comprehensive investigation of available COI sequence data, in order to gain insights into evolutionary rates and patterns of molecular evolution within Peracarida. **Methodology** A curated dataset of the world's marine (including brackish waters) peracarids was compiled on Barcode of Life Data Systems (BOLD), including new and previously generated sequences by the research groups authoring the current manuscript. Additionally, representative sequences of all peracaridean species publicly available on BOLD from marine environments across the world on 01/03/2019 were added. Only barcodes with a minimum length of 500 bp, without stop codons, and with indication of sampling location and taxonomic identification were used. Sequence edition and genetic analyses were performed using the software MEGA 7.0 (Kumar et al., 2016), BEAST 2.4.6 (Bouckaert et al., 2014) and the BOLD analyses tools (Ratnasingham & Hebert, 2013). The Barcode Index Number (BIN) system (Ratnasingham & Hebert, 2013) was used as delimitation criterion for assignment of molecular operational taxonomic units (MOTU) across the full dataset. **Results** Only 6727 of the 40289 publicly available DNA barcodes in BOLD, fulfilled our quality criteria. These barcodes belong to

571 valid morphospecies (274 genera and 109 families) and 812 Barcode Index Numbers (BINs). The orders Amphipoda and Isopoda were represented by the highest number of sequences (83.3% and 15.4% respectively) and the highest number of morphospecies (460 and 98 respectively). Within the order Amphipoda, the families Talitridae and Ampithoidae had the highest number of morphospecies (29 and 28 respectively), while within the order Isopoda, the families Idoteidae (22) and Sphaeromatidae (17) displayed the highest values. The family Hyalidae (order Amphipoda) had the highest proportion of BINs compared to morphospecies (216%). The overall mean intra-specific distance was 1.91% (max. 52.94%), congeneric 23.26% (max. 45.49%) and confamilial 26.20% (max. 59.68%). However, the mean distance among BINs was 18.81%. The family Cressidae (Amphipoda) displayed the highest mean congeneric values (35.68%), while the family Gammarellidae (Amphipoda) the lowest (11.40%). The mean GC content was 38.28% [24.96–50.49%], with the first codon position with the highest values (46.50%), followed by the second (42.54%) and the third (25.79%). The families Pseudidotheidae (Isopoda) and Lycaeidae (Amphipoda) displayed the highest and lowest GC content, respectively (43.85% and 27.49%). Major conclusions COI sequence data suggested an impressive amount of hidden diversity among marine peracarids, adding circa 40% more suspected species in relation to morphospecies. The most notorious taxa contributed disproportionately to the total number of potential new species, surpassing 10 hidden taxa in some cases and contributing with almost 1/3 of additional suspected species. Moreover, just 9 morphospecies contributed with 35% to the this “excess” species. GC content and genetic distances, in particular congeneric, varied considerably among families, suggesting appreciable differences in molecular evolution rates. Some families were considerably phylogenetically divergent from the remaining, which brings insights about taxonomic differences in evolutionary rates within this superorder.

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Keywords: Peracarida, DNA barcoding, meta-species, marine, Molecular variation

Community structure and habitat characterization of *Neoschrammeniella* cf. *bowerbankii* (Porifera, Corallistidae) aggregations in the Cantabrian Sea

Pilar Ríos, Francisca C. Carvalho, Francisco Sánchez, Elena Prado, Augusto Rodríguez, Joana R. Xavier, Teodoro Patrocínio and Javier Cristobo

The “lithistids” or rock sponges are an artificial group of demosponges that share the possession of a rigid skeleton, as a result of articulated spicules, named desmas. Actually are classified in Tetractinellida, Bubarida and Sphaerocladina Orders. *Neoschrammeniella* is a recent genus included in the family Corallistidae (Tetractinellida) that is known since the Jurassic being ~188 Ma the estimated age for its possible diversification. This

genus has a wide distribution and is known to occur in the Northeast Atlantic, Pacific and Antarctic Oceans. Here we describe for the first time the community structure of aggregations of the sponge *Neoschrammeniella cf. bowerbankii* in the Cantabrian Sea. This study is based on the analyses of visual transects using photogrammetric sledge (Fig.1) and samples collected by rock dredge (Fig. 2), in two geomorphological features, Le Danois Bank and the Corbiro Canyon of important conservation status as a Marine Protected Area (MPA) and as a Site of Community Importance (SCI). These sponge aggregations had similar ranges in both areas, 477-760m in Le Danois Bank and 520-760m in the Corbiro Canyon, and under similar environmental conditions. The temperature of water is 10.30–10.86°C in the Corbiro and 10.50–11.14°C in Le Danois Bank. The salinity range is 35.56–35.72 ppm in the Corbiro and 35.59–35.66 ppm in Le Danois Bank. The habitat is hard substrata, but bedrock in the Corbiro Canyon and boulders with mixed sediments in Le Danois Bank, but with much more density in the first one (200 live indiv./ha) than in Le Danois Bank (39.4 live indiv./ha). Several dead specimens whose siliceous skeleton persist after sponge were also observed and collected and appeared to also provide habitat to other macrofauna. The lithistid sponge aggregation may be subjected to gill nets impact in the Corbiro with anglerfish (*Lophius* spp.) as target species. *Neoschrammeniella cf. bowerbankii* are distributed mainly in the very vertical slope which is not appropriate for the fishing gear and usually the fishermen do not launch their gear in this area (data from logbooks and VMS, Punzón et al., 2016). In Le Danois Bank, the aggregation was subjected to bottom longlines targeting Alfonsino (*Beryx decadactylus*), Splendid alfonsino (*B. spendens*) and Blackspot seabream (*Pagellus bogaraveo*) between 1970 and 2011, date in which it was recognized as Marine Protected Area. Similar to the aggregated clumps of another lithistid species (*Leiodermatium pfeifferae*) recently recorded in the Mediterranean Sea, this discovery provides new insights into how the Mesozoic lithistid formations developed and functioned and is of great conservation relevance. Figure legends Figure 1. 3D reconstruction of video transect using Pix4D Mapper Pro. Green points show the camera's position. Figure 2. Samples of *Neoschrammeniella cf. bowerbankii* collected in the Corbiro Canyon.

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Keywords: VEMS, Porifera aggregations, Photogrammetry characterization, *Neoschrammeniella* habitat, Cantabrian Sea

Habitats characterization of circalittoral rocky bottoms of the Avilés Canyon System (Cantabrian Sea)

Francisco S. Delgado, Augusto R. Basalo, Maria G. Ballesteros, Elena Prado, Teodoro Patrocinio, Pilar Ríos, Antonio Punzon, Jose Rueda and Javier Cristobo

The area of the Cantabrian sea continental shelf located in the headwaters of the Avilés Canyon System (ACS) is characterized by rocky outcrops (Gómez-Ballesteros et al., 2014). This is mainly due to the sedimentary transport mechanisms associated with the ACS oceanographic dynamics. This area is currently declared as Site of Community Importance (SCI) in the context of the Natura 2000 network, mainly due to the existence of coral reefs (Sánchez et al., 2014). In this area operates some artisanal fisheries so that it is compulsory to know the vulnerable habitats spatial distribution in order to identify potential conflicts of use (Punzón et al., 2016). The LIFE+ INTEMARES project (www.intemares.com) has as main objective to ensure the effective management of Spanish Marine Protected Areas (MPA) net. For the elaboration, update and approval of Management Plans of ACS it is necessary to identify the conflict of uses between the conservation of vulnerable habitats and the fisheries. The INDEMARES, SponGES, ECOMARG and INTEMARES projects took place a series of multidisciplinary surveys in the area using different samplers for both sedimentary and rocky grounds (Fig.1). In this study, we have identified, characterized and mapped the different habitats and the communities that occupy the hard bottoms, using three analytic strategies: 1) Abiotic surrogate (unsupervised classification of environ-

mental scenarios); 2) Assemble and predict together (multivariate analysis) and 3) Predict first, assemble later (supervised classification, habitat suitability modeling), following the same criteria explained in Sánchez et al., 2017. The study of the main environmental variables that explain the settlement of sessile organisms was performed using the ISOCLUSTER tool, implemented in GIS software, and using depth and derivative variables from the bathymetry (slope, Bathymetric Position Index-BPI) together with the seabed characterization (facies). It is based on the Iterative Minimum Distance (IMD), which is the result of minimizing the variance within clusters as a way of optimizing partitioning efficiency. The results provide the estimated distribution of seabed areas that differ with respect to topography and substratum type for areas adjacent to earlier mapping sites. Six main habitat classes are identified from these abiotic data (Figure 2). The main explanatory variables for the hard bottoms habitat classification have been the BPI and the slope (Table 1). For the study of the communities of these rocky habitats we have been completed visual transects using a photogrammetric sled and stations accomplished with a rocky dredge which has allowed the estimation of the spatial distribution and density of the different species, following the same methodology explained in Sánchez et al., 2017. The most frequent species are of the following taxonomic groups: Porifera (21 species), Briozoa (13), Echinodermata (10), Polychaeta (8), Mollusca (7), Cnidaria (7) and others (8). With respect to the density of individuals the most abundant species have been: *Ophiothrix fragilis* (6.884 ind. by ha), *Leptometra celtica* (3.453), *Phakellia ventilabrum* (1.579), *Dendrophyllia cornigera* (0.379) and *Gracilechinus acutus* (0.084). The cup sponge (*Ph. ventilabrum*) and the yellow coral (*D. cornigera*) are the most representative structural species of the vulnerable habitat 1170 Reefs on the studied area, preferably dwells in very rough bottoms with high positive BPI fine scale. Habitat suitability models using environmental layers from acoustic methods are appropriate for the meso-habitats (10 mt to 1 km) or macro-habitat (1 to 10 mt) mapping. Taking into account the structural complexity of these rocky habitats, and to be able to characterize the micro-habitat (< 1 mt) and the behaviour of some typical species it is necessary a higher spatial resolution. We are testing the application of suitability models using environmental layers obtained from image analysis. From this methodology it's possible to obtain environmental variables equivalent to those of the acoustic methods (slope, rugosity, BPI, aspect, etc.) but with a few millimeter of resolution which will allow us to apply suitability models for microhabitats mapping. As an example, Figure 3 shows that small rocky ridges, identified in red by its positive BPI, constitute the preferred micro-habitat for filter-feeders species, in this case the brittlestar *Ophiothrix fragilis*. Figure captions: Figure 1. Study area and samples carried out to characterize the habitats and species distribution from INDEMARES, SponGES and INTEMARES surveys. Figure 2. Predicted spatial distribution of the six main habitat classes obtained from ISOCLUSTER analysis using bathymetry, slope, fine scale BPI and seabed characterization as environmental variables. Figure 3. Methodology process to obtain preferred micro-habitat of the brittlestar *Ophiothrix fragilis*. 3D model reconstruction from videotransects using photogrammetric analysis.

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Keywords: circalittoral habitats, marine protected areas, habitat suitability, Photogrammetry, Aviles Canyon System, biodiversity and ecosystem functioning

High resolution spatial distribution for the hexactinellid sponges *Asconema setubalense* and *Pheronema carpenteri* in the Central Cantabrian Sea

Augusto R. Basalo, Francisco S. Delgado, Elena P. Ortega, Pilar R. López and Francisco Javier Rodríguez

The study area, Central Cantabria Sea, is divided in two areas with very particular characteristics. The first one, Avilés Canyon System (ACS), is comprised of three different canyons, Avilés, El Corbiro and La Gaviera, each one with specific morphostructural characteristics. This system starts from the continental shelf, at 140 meters depth, and ends in the Biscay Abyssal Plain, at 4700 meters depth. The second one is a seamount, El Cachucho Marine Protected Area (MPA). The MPA comprises the Le Danois Bank and its inner basin, and is separated from the continental platform by the Lastres Canyon. This complexity, along with the current circulation patterns (González-Pola et al, 2012), makes these areas a very valuable diversity hotspot. El Cachucho MPA was included in 2008 on Natura 2000 especially because of the presence of large size sponges aggregations and gorgonian forests characterizing the area of study. As for ACS, it is being studied to become part of Natura 2000 Network because of the presence of vulnerable habitats included in 1170 Reefs habitat of the Annex I of EU Habitats Directive, specially deep water corals and sponge aggregations. The target species for the present study are the hexactinellid sponges *Asconema setubalense* and *Pheronema carpenteri*. For the identification and localization of the species requiring bedrock, *A. setubalense*, we analyzed the images captured during three different surveys: ESMAREC 0514, ECOMARG 0717, and SponGES 0617. Images come from the photogrammetric sled ROTV (Remote Operated Towed Vehicle) Politolana (Sánchez, F. and Rodríguez, J. M., 2013). In total, 80 transects were accomplished in the whole area to get the presence-absence data. As for *P. carpenteri*, we used data from bottom trawling and beam trawl, as well as the images captured by the photogrammetric sled. For the first results (Fig 2 and 3), Maximum Entropy Index (MAXENT) was used in to get the habitat suitability models of these species and get maps showing spatial distribution according to probability values. The environmental variables used for the MAXENT study were depth, slope, northness, eastness, rugosity and BPI fine scale extracted from a 32m x 32m grid high resolution bathymetry. In addition, abundance of the species studied was calculated based on photogrammetry and image scaling. For further analysis, a biogeochemical and physical approach will be used. As long as siliceous species of hexactinellida needs Si (dissolved silica) to form spicules, which provides support to the sponge, a layer containing silicate in depth is being prepared with the highest resolution possible. To assess dissolved silicate concentration in bottom waters (i.e., sea-floor), a forecast raster product from CMEMS (Copernicus Marine Environment Monitoring Service) has been used. This product covers IBI (Iberia – Biscay – Ireland Regional Seas) region with a horizontal resolution of 1/36° (~2km). An application of biogeochemical model PISCES, running simultaneously with the NEMO ocean model is used to generate the IBI ocean forecasts; generating the main biogeochemical variables, such as chlorophyll, oxygen, iron, nitrate, ammonium, phosphate, silicate, net primary production and the euphotic zone depth. Thus, the concentration of silicate will be extracted from this product using real depth extracted from the high spatial resolution bathymetric layer. Finally, to take into account the deep sea dynamics, a high energy dynamics associated with the tidal cycles layer will be used for the final model. Fig 1. Habitat suitability of the hexactinellid sponge *Asconema setubalense* using Maxent modeling. Fig 2. Habitat suitability of the hexactinellid sponge *Pheronema carpenteri* using Maxent modeling.

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Keywords: Le Danois Bank, El Cachucho MPA, MPA, Avilés Canyon System, deep-sea sponges, Habitat modeling

In-situ growth rate assessment of hexactinellid *Asconema setubalense* using 3D photogrammetric reconstruction in El Cachucho Marine Protected Area (Le Danois Bank, Cantabrian Sea)

Elena Prado, Francisco Sánchez, Pilar Ríos, Augusto Rodríguez-Basalo and Javier Cristobo

Deep-sea corals and sponges habitats are considered exceptional ecosystems from a biodiversity perspective. They form tridimensional complex habitats that have become a focus of conservation efforts in the deep sea. One of the principal role of sponges in the marine environment is linked to its filtration capacity, removing up to 95% of bacteria and particles from the water and 90% of dissolved organic carbon, thereby converting suspended particles and dissolved matter into food for other animals. Sponges seem to also play a role for the recirculation of carbon, silicon, and nitrogen. The presence of gorgonian forests and deep-sea sponge aggregations in the Le Danois Bank caused its declaration as the "El Cachucho" Marine Protected Area (MPA) by the Spanish Ministry of Environment, and its inclusion in the Natura 2000 network. Deep-sea sponge aggregations are considered vulnerable, so follow-up surveys are being performed to monitor their conservation in compliance with the EU Habitats Directive. These efforts in protect and conservation of vulnerable habitats have been linked to some objectives of SponGES project (H2020 grant agreement No 679849). SponGES is a research and innovation project and its goal is to develop an integrated ecosystem-based approach to preserve and sustainably use deep-sea sponge ecosystems of the North Atlantic. *Asconema setubalense* Kent, 1870, is a hexactinellid sponge and traditionally had been reported from greater depths (93-4270m) in the North Atlantic Ocean (Tabachnick, 2007). Recently this species was recorded first in the Mediterranean during the ROV exploration (> 250 m) of the Alboran Sea, the "Seco de los Olivos" (Pardo et al. 2011) and in the shelf of the Alboran Island, that provided the second Mediterranean record of this hexactinellid (Sitjà and Maldonado, 2014). Although there is not a dense population of *A. setubalense* in Le Danois Bank, its presence is confirmed by numerous ROV recordings in this MPA and has been one of the target habitats to be modeled in the different studies carried out there (Sánchez et al., 2017). The growth rates of the depth sponges provide valuable information about their biology and their ability to recover from possible impacts, for example the impacts suffered by fishing activity. However, the growth of hexactinellid sponges is hardly known, principally due to the inaccessibility of their preferred deep water habitat. No previous growth studies about *Asconema setubalense* in Cantabrian Sea were carried out and only a few of studies published any of them about *Asconema* spp. (Dayton, 1979; Leys and Lanzon, 1998; Marliave, 2015). We describe for the first time the growth rate of *Asconema setubalense* through the use of a photogrammetric methodology and 3D modeling in a non-invasive and novel approach. Structure-from-Motion (SfM) techniques from videos acquired in different dates along 6 years with ROTV, Politolana sled in El Cachucho MPA have been used. The cruises have been carried out in years 2014, 2017 and one more planned next July of 2019. With these data a multi-temporal study has been proposed. A complete 3D reconstruction of the deep-sea floor for each date with Pix4D Mapper Pro software has been achieved (Fig.1). Having a 3D point cloud of the study area allows us to propose a series of measures that were impossible to obtain until now. So in this 3D space the sizes (height and maximum diameter) of several *Asconema setubalense* specimens have been measured for each data set. The specimens present in all the years of study have different sizes, which in principle could be associated to different ages. To estimate the growth parameters (T_0 , K and L_∞) we use the methodology of Gulland and Holt. Finally, a growth curve using a Von Bertalanffy model equation has been achieved. The relationship between height and maximum diameters have been calculated (Fig.2) with a R^2 of 0.98. The results show a growth curve (Fig.2) in which the growth rate decreases as the individuals have larger sizes according to the results of Leys and Lauzon, 1998. So, taking into account the size indicators used in this study, this sponge reaches its maximum size over 50 years of age. This growth rate of the *Asconema setubalense* has been successfully obtained through this study in the central Cantabrian Sea. This type of methodology based on images does not cause damage or alterations on benthic communities and it is particularly necessary in vulnerable ecosystem studies and MPA monitoring. This study contributes not only to the improvement of the knowledge of this species, but is useful for the help of the implementation of monitoring plans of Marine Protected Areas, or the implementation of directives such as the Marine Strategies.

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Keywords: Underwater photogrammetry, *Asconema setubalense*, Marine protected area (MPA), deep-sea sponges, Sponge growth

Towards a standardized methodology for monitoring and assessing marine mobile epibenthic communities across spatio-temporal scales

Macarena Ros, Víctor López de la Rosa, Triana Revanales, Andrea Spinelli, Alejandro Fernández-Romero, Lucas Cervera-Currado and Jose Manuel Guerra-García

Habitat modification is one of the main drivers of global environmental change. Some of the most dramatic alterations of coastal habitats are the development of harbours and marinas. These artificial habitats have the potential to modify local ecological and environmental conditions and constitute critical entry points for the introduction of non-native species. However, efforts so far for understanding the consequences of these environmental impacts have focused primarily on sessile species that settle directly on the artificial structures. Mobile epibenthic communities (MECs) inhabiting the biogenic substrata provided by these species (basibionts) are generally overlooked, despite their key role in the trophodynamic of benthic ecosystems. This is mainly due to its small size, challenging taxonomy and to the absence of a standardized methodology specifically developed for the detection of this group of animals. The aim of this study was to design a standardized, easy-to-apply, low-cost and quantitative methodology that allows comparative studies of MECs associated with artificial habitats at different spatio-temporal scales, regardless of the basibiont to which they are associated. To address this aim, we developed and tested standardized passive collectors made of two types of commercially available plastic meshes folded three-dimensionally (hereinafter type A and type B). The type A had a higher volume, less compaction degree and required more processing time to extract the associated MECs from the mesh than the type B. Meshes were deployed in the water column at 1-meter depth through a rope anchored to the floating pontoon. Collectors were deployed for two periods of 1 and 3 months respectively, in three marinas of the Bay of Cádiz (Spain). A commonly used non-standardized methodology consisting in collecting potential fouling basibiont species was used for assessing the efficiency of the collectors in sampling MECs. Twelve collectors of each type were deployed for each deployment period in each marina. Overall, a total of 144 experimental collectors were analysed. After one month of immersion, the type A collected an average of 1924.69 ± 283.13 (mean \pm SE) individuals per unit belonging to 12 faunal groups, while the type B collected 595.66 ± 65.19 individuals per unit belonging to 10 faunal groups. After three months, the type A collected an average of 6060.36 ± 793.43 (mean \pm SE) individuals per unit belonging to 12 faunal groups, while

the type B collected 2465.00 ± 284.17 individuals per unit belonging to 11 faunal groups. Both type A and type B were more efficient in collecting MECs, both in terms of abundance and number of faunal groups, than the non-standardized methodology. This pattern was consistent across marinas and immersion periods. Based on their high efficiency and different physical properties of the two types of artificial microhabitat tested, its use as a sampling unit in the standardized methodology designed is discussed. This study provides scientific community with a standardized and quantitative sampling methodology for monitoring and assessing MECs associated with artificial habitats. This could contribute not only to increase knowledge on the biodiversity of epibenthic communities, but also to implement the use of these communities as a biological indicator of environmental impact in the context of marine environment management strategies.

Keywords: Biodiversity monitoring, fouling, invasive species, Recreational marinas, artificial structure

Mapping seaweed beds using multispectral imagery retrieved by unmanned aerial vehicles

Débora Borges, Isabel Azevedo, Luís Pádua, Telmo Adão, Emanuel Peres, Joaquim J. Sousa, Isabel Sousa Pinto, and José A. Gonçalves

Seaweed assemblages are present in rocky shores around the world, creating very productive ecosystems with high ecological and economic importance. These assemblages include a variety of structuring species that provide habitat, food and shelter for organisms from different trophic levels such as invertebrates, fish, and top predators, supporting complex trophic webs. Seaweeds are also considered a natural resource, for human use. Although traditionally seaweeds were used as fertilizer and animal feed in western countries, they are now considered a healthy food and are increasingly being incorporated in diets due to their recognised nutritional and functional properties. Therefore, an increased demand for biomass is being triggered by the development of new markets, due to seaweed bioactive properties, such as feed /food supplements and nutraceuticals/cosmetics. In situ conventional surveys for biomass assessment are time and resources consuming, calling for the development of innovative, reliable yet expeditious, imagery tools to map the intertidal and assess seaweed biomass available in the field, using remotely sensed images. The present work is being developed in the framework of the project SWUAV- Mapping the intertidal zone and assessing seaweed biomass using UAV images. This project aims to map seaweed beds and assess their standing stock, combining RGB, multispectral and hyperspectral images retrieved by unmanned aerial vehicles (UAV), with spectroradiometer data collected in the field. The basis of this approach is the discrimination of seaweeds by their pigmentation and grouped in green (Chlorophyta), red (Rhodophyta) or brown (Phaeophyceae) typologies. Within each group, it will be relevant to differentiate the main genus and if possible distinguish between species. Hence, a standard spectral signature will be obtained for assessing and remotely map the biomass available in the field for those discriminated seaweeds in a second stage of the project. Spectral signatures of several seaweed species were collected with a hand held spectroradiometer (ASD HandHeld 2: 325- 1075 nm) on a black background in several layers to obtain pure signatures. A preliminary linear discriminant analysis (LDA) over collected spectral signatures, performed after a band reduction operation carried out by using a recursive feature elimination (RFE) method, enabled to conclude that seaweeds are highly differentiable in what regards to both pigmentation typology and reproductive status (Fig 1). The influence of seaweed biomass variation (for species of green, red and brown seaweed) on the reflectance spectra was investigated through an experimental addition and removal of blades to and from a 53 cm² surface matching the spectroradiometer ground field of view (GFOV). The measurements ranged from one single blade to overfilling the GFOV with seaweeds blades, progressively adding several layers. Seaweed samples were subsequently dried at 60°C for 48 h and weighed (dry weight: DW). Biomasses were expressed by surface unit, in gDWm⁻² and plotted against the Normalized Difference Vegetation Index (NDVI) of each sample to build the relationship between these two variables per species (*Ulva* spp.: Fig 2). In parallel, a flight using an UAV equipped with RGB (as basis for photo-interpretation) and multispectral cameras (MicaSense RedEdge camera) was performed in February 2019 at Vila Chã rocky shore. All images were orthorectified, mosaicked and precisely georeferenced using a high accuracy GPS receiver, so that information of different sensors and seasons can be overlaid in a Geographic Information System. Using the multispectral data, we calculated the NDVI for the area covered by the UAV. The flight was set at 20 m height, yielding pixel sizes of 0.8 cm for the RGB and 1.6 cm for the multispectral, and supervised classification techniques were applied in QGIS to evaluate the extension of the following four ecosystem components: seaweeds; sand; mussels and rock; rock, barnacles and limpets. Three quadrat frames of 50x50 cm were used for the definition of training areas for each of the four classes. The validation of the classification process was performed by assessing its accuracy comparing the software output with

the groundtruthing observations of an extra quadrat per class. The information on the calculated NDVI for the seaweed beds in the studied area, is currently being crossed with data on species spectral discrimination and biomass/NDVI variation, so as to allow the remote assessment of the seaweed biomass. Similar flights will be performed in different seasons in order to assess seasonal variation of seaweed biomass and ecosystem components. Future work in the scope of SWUAV will also combine this information with hyperspectral data in order to develop a QGIS plugin for remotely assess and map seaweed biomass. This will be a valuable tool for rapid mapping and quantification of seaweed biomass, improving the knowledge of the local ecosystems, and contributing for efficient and sustainable management of marine habitats.

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Keywords: Semi-automatic classification, Seaweed spectral signatures, Biomass assessment, Mapping the intertidal, Remote sensors

Phase shifts from macroalgae to Zoantharia: effects on subtropical fish community.

Sergio M. Borges, Cristina V. Solano, Cataixa L. Batista, Adriana R. Hernández and Sabrina C. Martín

Nowadays climate change is the greatest driving-force behind most of the shifts observed in ecosystems all around the world. Probably its most evident consequence is the global rising of sea surface temperatures that affects marine life in many different ways. One of the most evident effects is the alteration of the latitudinal distribution of organisms. Tropicalization is the term used to define these changes in the distribution and abundance patterns of marine species that gradually move pole-wards as temperatures increase. The Canary Islands are affected by this phenomenon, and, besides the occurrence of new species from tropical regions, one of the main concerns is about native species that are now proliferating because of the warming waters. Zoantharians are one of these animals and, due to their capacity to greatly cover the substrata with colonies forming extensive mats, they are structuring rocky bottoms and generating a new kind of habitats that did not exist before in the archipelago. During the last decade, it has been verified the expansion of colonies of zoantharia (mainly *Palythoa caribaeorum* and *Zoanthus pulchellus*) found in the first meters depth of non-exposed rocky-shores. Expanding colonies are replacing the predominant macroalgae habitats and generating a new model of ecosystems previously absent in the Canary Islands. We planned a field research to study the effects of this zoantharian expansion in coastal fish communities found in those areas. We compared abundances and species composition of fish communities inhabiting zoantharia and macroalgae dominated habitats. We selected a total of 9 localities where we already knew about the presence of large extensions (>100 m²) of *P. caribaeorum* (3 sites) or *Z. pulchellus* (6 sites). These localities were distributed in 5 different islands of the Archipelago. In each site we studied two habitats: one dominated by zoantharia and the other dominated by macroalgae. We recorded species composition and abundance of benthic fish community, using a stationary visual method (n= 94) in which 25 m² were inspected for species identification and counts. In order to assess fish assemblage trophic structure, fish species were clustered by trophic groups into six categories: "Herbivores", "Planktivores", "Omnivores", "Microinvertivores", "Macroninvertivores" and "Piscivores". Differences in fish assemblages among habitats dominated by zoantharia and macroalgae were assessed by means of a permutational multivariate analysis of variance (PERMANOVA). Trophic group-specific abundances, species richness and total fish abundance were also analysed with permutational univariate ANOVAs. A total of 34 benthic and benthic-pelagic fish species were registered: 2 herbivores, 1 planktivore, 11 omnivores, 4 microinvertivores, 3 macroinvertivores and 13 piscivores. Most abundant groups were omnivores and microinvertivores with over 40 individuals / 25 m² (Table 1). Trophic group abundances showed significant differences between habitats dominated by *Palythoa* and macroalgae ($F = 5.64$; $p < 0.05$). 'Microinvertivores' was the most relevant group defining the differences between both habitats ($F = 6.12$; $p < 0.05$), showing higher abundances in macroalgae than in *Palythoa* dominated habitats (Table 1). In addition, ANOVA for total fish abundance showed significant

differences for factor habitat ($F = 10.37$; $p < 0.01$). As previously described, total abundances registered at *Palythoa* habitats were lower than at macroalgae dominated systems (Table 1). However, species richness analysis did not show differences among *Palythoa* and macroalgae habitats, neither did species abundance, species richness and total fish abundance among *Zoanthus* and macroalgae dominated areas. Results showed that habitats dominated by *P. caribaeorum* had a stronger effect upon fish communities. Analyses indicated that whenever this Zoantharia formed extensive mats over the substrata significantly less fish abundance occurred, especially evident in the case of the trophic group of microinvertivores. Growth strategies of this cnidarian species, covering large extensions of rocky bottoms (several m²) without leaving empty spaces in between, may explain these results. This means that macroalgae growing in between colonies are scarce, and consequently microinvertivore fish find reduced food availability in form of the small crustaceans and molluscs that are usually sheltered in macroalgae canopies. In fact, under *Palythoa* colonies there are a whole community of microinvertebrates but highly inaccessible or not explored by most of the fish community. The dominance of *Zoanthus pulchellus* in the substratum did not show to significantly affect fish communities. Growing strategy of the species is not as aggressive as in the case of *P. caribaeorum*. *Zoanthus* colonies are usually smaller, and the percentage of substrate coverage in studied areas was usually 30-40 % in comparison to 60-80 % coverage of *Palythoa*. Furthermore, their polyps are not so much embedded in the coenenchyme, appearing more isolated and making it even possible to find algae growing in between them. This would explain that fish assemblages find more feeding resources within this habitat and showed no differences between *Zoanthus* and macroalgae habitats. Considering that these kinds of zoantharia habitats are starting to proliferate in the Canary Islands due to the effects of climate change and local stressors, in most cases we were probably observing the early stages of phase-shifting processes from macroalgae to zoantharia dominated systems. In fact, many authors have reported about phase-shift processes at coral reef ecosystems changing to zoantharian systems, as a halfway stage before macroalgae proliferation. Some studies have assessed the effects over fish communities, reporting a decrease in the species richness but not in abundance, in contrast to results here presented. This could be explained because the Canary Islands are not coral reefs ecosystems, and therefore fish community resilience against this scenario is limited. This is the first report of a case of phase-shift from typically macroalgae-dominated habitats to Zoantharia dominated habitats in a subtropical region. These early observations about community-wide effects on fish assemblages are essential to understand the future changes undergoing in these subtropical regions, especially in a scenario in which they are becoming the new refuge for tropical species.

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Predictive model of competition between three species of saltmarsh plants: effects of sedimentary variables and CO₂ concentration

F. Xavier Niell, Miriam Ruiz Nieto, Rocío Jimenez and Raquel Carmona

Three species in the Palmones saltmarsh have displaced its frontiers in the last years (*Atriplex portulacoides*, *Sarcocornia marina* and *Artrocnemum glaucum*). To understand the mentioned displacements, several vari-

ables have been monthly used to control the photosynthetic capacity and the nutrient uptake.

Nutrient concentrations, salinity, redox potential in the interstitial water and, CO₂ concentration in the canopy have been controlled (the most direct related variable with climate change). The objective of this study was to compare the nutrient and CO₂ uptake within a realistic range of values observed in the field during 10 years and checked in the laboratory.

The values of each variable are classified in probability classes. The species that match more frequently with the environmental states take advantage on those whose needs are scarcely present in the saltmarsh. The “winners”, using competition terminology, are those being able to have the most efficient assimilation rates more frequently along all the sampling period of study.

The species that will benefit more from the changing environment states is *A. portulacoides*, clearly favored by the spring-summer hyper-eutrophication, recently increasing. *S. marina* is the most tolerant of the three species to extreme values of salinity and can endure salinities up to 90. *A. glaucum* takes advantage in environments with high concentration of oxidized nutrients, but the concentrations required for the oxidation of these nutrients does not happen frequently in the low and middle saltmarsh. Therefore, *A. glaucum* is excluded from the competition in these levels and remained in the upper saltmarsh.

The comparison of CO₂ uptake did not make any difference between the three species and currently did not revealed a factor of competition under the current concentrations. Following the present trends of CO₂ increasing concentration, *S. marina* will lead in growth with a future increase of this gas in the atmosphere.

The most selective variable is the range of high nutrient concentration and the second degree of importance is the increased salinity. Unexpectedly, CO₂ is not currently a decisive factor in competition

Keywords: Saltmarsh, halophytes, *Atriplex portulacoides*, *Sarcocornia marina*, *Artrocnemum glaucum*

Collapse of macrophytic communities in a eutrophicated coastal lagoon

Juan M. Ruiz, Maria Dolores B. Torrente, Rocío G. Muñoz, Aranzazu R. Segura, Jaime B. Esteller, Judit J. Casero, Lázaro M. Guirao, Pedro G. Moreno, Ignacio F. Navarro, Eugenio F. Nuez and Jesús M. Mercado

Marine ecosystems can experience sudden and unexpected catastrophic shifts to impoverished and undesirable states in response to environmental changes (Scheffer et al. 2001). Coastal habitats based on one or few engineer, foundation species such as those represented by macroalgae and seagrasses are particularly sensitive to these abrupt changes (Maxwell et al. 2016). In the present study we provide relevant information about the extensive and abrupt benthic macrophytes die-off recorded in one of the major Mediterranean coastal lagoons (Mar Menor; SE Spain) during an unprecedented period of turbid waters caused by a massive phytoplankton bloom (Figure 1). This relevant ecosystem remained in a clear-water state dominated by submerged vegetation during decades (Belando et al. 2019), despite the huge amounts of nutrients delivered into the lagoon from intensive agriculture practices (Velasco et al. 2006) and the limited water exchange of this semi-enclosed water body. The high resilience of this ecosystem to increased human pressures was likely associated to the existence of feedback mechanisms typically associated to benthic vegetation (e.g. high capacity to remove nutrients and particles from water column; Maxwell et al. 2016) and the phenotypic plasticity of the dominant species to buffer population responses to environmental changes (Marín-Guirao et al., 2019). However, the turbid-water period could have surpassed the environmental thresholds (i.e. light reduction) beyond which dampening mechanisms fail and vegetated seabed can rapidly shift to an unvegetated, bare state. Figure 1. Upper panel: evolution of chlorophyll a in the Mar Menor lagoon between 1980 and 2018, obtained by spectrophotometric determination from seawater samples; Central panel: evolution of chlorophyll a in the period 1998-2018 obtained from analysis of satellite images (the red line correspond to values obtained from water samples determinations); Lower panel: evolution of water turbidity (light attenuation coefficient k_d , m⁻¹) and percentage of surface irradiance obtained at 3 m depth by direct measurement of PAR irradiance using a submersible photometer. Source: IEO-MMEM project, unpublished data. The aim of this study was to provide precise and robust evidence of the magnitude and dynamic of the apparent regime shift experienced by the Mar Menor lagoon, as well as to get some insights about factors that have driven such abrupt changes. We used standard methods of marine vegetation mapping (i.e. a combination of lineal transects using SCUBA diving and trawled underwater videocamera and satellite images; Short & Coles 2001) to obtain georeferenced and precise distribution limits of the main types of submerged vegetation (i.e. monospecific and mixed

meadows of the aquatic angiosperms *Ruppia cirrhosa* and *Cymodocea nodosa* and the chlorophyte *Caulerpa prolifera*). A first sampling approach was performed in autumn 2016 and a new sampling campaign was performed about one year before (summer 2017) using both direct and indirect visual methods. Georeferenced data was integrated in a Geographic Information System (© ArcGIS 10.2) to obtain interpolated maps of macrophytes distribution and to estimate the surface area occupied by each vegetation type. The resulting maps were compared with data obtained by this research team using the same methodology in 2014, just before the turbidity event (Belando et al. 2019; Figure 2). Figure 2. Maps of macrophyte communities of the Mar Menor lagoon obtained in 2014 by Belando et al (2019) and in 2016 and 2017 in this study. The red line showed in the 2017 map correspond to the maximum depth limit of macrophytes on that year. Map legend: *C. nodosa* (orange), *C. prolifera* (dark green), mixed meadow *C. nodosa*-*C. prolifera* (green), *R. cirrhosa* (pink) and silty unvegetated bottoms (yellow). Our results showed total absence of macrophytes beyond a depth limit between 1-2.5 m in autumn 2016 (Figure 2), evidencing that most areas in the lagoon have shifted to an unvegetated state characterized by muddy and silty sediments (Figure 3). This represents a massive total loss of 86.2% of the surface area covered by macrophytes in 2014. The remaining vegetated bottoms were all concentrated in the very shallow areas of the lagoon, suggesting that the light limitation favored by turbid waters could be a primary factor involved in this sudden, catastrophic shift. This is consistent with the drastic reduction of PAR irradiance recorded during at least 9 months in 2016 (i.e. less than 5% of subsurface downward irradiance; Figure 1), when light levels were far below the minimum requirements for the dominant macrophytes *C. nodosa* and *C. prolifera*. Despite this evidence, other environmental factors such as the temperature anomaly recorded in 2015, which could have favored the phytoplankton bloom initiation, may have also contributed to the subsequent loss of submerged vegetation. Figure 3. Comparison of images obtained in a fixed sampling site at 4-5 m depth showing the macrophyte-dominated state documented in 2014 (left) and the shift to an unvegetated state dominated by highly anoxic silty sediments in 2016 (right). In the period 2016-2017 the total vegetated area increased by 4,421 hectares, which represent a recovery of 38.9% of the area lost in the precedent period. Most of this recolonized area corresponded to monospecific meadows of *C. prolifera* between the vegetation depth limit recorded in 2016 and the 4.5 m isobaths (Figure 2). This rapid recolonization can be explained by the recovery of water transparency during the macrophytes growing season (from March to July 2017; Figure 1) and the very fast growing rates of this opportunistic alga. By contrary, the seagrass *C. nodosa*, with considerably lower growth rates, only recolonized 0.87% of the lost area during the same period. This work has evidenced that macrophyte-dominated marine ecosystems can follow a non-linear dynamic response to sustained eutrophication, experiencing a sudden collapse after a long period of apparent stability. The ecosystem shifted to an unvegetated state once a critical environmental threshold has been surpassed, even in a single disturbance event (phytoplankton bloom). After collapse, our results suggest that the rate and fate of the recovery path up to the former vegetated state will depend on a complex interplay between the inherent characteristics of the species, recovery of water-column conditions and climatic variability. Therefore, the patterns of macrophyte community recovery could be complex with the potential to follow nonlinear dynamics or “hysteresis” that can lead to bistability in the ecosystem (Scheffer et al. 2001). Moreover, the rapid recolonization rate showed by *C. prolifera* could represent a positive feedback mechanism favoring the recovery process, but the lack of such response by the seagrass *C. nodosa* means that ecosystem could remain in a state with lowered resilience and hence more vulnerable to future disturbance events.

Acknowledgements

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Keywords: Eutrophication, Ecosystem shift, Nonlinear responses, *Cymodocea nodosa*, *Caulerpa prolifera*

Chemical Characterization of a New Acidified Region to Study Ocean Acidification in Subtropical Ecosystems

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Human activity is generating an excess of atmospheric CO₂, creating what we know as ocean acidification, which it is predicted to cause important changes in marine ecosystems. Until recently, most of the research on this phenomenon has been carried out under laboratory conditions, with small-scales, using few representative species and live cycle stages. These limitations raise the questions about reproducibility of the environment, including indirect effects and synergies. One way to solve these problems, inherent to laboratory experimental procedures, is by conducting studies in natural areas where expected future pH conditions already occur, as is the case of CO₂ vent systems. Here, we present a new natural CO₂ system located off the southern tip of La Palma Island, in an area called Fuencaliente (Canary Islands, Spain), that can be used as an analogue to study the effects of ocean acidification on the marine biodiversity. However, several factors need to be considered a priori, such as the characteristics of CO₂ emissions, pH gradients, alkalinity, the replications of seeps and the influence of other gases or natural substances. We show the chemical characterization of the carbon system off the coast of Fuencaliente using a VINDTA system, as well as the concentrations of metals and macroconstituents (K, Na and Ca) analyzed using an optical atomic emission spectrometry with inductively coupled plasma (ICP-OES), and nitrate, phosphate and silicate measured using a Technicon AutoAnalyzer II. After the exploration of the area, several emissions points have been found with similar chemical features and without harmful elements for life. Although this kind of acidified systems may be rather dissimilar from future oceans' conditions, this area of La Palma island is an exceptional spot to study the effect of natural acidification on marine biodiversity. These studies can then be used to understand how life have persisted through past Eras, with higher atmospheric CO₂, or to predict the marine ecosystem consequences of the tomorrow oceans.

Keywords: CO₂ vent, Atlantic Ocean, volcanic activity, Shallow rocky habitat, Natural laboratory

History of red coral (*Corallium rubrum*, L. 1758) change in biometric parameters and carbon retention capacity: a meta-analysis in NW Mediterranean

Miguel Mallo, Patrizia Ziveri, Victoria Reyes-García and Sergio Rossi

The lack of published long time-span and geographically wide data of red coral (*Corallium rubrum*; Linnaeus, 1758) is limiting our capability of determining its ecological historical role. This study is addressing this limitation by compiling historical *C. rubrum* biometric ecological data to tackle population trends and carbon retention capacity and determine how the Mediterranean population changed over time. To achieve this goal, we performed a meta-analysis using quantitative and qualitative data obtained from academic and grey documents retrieved from scientific web browsers, scientific libraries, requests to scientists, and from the references of the pre-selected documents from all previous sources. From this documentation, first we identified, and then compiled information on red coral's most reported biometric parameters within a depth limit of 60

m: basal diameter, height and weight inside the Catalan Sea and the Ligurian Sea. For both regions, yearly averages were obtained for each parameter. We also estimated *C. rubrum*'s potential area, colony numbers, total weight, carbon input, and carbon retention in its skeleton. Red coral's basal diameter, height and biomass values decreased in both regions until the 1990s. Then, from the 2000s, the value of such parameters increased, surpassing the levels of the 1960s (Liguria) or slightly lower than the 1980s (Catalan Sea). The carbon flux difference between the oldest (1960s: Liguria; 1970s: Catalan Sea) and the lowest (1990s) biomass value is nearly double. Recovery trends in *C. rubrum* observed in the last decades are biased due to the data from protected colonies concentrated in few areas, leaving large unprotected coral areas without quantitative information. The shift in the decreasing trend observed in the 1990s coincides with the exhaustion of coral fishing banks and the Mediterranean trawling ban. The recovery of *C. rubrum* signals the effectiveness of protection measures. Recovery in the Catalan Sea is lower than in the Ligurian Sea due to more frequent poaching events in the former, where the coral is of higher quality. *C. rubrum* mitigation capacity for climate change (in the form of stored carbon) has drastically changed in few decades. Probably the red coral nowadays, in these limited protected areas, is a pale reflex of what it was only one century ago all over the Mediterranean Sea.

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Keywords: *Corallium rubrum*, Red coral, Mediterranean, historical marine ecology, Catalan Sea, Ligurian Sea,

Macroalgal diversity along the North Portuguese shores

Marcos Rubal and Puri Veiga

Rocky shores are frequent habitats along the North Portugal coastline. Macroalgae are one of the most diverse and ecologically relevant assemblages on rocky shores. The aim of this study is to explore the diversity patterns of macroalgae along the North of Portugal. Ten rocky shores were studied: Moledo, Areosa, Viana, Amorosa, Belinho, Labruga, Mindelo, Cabo do Mundo, Foz and Aguda. At each one of these rocky shores, three tidal levels were sampled (i.e. high, medium and low). At each tidal level, three different sets of three quadrats (20 x 20 cm) were scraped from the exposed substrate (tidepools were not considered). In rocky shore studies, a single quadrat covering only 0.04 m², is known to sample only a small fraction of the macroalgal species at a site because of small-scale spatial variation. For this reason, at the present study, data analyses were done on species abundance data pooled over 3 replicate quadrats set (0.12 m²), which was considered as 'sample'. Based on the samples, we calculated the total number of species, the Shannon index, using the biomass of each species as proxy of their abundance. We also considered the number of exclusive species, those that only were recorded on a single shore during the sampling. A total of 83 species were identified. Viana was the shore with higher number of species (46) while Moledo showed the lower number of species (21). Viana also showed the higher number of exclusive species (6) while Cabo do Mundo did not harbour any exclusive species. However, results of the Shannon index showed its higher values at Aguda (2.55) and the lower at Areosa (1.6). No clear latitudinal pattern on macroalgal biodiversity was found despite the clear latitudinal gradient in urbanisation with very populated areas in the south (e.g. Vila Nova de Gaia, Porto, Matosinhos) and lower population density in the north (e.g. Caminha, Viana, Esposende). The presence of the main river discharges (Minho, Lima and Douro) did not seem to be a major driver on the distribution of macroalgal diversity. Therefore, local drivers at the shore scale seem to be the main responsible for shaping the diversity of macroalgal assemblages.

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Keywords: North Portugal, rocky shore, macroalga, Biodiversity, Atlantic Ocean

Deep water fish ontogenic changes in habitat preferences in several NE Atlantic ecosystems

Alberto Serrano López, Antonio Punzón Merino, Francisco Sánchez Delgado, José Manuel González Irusta, Ana de la Torriente Díez and Marián Blanco Ginér

Seamounts, caps, banks and canyons have been described as essential habitat for several fish species. Fish can partition habitat resources between species as well as between size classes within a species (ontogeny). These ontogenic changes in distribution of mobile animals may reflect decisions on how to balance conflicting demands associated with foraging and avoiding predators and competitive exclusion. In this paper, ontogenic shifts in habitat preferences of fish are analyzed in different ecosystems: Le Danois Bank, Galicia Bank, Avilés Canyon and the northern Spanish shelf break and upper slope. Data were obtained from 15 multidisciplinary surveys carried out in 2003, 2004, 2008, 2009, 2010 and 2011. Fish species matrix was reduced only to those species with biomass higher than 1% in one of the ecosystems. These species have been separated in 10 cm size classes. To study the combined effect of environmental variables on fish assemblages, a Canonical Correspondence Analysis (CCA) was performed. Variables used in the analysis were depth, near-bottom temperature and salinity, sedimentary characteristics (weight percentage of gravel and coarse sands, medium and fine sands, silt, and weight percentage of organic matter), and biogenic complexity (as biomass of large sessile species). Most of fish species follow a bigger-deeper strategy. When they are juveniles live in shallower depths and when they grow up, move to deeper waters, mainly in canyons and seamounts. Most of these species are eurybathyal. Other species inhabit only on the shelf. They are slope estenobathyal species. The distribution of 6 species (3 eurybathyal and 3 estenobathyal) were modelled using General Additive Models (GAMs) in a Distribution Models framework. For each species we modelled several size classes, showing the differences in distribution and ecological niche. Our results highlight the importance of canyons, seamounts and banks are essential fish habitats, offering nursery areas and shelter for reproducers, and providing different ecological niches which reduces competition and promotes co-existence between species and also between intraspecific age groups.

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Keywords: deep sea, size structure analysis, habitat complexity, seamounts, canyons, Deep-water fish, habitat partitioning

New data of Tetractinellida (Porifera) in Aviles Canyon system and Le Danois Bank (Cantabrian Sea). Results of the SponGES Project

Javier Cristobo, Pilar Ríos and Paco Cardenas

The Avilés Canyon System (ACS) and Le Danois Bank (LDB) are two of the seven case studies (CS4 and CS5) in the European Project SponGES (Deep-sea Sponge Grounds Ecosystems of the North Atlantic: an integrated approach towards their preservation and sustainable exploitation). The ACS is a complex canyon and valley system designated since 2014 as a Site of Community Importance as defined in the European Community Habitats Directive. The complex comprises three main canyons of different morphostructural character: La Gavierra, El Corbiro and the Avilés Canyon. Le Danois Bank is a large seamount that rises abruptly from the Bay of Biscay abyssal plain at nearly 5000 to 425 m at its summit. This is the 1st Marine Protected Area in Spain since 2008. SponGES_0617 and ECOMARG_2017 were the last expeditions for prospected sponges grounds in these areas, which were explored before during INDEMARES and ECOMARG projects. Samples were collected with Rock Dredge and Beam Trawl during SponGES_0617 expedition while in ECOMARG_2017 we used Photogrammetric Sled (PS) and ROV for recording and collecting the fauna. In the 1st expedition we visited 29 stations between 240 and 1525 meters. 16 were prospected with Rock Dredge and in these areas, sponges

are the most abundant phylum, while in sedimentary bottoms of 13 stations prospected with Beam Trawl, Echinodermata was the most important. The sponges of Order Tetractinellida in the sampled area, represents 33 % of total of samples, 21% of which are "Lithistides" principally of the Families Corallistidae and Siphonidiidae. Species of Tetractinellida of this communication belongs to Suborders Astrophorina and Spirophorina; in the last, only one species of Genus Craniella is included. Within Astrophorina 6 Families were represented: F. Geodiidae Gray, 1867, F. Pachastrellidae Carter, 1875, F. Theneidae Carter, 1883, F. Thrombiidae Sollas, 1888, F. Calthropellidae Lendenfeld, 1907, and F. Vulcanellidae Cárdenas, Xavier, Reveillaud, Schander & Rapp, 2011. In the Family Geodiidae, we had found 8 species. *Geodia nodastrella*, *G. pachydermata*, *G. anceps* and *Penares helleri*, are well known and consistent with the descriptions made by other authors, but only the 1st is previously documented in this area. The genetics studies of others species such as *Geodia cf. spherastrella* or *Erylus cf. nummulifer* are still pending to complete the description. *Caminella pustula* is know only from the south of the Azores archipelago and Le Danois Bank. The most characteristic species in this area is the *Geodia cf. barretti*, filmed many times by ROV and PS in other expeditions, but never collected directly until last ECOMARG_2017. Its habitus is similar to *Geodia barretti*, normally with smooth clear surface appearance. In our samples they have regular long hispidity caused for triaene spicules and founded too by Cardenas in the specimen of New England seamounts, but in this case they have short hispidity. We have too found small differences in spicules of our samples but it's necessary to make more chemical and genetics test for identifying these Cantabrian species, that somehow represent a separate population. In Family Pachastrellidae, we have 5 species in the area, being *Pachastrella ovisternata* the most frequent and very characteristic, the presence of a *Pachastrella cf. nodulosa* that requires genetics testing to know if is the same as *nodulosa*. In our samples, it is very interesting the presence of *Hexadella*, covering the surface of the sponge completely. Others species present are *Characella pachastrelloides* and *Nethea amygdaloides*. One of the most interesting results are record of a the probably new species of *Triptolemma* genus. In the Family Theneidae, we have one very abundant species, *Thenea schmidtii*, not documented in this area before, maybe because in previous expeditions she was automatically identified as *Thenea muricata*. Curiously, the small Family Thrombiidae with only 2 Genus, provide us 1 new Genus and 2 new species for science. One of them was been discovered before us, by Kelly and other authors in the South of Portugal. We have found at least 10 samples in 3 stations in Le Danois Bank between 650 and 676 m deep. The other new species of new genus was founded in Le Danois Bank too. This species is characterized by oxeas of 2 sizes, spiny dichotriaenas and acanthodichomesotriaenas. We have found 2 species of the Family Calthropellidae; *Calthropella geodioides*, documented before in this area by us in La Gavierra Canyon, while *Calthropella durissima* until now was only documented in the Azores Islands. Together with our samples, there is a wide distribution to the Cantabrian Sea in Le Danois Bank at 660 m. The samples found in this area from the Family Vulcanellidae are the very well known *Vulcanella gracilis* and *Poecillastra compresa*. We conclude that Le Danois Bank is a hotspot of biodiversity also in the porifera phylum, as already shown in other zoological groups. Figure 1. Map showing the main operations during the SponGES 0617 cruise to the Cantabrian Sea.

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Keywords: Biodiversity, SponGES Project, Taxonomy, Bathyal, Sponge aggregation

Distribution patterns of intertidal zoantharian species in the Canary Islands and their relation with ocean temperature

Cataixa L. Batista, Sonia Fernández Martín, Sergio Moreno-Borges, Adriana Rodríguez and Sabrina Clemente

Global warming is driving changes in the distribution patterns of many species, leading to a tropicalization and meridionalization of the biota. In this context, populations of some marine species are in current regression while others favoured by temperature increases are expanding their populations. Such is the case of benthic cnidarians belonging to the order Zoantharia and suborder Brachycnemina, whose populations are able to cause phase-shifts in corals reefs ecosystems (Cruz et al. 2016). Marine assemblages at the subtropi-

cal region of the Canary Islands consist of a combination of both temperate and tropical species (Brito et al. 2005), mainly due to the east-to-west seawater temperature (SST) gradient that naturally exists throughout the Archipelago (23–25°C in summer) (Figure 1a). Therefore, it constitutes a unique emplacement to study any biota reorganization processes due sea water warming. The aim of this study was to create a baseline of the distribution of intertidal zoantharian populations in relation to the temperature gradient along the Canary Islands. Surveys were conducted during 2015–2016 throughout the Archipelago at intertidal rocky platforms where zoantharian species could inhabit (Figure 1b). Surveys consisted in exhaustive search through the entire study area during monthly spring low tide by at least two people. Differences in zoantharian assemblages among the different islands were assessed by means of permutational multivariate analysis of variance (PERMANOVA). Species-specific abundance analyses were also performed with permutational ANOVAs. In all previous analyses, the size of the intertidal rocky platform surveyed at each location was taken into account as a covariable. Five intertidal Zoantharia species belonging to suborder Brachycnemina were recorded along the Archipelago: *Palythoa* aff. *clavata*, *P. caribaeorum*, *P. grandiflora*, *Zoanthus pulchellus* and *Isaurus tuberculatus*. Species identifications were sensu López et al. (2019). Additionally, 15 specimens of an unknown species were registered in Matas Blancas, Fuerteventura Island (Figure 1b). Although genetic analyses are necessary for an accurate species identification, external morphological features matched *Terrazoanthus* Reimer & Fujii, 2010 (suborder Macrocnemina), constituting the first record of this genus in the Canary Islands. A total of 2208 zoantharian colonies were recorded, being *P. aff. clavata* the dominant species (1754 colonies) that was registered in all islands (Figure 1b). The analysis of the abundance and composition of zoantharian assemblages showed a significant effect of factor "Island" ($F= 2.05$; $p=0.024$), highlighting significant differences in assemblages found in the coldest eastern island of Lanzarote (Figure 1a,b), in contrast to the other islands of the Archipelago (Figure 1b). Species-specific analyses of *P. aff. clavata* showed a significant effect of the interaction between factor 'Island' and covariable "Platform size" ($F= 5.09$; $p=0.003$). Densities found in La Graciosa were significantly higher than in the rest of islands, while abundances within Lanzarote were significantly lower (Figure 1b). Despite rocky platforms of La Palma, La Gomera and El Hierro are noticeably narrower than in the rest of islands, which reduces habitat availability, not differences in the density of *P. aff. clavata* were detected between these islands and Tenerife, Gran Canaria and Fuerteventura. In the case of the latter islands, the species also showed warm waters affinities showing higher abundances restricted to the warmest locations as well as in the widest intertidal platforms (Figure 1a,b). In the case of *P. caribaeorum*, no effect of "Island" but a significant effect of "Platform size" ($F= 7.73$; $p=0.049$) was detected over abundances. The two largest populations of the species were recorded in Lanzarote and Fuerteventura (Figure 1b) in extremely large and elevated rocky platforms which, combined to the wide tidal amplitude experienced in these islands (up to -1.8 m), leaves many tidepools separated from the open ocean and heating for several hours during low tide. These particular characteristics may explain why thermophilic *P. caribaeorum* is able to inhabit in the intertidal zone of the eastern colder islands of the Canaries. What is more, the other intertidal population of *P. caribaeorum* is almost restricted the warmest western coast of Tenerife, with only one extra colony that has been recently found in the southeastern coast (Figure 1b). This may be the result of a recent expansion of this species related to sea water warming. The distribution of *I. tuberculatus* did not show differences between studied islands, but a marginally significant effect of "Platform size" ($F= 3.70$; $p=0.073$) was observed. Discrete colonies were restricted to the low intertidal of the largest rocky platforms of Tenerife, Lanzarote and Gran Canaria, locations characterised by high hydrodynamic levels. *P. grandiflora* and *Z. pulchellus* were only recorded in Tenerife and, although *P. grandiflora* was registered in the warmest coast of the island, its relation to SST cannot be addressed since only one population was found. *Z. pulchellus* seemed to be also restricted to specific locations with the two largest populations recorded in the northern coast of Tenerife (Figure 1b), covering huge extensions over rocky platforms. We found clear distribution patterns related to the SST gradient throughout the Canary Islands only for intertidal populations of *P. aff. clavata* and *P. caribaeorum*. Other factors such as specific habitat features or interspecific interactions may be responsible for the abundance of other zoantharian species. Therefore, effective monitoring programs are recommended only for *P. aff. clavata* and *P. caribaeorum* populations in order to early detect any possible population expansions or even phase-shifts mediated by sea water warming that might lead to significant changes in ecosystems dynamics. Figure 1: Image of sea water temperatures (SST) recorded around the Canary Islands in summer time generated by satellites AVHRR/NOAA, showing the east-west gradient due to the influence of the Saharian upwelling (a). Distribution and abundances of zoantharian species around intertidal habitats throughout the Canary Islands: La Palma (LP), El Hierro (EH), La Gomera (GO), Tenerife (TF), Gran Canaria (GC), Fuerteventura (FV), Lanzarote (LZ) and La Graciosa (LG)

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Keywords: Coastal zone, Bioindicator, *Palythoa* spp., ocean warming, Monitoring

Using biological traits to assess fishing gear impacts in the seabed. The INTEMARES experience

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The application of Biological Traits Analysis to assess fishing gear impacts in the sea bed is reaching growing importance in the last years (e.g. Tillin et al., 2006; De Juan et al., 2007; De Juan and Demestre, 2012; Foster et al., 2015; González-Irusta et al., 2018) driven by the implementation of the ecosystem approach into the management of the marine ecosystems. Most of this effort has been focused on analyzing trawling impacts whereas the impacts of other gears such as longline or gill nets have not been analyzed with the same intensity despite being widely used on Vulnerable Marine Ecosystems (VMEs). In the framework of the INTEMARES project (www.intemares.es), the impact of three different gears (otter trawl, gill nets and longlines) have been analyzed in the benthic communities of the Aviles Canyon System, a Site of Community Importance (SCI) of the Spanish marine Natura 2000 network. The soft and hard bottoms of the studied area were sampled using a wide range of methodologies (beam trawl, photogrammetric sledge, box corer, rock dredge) in sites with different levels of fishing effort for each analyzed gear. The fishing effort was determined using data from the Vessel Monitoring System (VMS) of the fishing boats, which provides boat's location and other relevant information every 2 hours allowing to delineate fishing effort maps with a resolution of 500 m (Punzón et al., 2016). The sampled benthic species (excluding fishes) were analyzed using the same traits and scores applied in the BESITO index (González-Irusta et al., 2018) which includes; size, longevity, attachment type, benthic position, flexibility, fragility and feeding habit. For each trait, scores from 1 to 4 were assigned, being 1 the less sensitive category to the trawling and 4 the most sensitive category. A complete description of the traits and the scores can be consulted in González-Irusta et al. (2018). Gill net and trawling impacts were apparent in most of the analyzed traits (Figures 1 and 2 respectively) whereas longline impacts had a less clear effect on benthic species. Some traits, such as longevity or size showed a common negative response to trawling and gill net impacts whereas others showed a specific response to each gear (e.g. feeding habit or motility). The results shown in this work are a first step in the development of new metrics, which will allow to extent the BESITO index approach to other fishing gears such as longline and gill net. The development of common methodologies to assess fishing impacts of different gears is a necessary step to allow the analysis of cumulative impacts, a key point in the implementation of the ecosystem approach and its legislative frame (Marine Strategy Framework Directive, Habitat Directive, Marine Spatial Planning Directive). FIGURE CAPTIONS Figure 1. Gill net impact. Evolution of each trait category relative abundance (%) across a gill net impact gradient with five categories; Very low effort, low effort, medium effort, high effort and very high effort. Trait categories: score 1 (light blue), score 2 (dark blue), score 3 (light green), score 4 (dark green). Figure 2. Trawling net impact. Evolution of each trait category relative abundance (%) across a gill net impact gradient with five categories; Very low effort, low effort, medium effort, high effort and very high effort. Trait categories: score 1 (light blue), score 2 (dark blue), score 3 (light green), score 4 (dark green).

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Keywords: Marine protected area (MPA), vulnerable marine ecosystem (VME), fishing impacts, gillnet and long

Simulating trophic impacts of recreational fishing scenarios on two oceanic islands using Ecosim

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A mass-balance trophic model was built to describe the impact between 2006 and 2016 of the different fleets that operate on the marine ecosystem of the Tenerife-Gomera islands. Thirty-one functional groups were defined, corresponding to benthic, demersal, and pelagic domains, also including the deep scattering layer (DSL) and detritus. Keystone index and mixed trophic impacts matrix showed a bottom-up control in the benthic-demersal area while in the pelagic zone intermediate predators such as cephalopods play a bigger role in the ecosystem. Toothed whales, turtles, oceanic sharks and tunas were identified as a potential keystone species in the pelagic domain, and sparids, epidermials fish, and cephalopods also were considered keystone groups in the benthic-demersal domain.

The assessment of the impact that recreational fishermen exert on most groups is highly dependent of methodology used to collect information of captures and effort from anglers. When the most conservative scenario is considered, the impact of recreational fishing on the ecosystem would hardly be relevant in the short-term. However, if considering two other studies based of on-site survey, the biomass of fish groups, mainly serranids, sparids and herbivorous, will greatly decline, coinciding with the overfishing scenario described for the Canary Islands in the scientific literature.

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Keywords: Ecological Indicators, Ecosim, Fishing impact, Network analysis, Recreational fishing, Trophic model

Investigation of juvenile Angelshark (*Squatina squatina*) habitat in the Canary Islands to inform protection of this Critically Endangered species.

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A unique stronghold for the Critically Endangered Angelshark (*Squatina squatina*) is found in the Canary Islands, where the species is still frequently encountered by divers and fishers. This provides a great opportunity to conduct research into Angelshark ecology, as limited understanding of Angelshark habitat use, movement and site fidelity at is a major factor preventing effective species protection and conservation. A key focus for Angel Shark Project: Canary Islands (ASP:CI) (a collaborative initiative between Universidad de Las Palmas de Gran Canaria, Zoological Research Museum Alexander Koenig and Zoological Society of London) is the investigation of juvenile Angelshark habitat in the region and identification of Angelshark nursery areas. The definition of shark nursery areas varies greatly in scientific literature, but ASP:CI have developed a multidisciplinary approach (using citizen science sightings, satellite imagery, focal surveys and mark-recapture techniques) to test the three nursery area criteria outlined in Heupel et al. 2007. Research over the last four years has identified that juvenile Angelsharks are present across a number of shallow, sheltered beaches in the Canary Islands. The team have identified 2 confirmed nursery areas, 13 potential nursery areas and 21 beaches where juvenile Angelsharks are observed. Mark-recapture work at the largest confirmed nursery area, Playa de Las Teresitas in Tenerife with 424 sharks tagged confirms that juvenile Angelsharks remain present for up to 15 months. A comparison of prey species composition was carried out between the different studied areas showing that the sand smelt (*Atherina presbyter*) was the most abundant species in 13 of the 15 areas studied, thus establishing a possible direct relationship between the two species. In addition, four species of the sparidae family were among the eight most abundant species. Data were used to develop a Guidance Document on the identification and protection of juvenile Angelshark habitat in the Canary Islands, which identifies eight key recommendations to improve protection of these areas.

Keywords: Angelshark, juvenile, nursery areas, Canary island, mark-recapture

Stock structure of the common mora in the mid-North Atlantic (Azores archipelago)

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With the spatial expansion of bottom longline fishing effort in the offshore areas of the Azorean Exclusive Economic Zone (EEZ) during the last decades, the common mora (*Mora moro*) became a commercially exploited resource in the Azores (mid-North Atlantic). It is the most important demersal species caught in deep waters between 700 and 1,200 m. It presents a relatively sedentary behaviour, supporting the possibility of the existence of local populations constituting different management stocks in the Azores. However, it was not possible until now to define if the Azorean component of the population can be considered as a discrete fishery management unit (FMU). The stock structure of this species remains poorly understood in the Azores region. Consequently, there is no analytical assessment for this resource. In this study, we summarized the detailed information on the catch, fork length, weight, sex, and maturity from targeted surveys in the Azores waters, complemented by fishery data, over the past c. 25 years. This study results from a recommendation of the International Council for the Exploration of the Sea (ICES) working group on the biology and assessment of deep-sea fisheries resources (WGDEEP), with the objective of examining the population structure of *M. moro* in the Azores archipelago based on all available data to test the hypothesis that the stock of this species in the ICES Subarea 10a2 represents a FMU. Results are presented and possible assumptions and strategies for the assessment are discussed.

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Keywords: Mora moro, Commercial fish, population structure, assessment, Management, deep-sea

Long term evaluation of spearfishers tournaments in the Canary Islands

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Recreational fishing shows a growing number of followers, but at the same time it is a difficult fishery to assess, due to the lack of records of the activity and captures. In the present work it has been analysed the information collected from 118 spearfishing tournaments held in the Canary Islands between 2007 and 2017. The number of recreational anglers in the archipelago has increased over 50% in this decade, passing from 57,000 in 2007 to 114,000 in 2011. Currently the number of recreational fishing licences is about 90,000, 6.8% of which are spearfishermen. The levels of catches vary between islands and years analysed, with a catch per unit of effort that oscillated between 0.06 and 2.11 Kg/spearfisher/hour ($X=0.62$, $SD=0.34$), and between 0.2 and 2.1 fishes/spearfisher/hour (between 15 and 343 fishes per tournament). Moreover, spearfishers show a great selectivity toward a few species, highlighting the seabreams and parrotfish. This makes the evaluation of the fishing tournaments a useful tool to know the impact of this activity and better understanding of their role on the status of fish stocks in the Archipelago.

Keywords: Recreational fisheries, Canary island, spearfishing, Tournament, Archipelago

Effect of diet on growth and development of the jellyfish *Phyllorhiza punctata* (Cnidaria, Scyphozoa) in laboratory conditions

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In recent years, interest in jellyfish rearing has increased for many purposes, such as scientific research – considering their major impacts on marine ecosystems and their venom's properties, and more recently for ornamental aquaculture. Although their crescent value, the maintenance of these delicate organisms requires specific methods and even with the latest technology available, breeding most of jellyfish species is still a challenge. Studies on the nutritional needs and optimal rearing conditions for jellyfish are still very incipient and require several technical and scientific advances, in order to allow an optimal growth and development of these organisms in captivity. The aim of this study was to evaluate the effect of four different diets on the growth and development of adult specimens of jellyfish *Phyllorhiza punctata* (Cnidaria, Scyphozoa), an emerging species on the ornamental trade, indigenous to the tropical western Pacific Ocean and considered an invasive species on several coastal waters worldwide. For the experiments, 120 juveniles of *P. punctata* were cultivated in Kreisel tanks connected in a recirculation water system (one individual system with 3 replicates of 10 specimens for each diet). All diets consisted in the supply, four times a day, of *Artemia franciscana* metanauplii enriched with SELCO, with the daily addition of a different supplement for three of the diets. Diet M with a supplement of mashed mussel (*Mytilus edulis*), Diet H with hake (*Merluccius merluccius*) and Diet S with soybean (*Glycine max*). Control diet consisted of exclusive feeding with *artemia metanauplii* enriched with SELCO, four times a day. The experiments lasted for 62 days. The results obtained for the growth of all the analyzed individuals revealed higher values of bell diameter for the Diets M, H and S (5.22 ± 2.44 cm, 4.99 ± 2.14 cm and 5.32 ± 2.87 cm, respectively), when compared to the control (3.04 ± 1.91 cm), with statistically significant differences between control and Diets M and H (Kruskal-Wallis, p -value = 0.039; Dunnett's test, p -value < 0.05), although Diet S shows a relatively higher growth. However, due to the growth similarity for Diets M, H and S in this study, there are no statistically significant differences between the three (p -value > 0.05). When

analyzing the growth of bell diameter excluding deformed specimens, the control diet still shows the lowest growth (4.24 ± 2.15 cm), and exhibits statistically significant differences between Diets M, H and S (6.73 ± 2.34 cm, 5.96 ± 1.28 cm and 8.52 ± 2.43 cm, respectively) (ANOVA, p -value = 0.04, Dunnett's test, p -value < 0.05). Although no significant differences were observed between Diets M, H and S (p -value > 0.05), the differences between the growth with these diets are noticeable. Diet S provides the highest and fastest growth of the bell diameter as well as a more representative morphology of the species in the wild. These results suggest that this species has the capacity to feed on organic matter of both animal and vegetable sources, which represents an important contribution to the improvement of rearing and nutrition techniques of jellyfish in aquaculture. However, further studies are needed in order to better understand the nutritional needs of most jellyfish, as well as the effect of the diet on their growth and physiological development.

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Keywords: Diet, Growth, Scyphozoa, Ornamental aquaculture, Medusae, Soy (*Glycine max*)

Environmental Education, Citizen Science and Dissemination of Science in a coastal Atlantic community: the role of the CMIA of Vila do Conde

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The Center for Environmental Monitoring and Interpretation (CMIA) of Vila do Conde is a municipal infrastructure with scientific coordination of the Interdisciplinary Center of Marine and Environmental Research (CIIMAR). As a promoter of Environmental Literacy and Science Communication, the main objective of the center is the development of awareness and environmental education actions for the society. Additionally, the center is also involved in the monitoring of several environmental descriptors, contributing for the improvement of the environmental status of the regional area. CMIA focuses its activities on four complementary areas, such as research, monitoring, environmental education and scientific dissemination, contributing to a change in behavior, always promoting environmentally friendly options and environmentally sustainable attitudes towards today's society. CMIA promotes actions of environmental awareness and dissemination of science through the development of scientific exhibitions and pedagogical activities inside and outside, alerting the public to the environmentally threatened realities, seeking to respond to environmental problems through the stimulation of discussion forums and training courses. Thematic exhibitions are communication and dissemination vehicles of science, and one of the tools used in the area of environmental education, mainly as a way of diffusing science to a diverse public. CMIA exhibitions use a visual language to show important aspects or situations of the environment in diverse scientific areas, addressing different themes from different points of view. Some of them like "Journey to the Deep Sea", "Pollution of the Seas: The Heritage of Humanity?" or "Discovering the World of Marine Macroalgae!" approach themes related to the Ocean, its problems or its biodiversity. After being exposed in CMIA, CMIA exhibitions are available free of charge to other centers, agencies and entities that carry out activities related to education and environmental dissemination, increasing in this way the scope of CMIA, both geographically and in regard to the number of persons covered. By executing a non-formal education, CMIA seeks to take into account the personal needs of its visitors as well as the school community. It presents a strategy based on the promotion of a high diversity of activities, aiming different audiences, from pre-school to adults, thus involving all civil society. Moreover, to develop the critical spirit of the participants, various modalities are presented with the objective of raising awareness on environmental issues, both in the space where our visitors are inserted and at a global level, enabling them to understand and overcome their limitations and possibilities of action. In this way, our activities take place both in our facilities and in commuting to and from school, either individually or as a group, on ordinary days or in the form of a celebration of ephemeris (days and theme weeks). The non-formal nature of the educational actions carried out by CMIA makes its activities widely requested, having much to offer to formal teaching institutions to complement its activities, allowing the learning of scientific concepts and environmental values

in a more playful context. Another of CMIA actions are the nature exploratory walks as “Marine Biodiversity”, “Discovering the Coast” and “Discovery of the dune flora”. Although these were one of the activities that were implemented in the early days of CMIA, it was only in 2013 that they emerged with more strength, becoming from that moment a great asset of the team. CMIA felt the need to make its activities more interactive and we verified that as we were offering different nature exploratory walks, they had immense support from the community in which they are inserted and within all age groups. Nature exploratory walks are activities that can be established as an instrument for the development of citizenship through environmental education, social awareness and sensitization to socio-environmental problems of the community. These actions contribute for the search of solutions through critical reflection, enabling the development of participative activities of citizen science. These exploratory walks allow the identification and classification of different species and their importance for the biophysical balance, allowing the participants to realize that all areas and especially protected areas have a fundamental role for the different species, making the participants aware of the role of citizen science and the role that each of us plays on the protection of these habitats and species. CMIA also invests in several activities that allow an adult audience in the beginning and / or career progression to improve its knowledge and development of personal, civic and social skills in various scientific and environmental related fields. In the summer, CMIA works as a Blue Center for the Blue Flag programme, performing playful-pedagogical games (Ocean Clean or Discovering the Oceans) and promoting various workshops both in its facilities and in the beaches of Vila do Conde awarded with the Blue Flag, adopting a proactive role in the development of attitudes and bases for a more responsible citizenship. CMIA intends to maintain the diversity of pedagogical activities offered to the public, contributing to increase the scientific literacy of the population, disseminating the importance of environmental and ecological values, providing information, training and education to all. We intend to promote the involvement in environmental issues, so that together with the population we can contribute to the maintenance and promotion of the environment.

Keywords: Environmental Education, Public mobilization, citizen science, Scientific literacy, Science dissemination

Funchal Marine Biology Station: 20 years of research and promotion of Madeiran and Macaronesian marine biodiversity (NE Atlantic Ocean)

Mafalda Freitas and Manuel J. Biscoito

The Funchal Marine Biology Station, on the Portuguese island of Madeira, is a research centre owned by the Municipality of Funchal and dedicated to research and education in marine sciences. It was inaugurated on September 28th, 1999 and its main goals are to develop the sciences and technologies of the sea, particularly, biology and ecology of coastal and deep sea waters. The Station is the base for the Marine Biology team of the Natural History Museum of Funchal and the Faculty of Life Sciences of the University of Madeira. These two teams participate in several research projects including studies on the fish and crustacean diversity of Macaronesian deep waters, coastal biology and ecology, biology and migration of sea turtles and marine pelagic birds' biology and migration. Simultaneously, the Station also carries out educational activities to promote knowledge about sea habitats and environmental education, either in an institutional way, or working in collaboration with other public and private entities. During the last 20 years the Station has contributed with 46 scientific papers, 20 research projects, 30 scientific campaigns and expeditions, 14 didactic publications, 2 temporary exhibitions, and has received more than 12,000 visitors in guided tours. The didactic activities in 2018 are the object of this presentation.

Keywords: Funchal Marine Biology Station, research , promotion, Madeira Island, Marine Biodiversity, Didactic activities

Improving perception and acceptability of aquaculture through hands-on experimental activities for schools

Marta V. Correia, Helena Peres, Marisa Almeida and Laura Guimarães

World fish production peaked at around 171 million tonnes in 2016, with aquaculture accounting for 47% of the total, or 53% if we exclude non-food uses (including processing for fishmeal and fish oil) (FAO, 2018). Aquaculture offers enormous potential to meet the demand for food associated with the global population growth and is an alternative to the exploitation of natural resources. Natural resources have stagnated in recent years due to intensive fishing, which has led to a reduction of the fishing resources populations, and due to the fisheries restrictions implemented as a measure for the conservation of fish stocks. Furthermore, fish consumption should be stimulated as it helps to prevent the onset of cardiovascular diseases and contributes to the reduction of risk factors for diseases associated with obesity, being also an important component of the Mediterranean diet. This growing importance of aquaculture comes at a time when the world becomes more aware of environmental issues and consumers have become more attentive, increasingly demanding safer products (Bacher, 2015). Thus, despite all the efforts made in the last decades to improve sustainable aquaculture practices and the technological innovations implemented in this area, the resources produced in aquaculture are still socially less accepted and associated to a very negative public perception. So, new approaches are needed to increase aquaculture social acceptability, being this one of the aims of the current project. To improve public perception of aquaculture it is also important to increase the scientific knowledge of the society about the ocean, i.e. Ocean Literacy. On this subject, young people are a key target for their importance in future socioeconomic development. The Ocean is an important source of countless resources and services of inestimable value. Its protection is therefore essential and a global priority. Although crucial for its protection, integrated understanding of the influence of the Ocean on mankind and the influence of mankind on the Ocean is still rarely addressed in school curricula or in complementary activities offered to young people. It is, therefore, necessary to promote a comprehensive discussion about integrated knowledge of the Ocean, particularly to the new generations. Through the development of experimental activities to be carried out by students in their schools or during visits to CIIMAR we intend to increase Ocean Literacy and address a wide variety of topics related to the Ocean, including those related to aquaculture and aquaculture products. One of the activities provided is on the topic Integrated Multi Trophic Aquaculture (IMTA). This activity allows students to build a laboratorial IMTA system and to recognize it as a nature-based solution for the sustainable exploitation of marine resources, by minimizing the impact on the environment and fostering economic valuation through the diversification of products. With this hands-on experiment, students work essential aspects of biodiversity, concepts of trophic webs and eutrophication associated with the discharge of effluents enriched in inorganic nutrients, as well as principles of circular economy improving their perception and acceptability of aquaculture. A questionnaire was developed to assess if the involvement on the IMTA hands-on experimental activity had the capability of increasing Ocean Literacy and knowledge about aquaculture-related topics and concepts in our participant students and understand if the importance they attribute to the Ocean has changed in any way after the activities. A total of 60 students came to perform the activities at CIIMAR for one week during scientific holiday weeks organised for them in the Christmas, Easter and Summer holidays of 2016 and 2017. Students were from the 3rd cycle of basic education and high school of different schools located in northern Portugal. In each week students did five different activities related to topics of Marine Sciences, including the IMTA experimental activity. The questionnaire was based on items with a Likert scale response and multiple-choice questions and was distributed at the beginning and at the end of the week of activities. The results of these questionnaires showed that the knowledge regarding several of the topics related to aquaculture and fisheries had increased after the week of activities. For example, the percentage of respondents that, after the week of activities had assumed that they knew what “Fishing Stocks” were increased from 18 % to 68%, with the option “I’ve never heard of it” decreasing from 47 % to 10%. Results also showed that the importance the participants attribute to the Ocean also increased. For instance, to the question “How important is the Ocean to you?” the percentage of answers “Extremely Important” increased from 35 % to 43 %. In conclusion, these hands-on activities can be a valuable tool to increase Ocean Literacy, teach environmental sustainability and other ecological concepts and allow students to understand the concepts and the technology behind aquaculture. These activities can, therefore, increase the knowledge, perceptions, and acceptability of aquaculture and its products.

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Keywords: Public perception of aquaculture, Ocean literacy, Hands-on activities, Questionnaires, Integrated multi trophic aquaculture

The Importance of Environmental Education Activities Assessment: the “School Of Nature” Project Case Study

Leonor Cruz, Liliana Vasconcelos, Susana Pereira, Soraia Castro, Marisa Gomes, Fábio Faria, Marta Pascoal, Pedro Gomes

Environmental Education (EE) is an area that has been gaining importance in today's world, by its important role in the transmission of knowledge, skills, attitudes and values for a conscious and responsible citizenship. Through EE, the individual takes on certain behaviors and internalizes a certain set of values that will gain “life” through attitudes. EE, by its objectives, aims essentially at the acquisition of knowledge and, consequently (we hope...), the change of values and attitudes (Vasconcelos, 2013).

Since 2011, the Viana do Castelo Environmental Monitoring and Interpretation Center (CMIA-VCT) has been developing, among other initiatives, the School of Nature environmental education project. This is an educational project that aims to bring young school communities (and indirectly the community in general) closer to the natural heritage and Natura 2000 sites in the region, encouraging and fostering knowledge and respect for and appreciation of biodiversity and the local natural ecosystems (the sea, the river and the mountain). As a well-defined work methodology and with a set of educational resources prepared specifically for the target age groups of the local educational project, until 2019 it has involved 2 899 students, 162 classes, with 387 field events and 255 classroom activities and study visits.

There are numerous on-going environmental education projects in Portugal, but few of them carry out the necessary evaluation of its effects on the participants. Evaluation is a fundamental step in order to correct the course of action, to reinforce its positive aspects or even to define new paths and improve the quality of the actions developed. (IPAMB, 2000). In this sense, in the 2011/2012 academic year, the CMIA-VCT developed a study to adjust an evaluation methodology that would allow the analysis of the extent to which this educational project promoted the acquisition of knowledge, as well as changes in behavior and attitudes in the students involved. This study allowed us to improve the methodology of the educational project itself and the pedagogical interventions over the following academic years.

The assessment focused on applying “pre-test” and “post-test” questionnaires to each of the students involved in different project phases. The pre-test was performed at the beginning of the first school period, before starting any kind of activity or approach of concepts. The “post-test” was applied in the third school period, at the end of all the pedagogical interventions of this educational project. The questionnaires were developed according to the target age range, the syllabus of the subjects of the natural sciences areas and the concepts that will be explored along the various pedagogical interventions. They have exactly the same structure and content so that an analysis of the impact the project may have had on different areas of student learning process was possible. The same set of questions were made, with the possibility of 2 types of answers: a) closed ones, conditioned to the type of answer to be given and with a clearly quantitative assessment; b) open ones, allowing the analysis of other concepts and also the student's perception about nature conservation in general and the importance of studying and protecting natural values in particular.

From the overall analysis of the results it was found that the contextualization of learning, provided by pedagogical interventions, in the context of classroom and fieldwork, contributed positively to a significant conceptual change (figure nº.1). As the age of the students increases, it was found that the students' level of pre-knowledge about the subjects also increased and, therefore, the differences after the pedagogical interventions were minor (figures 1a to 1d). Regarding the assessment of attitudes, it can be concluded that the activities developed under the environmental education project did not significantly influence the student's

predisposition for the preservation of the ecosystems under study.

All themes revealed statistical significant differences between the pre- and post-tests. The theme of the forest was the one that always represented the smallest amplitude in the correct pre-test and post-test answers, being also the theme with the highest percentage of correct answers obtained. The results of the themes associated with the coast (rocky beach and dune systems) showed a remarkable difference between initial and final evaluations, revealing that near-sea communities do not yet have the desired ocean literacy.

The proximity to the sea is not necessarily synonymous with recognizing its ecosystems as a natural heritage full of particularities and conservation values. Primarily, these natural spaces are valued for their landscaping and bathing function and not necessarily for all the biodiversity contained within and the extreme abiotic factors that characterize them. As for the river theme, which usually has a larger range in the pre-test and post-test answers, it is considered that the typology of activities developed is very well received by students and teachers. However, the theme in which the project was focused was not very familiar to most teachers and the contents to be explored were not initially associated with the ecosystem concerned.

Being one of the objectives of this project to ensure that each class “exhaustively” explores a particular environment - sea, river or forest - the assessment tool that was carried out has proved to be fundamental in the optimization of the intervention methodologies and processes developed and put into practice. In addition to the reinforcement and special attention that needs to be devoted to topics such as the coast and the riparian ecosystems, it was recognized that secondary students needed a different approach, as they, in general, presented high percentages of correct answers in the post-tests.

As a result, a new educational project called “Sea Scientists” was conceived and implemented, hoping to lead the older students to a higher level of demand. As this project was implemented only in the academic year of 2018/19, there are yet no results available.

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Keywords: Nature 2000; Environmental Education;

Acquired knowledge about Ciguatera Fish Poisoning in the Canary Islands population

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Ciguatera Fish Poisoning (CFP) is a human food poisoning caused by the consumption of fish that have toxins, commonly known as ciguatoxins. The precursors of these toxins are naturally present in the marine environment inside certain microscopic algae (dinoflagellates) and are transferred to the fish through the food chain. These toxins are not eliminated by any preservation or cooking technique. The only way to know if a fish is a carrier is through laboratory analysis (few days). According to Friedman et al. (2017) the CFP is the main disease caused by marine toxins worldwide. It is estimated that between 20,000 and 50,000 people suffer CFP annually (Marcus, 2018) although it is believed that there are many more because it is difficult to diagnose due to the variety of symptoms that presents (gastrointestinal, neurological and cardiovascular symptoms). The treatment is symptomatic and the patient improves in days or weeks, sometimes persists months or even

years, death is rare. In Europe until 2004 the CFP was only related to imported cases (travelers or fish from other latitudes). From 2004 to 2018 one hundred and twenty-two CFP have been reported in the Canary Islands (CI) (Spain). Madeira and the Selvagens Islands (Portugal) have also reported human cases (Vale, 2011). In the CI the CFP is considered an emergent notifiable disease although the risk is small due the low annual sick rate (Nuñez et al., 2012). In the CI since 2009 the catches of professional fishermen are being monitored for certain species and weights while in Madeira and Selvagens Islands some fishing restrictions have been implemented. Also since 2009 in the CI several projects and initiatives are being developed. These try to provide knowledge on CFP human epidemiology, ecology of microalgae, potentially affected fish, toxin detection methods, etc. According to Sanchez-Henao et al. (2019) the CI is already an area of endemic expansion of the CFP. Educating society is a critical component in the prevention of CFP, as it improves the ability to diagnose cases in humans (Friedman et al., 2017) and allows consumers to make informed decisions about food choices (FAO/WHO, 2016). The objective of this study was to assess the knowledge and perceptions of the CI population about CFP. Methodology The universe was the CI population. A not proportional allocation sample to the population size of each island was selected to reduce the level of error in the non-capital islands and to guarantee the reliability of the data in the estimates at the island level. Telephone surveys (n 1825) were conducted in 2018 (July, August and September) by sex, age and municipality quotas (error 2.3%, confidence level 95.5%). Results 87.2% of the population consumes fish on a regular basis. Fresh fish (multi-response) is the most consumed (81.2%), then frozen fish (53.6%). Fish caught by non-professional fishermen is consumed by 14.1% (some islands 40% is reached), of these, 79.4% do not consider the consumption of fish from this source as a health risk. To the question 'do you know what ciguatera is?' 89.6% of the population (n 1825) points out 'does not know or has not heard' of the ciguatera, the 3.8% 'do not know, but have heard' and 6.6% say 'yes'. From the interviewees who report knowing or having heard about, that is 10.4% of the total: 65.8% state that they have no information on human cases, 56.5% state that CFP have relevance in public health, 44.6% indicate that the cause is because the fish consume toxic algae while the rest declare not knowing or give wrong answers, 11.5% declare that the ciguatera can be eliminated (open answer) being freezing the main choice (55.2%), 12.9% notes that exist regulation to comply. 82% of the total (n 1825) wishes to receive more information about the ciguatera. According to the Regulation (EC) No 178/2002 about food law and safety the 'risk analysis' means a process consisting of three interconnected components - risk assessment, risk management and risk communication - and it applies to global food security manner. Among others, the objective of 'risk communication' is to improve the effectiveness of the risk analysis process among all interested parties. The monitoring and evaluation of the effectiveness of the communication of risks in food security is essential to knowing if the audience receives, understands and responds adequately. One of the methods of monitoring established by FAO/WHO (2016) is through targeted surveys. Therefore, this work can be an instrument to know the impact on public opinion of the communication actions that several entities develop in the CI. Conclusion After 10 years of starting various projects and initiatives related to CFP only 4.7% of the population shows some valid knowledge of how ciguatera is produced, 3.6% declare to have knowledge about human cases and 1.3% declares that there are regulations to apply. Therefore, in the framework of risk communication it is necessary to design a program to achieve a successful implementation based on risk analysis considering the main stakeholders (of course including consumers). It is necessary that the different institutions work together (EFSA, 2017).

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Keywords: Ciguatera fish poisoning (CFP), Acquired knowledge, Telephone survey research, Risk Communication, Canary Islands - Spain

Combining artificial substrates, morphology and DNA metabarcoding for investigating macrozoobenthic communities in NW Iberia

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Problem statement: Assessment of species composition in zoobenthic communities is a key element of bio-monitoring in marine waters. However, marine biodiversity assessment in large-scale biomonitoring programs is challenging because it is commonly based on morphological identifications, which are time-consuming and require taxonomic specialists. The emergence of a new generation of technological DNA-based tools is transforming our ability to study patterns and trends of biodiversity. DNA metabarcoding provides the opportunity to improve accuracy and throughput compared to morphology-based assessments. Although some methodological optimization is still required, high-throughput techniques coupled with standardized sampling strategies accurately improve the ability to monitor marine communities with unprecedented potential for precision and detail. Different artificial substrates deployed in coastal areas may selectively influence and enhance the colonization of different species determining the resultant community. The creation of permanent monitoring stations using artificial substrates provides an alternative to easily and routinely assess biodiversity. Objective: Here, we test the influence of different types of artificial substrates on the composition and structure of marine macrozoobenthic communities. In order to achieve this goal, we combined morphology and DNA metabarcoding approaches to monitor coastal macrozoobenthic communities and investigate the short-term and seasonal patterns of colonization in artificial substrates. Methodology: Three different types of artificial substrates were used: slate, polyvinyl chloride (PVC) and granite. In December 2016, 16 replicates of each substrate were randomly deployed close to the dock of Toralla Island (Vigo, NW Spain). After 3, 7, 10 and 15 months, four replicates of each substrate were randomly removed. Three replicates of each substrate were used for morphology and one replicate of each substrate was used for DNA metabarcoding. The mobile and sessile fauna were separated and preserved for subsequent analysis. DNA amplification was performed for an internal region of the COI barcode (~313 bp) and for the V4 region from 18S rRNA gene (ca. 400 bp). Results: Compared to morphology, DNA metabarcoding retrieved more taxa and higher taxonomic resolution. However, some of the species identified with morphology were not detected using DNA metabarcoding (e.g. *Pecten maximus* and *Halocynthia papillosa*). While sampling strategy, samples processing or biomass can explain some of these cases, attention should be paid for the non-detection with DNA of very representative species, like the *balanus Perforatus perforatus*. Compared to the other substrates, slate replicates resulted in a higher colonization of target taxa and granite replicates have the lower rate of species detected. Some taxa revealed different time-patterns to colonize the substrates (e.g. *Ampithoe rubricata* and *Hiattella arctica*) as well as variations in taxonomic diversity among substrates and seasons. While some species were evenly detected in all/nearly all of the substrates and time combinations (e.g. *Obelia geniculata*/*Jassa slatteryi*), some taxon were time-specific (e.g. *Limacia clavigera* only detected in second recruitment time - after 7 months; *Dexamine spinosa* detected in all substrates but only in third recruitment time - after 10 months) or randomly detected with variations in taxonomic diversity among substrates and seasons (e.g. *Myrianida prolifera*). The adopted approaches also demonstrated to be efficient to detect non-indigenous species (e.g. *Perinereis aibuhitensis*: a commercially very important polychaete also used for biomonitoring studies). Major conclusions: In general, artificial substrates promote macrozoobenthic colonization and coupled with molecular tools capture the composition of natural community. This highlights the importance of the adopted sampling strategy to be used in biomonitoring programs to capture benthic biodiversity. The results also illustrate the influence of substrate and season in the recruitment of zoobenthos. Although DNA metabarcoding detected a higher number of taxa and with more resolution, the two methods applied were somewhat complementary in their ability to detect benthic species and both should be used to avoid missing relevant taxa. This project provided an opportunity to yields insights for a better comprehension of fundamental ecological processes in macrozoobenthic communities and opening new perspectives to be used in other studies to supply more extensive, detailed and rigorous data for marine resource management and biodiversity conservation in coastal ecosystems.

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Keywords: DNA metabarcoding, High-throughput sequencing (HTS), biomonitoring, Artificial substrates, Marine macro-invertebrates

Metabarcoding the benthic boundary layer: the role of sampling method and marker characteristics in the DNA signatures obtained at the interface between benthos and plankton

Adrià Antich, Creu Palacín, Dani San Roman, Owen S. Wangensteen and Xavier Turon

The study of community structure is crucial for monitoring and managing marine ecosystems. Metabarcoding has been used to assess the biodiversity of benthic and planktonic marine communities. However, the DNA dynamics in the benthic boundary layer has not been analysed yet. The signal recovered in this transition depends crucially on the type of sampling performed: community DNA (comDNA) vs environmental DNA (eDNA), and the characteristics of the primers used to generate amplicons: universal vs group-specific primers. The aim of this study, performed in the Cabrera National Park, is to characterize with metabarcoding two different benthic littoral communities (photophilic and sciaphilous) and the adjacent water column at four distances (from 0 centimetres to 20 metres) from the benthos. We compared the signal obtained from comDNA sampled by quadrat scraping in the benthos and eDNA obtained from the water by filtering at 0.22 µm. We used two different primer sets, one universal (COI, Wangensteen et al. 2018), and a second one specific for vertebrates (12S, Miya et al., 2015) targeting the fish communities. The results of both primers were markedly different. With COI, only 14% of the reads obtained in the water could be assigned to eukaryotes, as opposed to ca. 99% in the benthos. A high diversity was found, with 1,883 MOTUs detected in the benthos and 839 in the water. The structure of the communities in benthos and water was markedly different both in terms of main groups and MOTU composition, and significant differences between photophilic and sciaphilous samples (both in benthos and water) were also detected. The interaction interchange between benthos and water was weak, with only ca. 2% of MOTUs shared. The number of shared MOTUs decreased from the benthic boundary to distances of up to 20 m. With 12S, 31 fish MOTUs were detected, and ca. 30% of them were shared between benthos and water. Again, the highest number of shared MOTUs was found adjacent to the benthos and the percentage diminished with distance. This marker showed significant differentiation between photophilic and sciaphilous communities, but not between benthos and water samples. By ecological categories, benthic fish were more abundant in the benthos and water column close to it, while pelagic fish were more abundant in the external samples and benthopelagic fish were ubiquitous. We conclude that the benthic signal is highly local and cannot be adequately assessed in water samples, even adjacent to the benthos, due to fast dilution of benthic DNA. Moreover, unspecific amplification rendered the universal primers poorly efficient for eukaryote amplification in eDNA samples rich in prokaryote DNA such as those from the filters. The specific primers performed better, but high stochasticity in fish DNA presence in the samples makes it necessary a high level of replication to obtain reliable estimates of fish diversity in both benthic and water samples.

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Keywords: COI, 12S, metabarcoding, Benthic boundary layer, Community DNA, eDNA, Biodiversity

Molecular and morphometric combo reveals extraordinary hidden diversity in European Polychaetes from the Phyllodocidae family

Marcos A. Teixeira, Arne Nygren, Ascensão Ravara and Filipe O. Costa

With the rise of the molecular tools, the combination of both molecular and morphological data is becoming the typical way to describe new species. Over the last decade the diversity of the family Phyllodocidae (Annelida, Polychaeta) increased due to the discovery of multiple species complexes. Polychaetes are one of the most prominent bioindicator groups and well represented class of organisms among the marine benthic invertebrates. However, many of these complexes are not formally described following their discovery, persisting as unnamed species that are seldom recognized in research and conservation programs. *Eumida sanguinea* was found to be a false cosmopolitan species since Nygren & Pleijel (2011) detected 9 new lineages distributed from the NE Atlantic to the Mediterranean and described 7 of them based on the pigmentation pattern and sequences from mtCOI gene and the nuclear ITS region. Similar to the previous example, *Eulalia viridis* is now a described complex of two species, but novel molecular data shows an impressive hidden diversity around the European NE Atlantic and the Mediterranean questioning once again the cosmopolitan status of another polychaete species. True cryptic species have no consistent morphological differences, however some slight dissimilarities in the size and shape of key morphological characters can be further explored by quantitative morphometric analysis to complement the molecular data. Resorting to multivariate statistical analysis and the incorporation of statistical dissimilarities derived from the SIMPER routine of the PRIMER software, we have been able to separate similar species through graphic visual clusters and calculate the proportions of the morphological characters that most contribute to the differences between lineages. In this study we introduce two new *Eumida* lineages from Great Britain, by using the mtDNA COI-5P and rDNA ITS/28S sequences, to add to the already large *Eumida sanguinea* species complex, while complementing it with morphometric data against three other described lineages from the complex. By applying four different MOTU clustering algorithms to the COI-5P sequence data, we also revealed five additional lineages within the *Eulalia viridis/clavigera* complex with 17.3% mean distance (K2P), where one of the them is exclusive to the Madeira Island (Portugal). In addition, one of the three Mediterranean lineages does not display the characteristic green colour found in the complex and might belong to a new undescribed *Eulalia* species. Due to their abundance, cryptic species can in no way be neglected if we want to correctly assess species diversity and distribution, understand biogeographic patterns, or to keep track of natural or anthropogenic induced changes in the marine communities. Hence, since species complexes may have distinct biological characteristics and different sensitivity to pollution and environmental stresses, failure to recognise this hidden biodiversity may compromise the accuracy and the interpretation of biomonitoring, ecotoxicological and bioaccumulation data. DNA barcode data obtained as a concurrent product of the efforts dedicated to the study of cryptic species can also be used in conjunction with high-throughput sequencing technologies for biomonitoring programmes or other relevant ecological researches.

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Keywords: Polychaeta, cryptic species, Integrative taxonomy, DNA barcode, Morphometrics

Patterns of spatial and temporal variation in estuarine meiofaunal communities assessed through DNA metabarcoding: a case study in the Lima estuary (NW Portugal)

Maria Fais, Sofia Duarte, Pedro E. Vieira, Ronaldo G. Sousa, Mehrdad Hajibabaei, Carlos A. Canchaya and Filipe O. Costa

Meiobenthic organisms are key components of estuarine environments, providing invaluable functions and services. In particular, meiofaunal organisms participate actively in nutrient cycles and energy flux, supporting higher trophic levels, as well as the stabilization of sediments. Due to their small size (between 30 µm and 1mm) and high dispersal potential, meiofauna was considered to have a ubiquitous distribution. However, with the recent progress of DNA-based technologies, in particular, DNA metabarcoding, the cosmopolitan meiofauna paradox is being questioned, suggesting that meiobenthic communities can comprise also endemic and narrow-range species. Several studies on the spatial variability of meiofauna at small-scale (within meters) and at meso-scale (meters to km) have been carried out in an attempt to elucidate community structure, both using traditional approaches (such as morphological identification) and DNA-based tools. All these studies focused on the importance of environmental features (i.e., salinity, sediments' size, percentage of organic matter) in the distribution of particular taxonomic groups, as well as of their bio-ecological characteristics, which may overall explain the spatial structure of the meiobenthic communities. However, little attention has been given to the temporal variation of meiofauna communities in estuaries, and to the best of our knowledge, no research on the spatial-temporal dynamics of Iberian estuarine meiofauna has been yet performed using DNA-based tools. Although DNA metabarcoding is assumed to i) allow a faster detection, ii) identify hard morphologically-intractable meiobenthic organisms or communities, and iii) target different taxonomic groups simultaneously, further refinement is required to better tailor this method for meiofauna profiling. A well-defined choice of marker loci and primer pairs, and the design of robust protocols for sampling effort and for the bioinformatics data processing and analysis is needed to avoid the increase of technical bias during the workflow. The main goal of the present study was to investigate spatial and temporal variations of the estuarine meiofauna communities using DNA metabarcoding. Sediments were collected in four sampling stations in the Lima estuary (North-West of Portugal), in June 2017 and June 2018, from the first 5 cm of sediment directly to 50 mL sterile falcon tubes (3 cm ø). Three samples were collected at the high and middle intertidal zones in each sampling station. For each site and yearly sampling campaign, we assessed salinity, total organic matter (TOM) and grain size. DNA was extracted from 10.0 g (± 0.50 g) of sediment from each collected sample, using the DNeasy PowerMax Isolation kit (QIAGEN®). Two different primer pairs were used for the production of amplicon libraries and their high-throughput sequencing (Illumina-MiSeq): a sub-region from the mitochondrial cytochrome c oxidase gene (COI) and the V4 hypervariable region of the nuclear ribosomal 18S rRNA gene (18S). Raw reads were quality-filtered by the removal of sequencing adapters and trimming of low-quality bases. Further de-multiplexing and read processing were carried out in mothur, using customized procedures. Reads were clustered into OTUs using a 97% similarity threshold. Then, the representative sequences of each OTU were BLASTed against the GenBank non-redundant nucleotide database and taxonomically assigned in MEGAN v.6.13. In terms of OTUs richness and turnover, our results showed that our communities globally changed along space and time. Meiobenthic structure, in general, displayed well-separated temporal and spatial clusters, with some generalist OTUs occurring along with the stations for both sampling years. Qualitatively, the two primer pairs detected different taxonomic groups attributed to meiofauna in a complementary manner. Nematoda, Platyhelminthes, Annelida, Arthropoda Crustacea, Amoebozoa and Ciliophora were the most abundant phyla, with different temporal and spatial proportions. Our results indicated that Lima estuarine meiofaunal communities are strongly influenced by the salinity, sediment grain size, and total organic matter, affecting meiofauna distribution among the sampling stations. Moreover, further differences on a time scale were found between the sampling years, probably as a consequence of greater rainfall during 2018 (<https://www.pordata.pt>). With a careful sampling design, laboratory and data analysis protocols, DNA metabarcoding can constitute a powerful approach for meiobenthic communities profiling, although further efforts are required for preparing dedicated meiofauna references library.

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Keywords: Meiofauna, estuarine and coastal ecology, spatial and temporal distribution, eDNA metabarcoding, machine learning

Phylogeography of the deep-sea morid codling *Lepidion lepidion* reveals the presence of two ancient atlantic/mediterranean lineages.

David Barros-García, Elsa Froufe, Rafael Bañón, Juan C. Arronte, Francisco Baldó and Alejandro De Carlos

Historically, deep-sea habitats have been considered highly homogeneous, leading to the generalized idea that the species inhabiting them have enormous distribution ranges with little or no opportunity for speciation processes. This idea has remained unchanged until recent times because these environments have remained practically unknown, mostly due to budget constraints and technological infrastructures required to obtain good sample coverage. Nowadays, it is known that the seabed has a varied relief, with different seascapes and variable orography, which could set the conditions for a higher production leading to an increased biodiversity. This is the case of specific highly productive habitats such as seamounts or submarine canyons that work as isolated islands of endemism or even like stepping stones for dispersion depending on the oceanographic conditions and the biology of the species under study. In the present investigation, different questions regarding the genetic diversity of deep-sea morid cod *Lepidion lepidion* have been answered. In order to achieve that, four different habitats throughout its distribution area have been sampled: three in the north-eastern Atlantic Ocean (Aviles Canyon, Galicia Bank and Porcupine Seabight) and one in the western Mediterranean Sea (Balearic Basin) (Fig. 1). Figure 1: Sampled sites of *L. lepidion* (AC: Aviles Canyon; BB; Balearic Basin; GB: Galicia Bank; PS: Porcupine Seabight) Genetic analyses were carried out using three mitochondrial (COI; 16S; NAD2) and two nuclear (RAG1; ZIC1) markers. Neutrality tests and mismatch distributions indicated past events of demographic expansion in the western Mediterranean Sea and the north-eastern Atlantic Ocean. However, north-western of the Iberian Peninsula (Aviles Canyon and Galicia Bank) showed higher values of genetic diversity and typical features of long term stable populations. Discriminant Analysis of Principal Components (DAPC) indicated the presence of two different and partially mixed nuclear lineages, suggesting isolation followed by secondary contact between individuals from the Atlantic Ocean and the Mediterranean Sea (Fig. 2). Figure 2: Lineage membership probability for each individual of *L. lepidion* (AC: Aviles Canyon; BB; Balearic Basin; GB: Galicia Bank; PS: Porcupine Seabight). In general, no genetic structure was found, with only moderate differentiation in Aviles Canyon (NW Iberian Peninsula). This could be explained by the asymmetric migration occurring in this specific location, perhaps due to the prevailing oceanographic conditions in the area. The Galicia Bank seamount seems to play a key role in the connectivity between the Mediterranean Sea and the north-eastern Atlantic Ocean. Finally, the results suggest the genetic connectivity of *L. lepidion* likely to be due to the pelagic stages, and ultimately to the variations of the oceanographic conditions over time.

Keywords: DNA, mtDNA, Seamount, Submarine canyon, demographic history

Lipidomics of solar-power animals: a tool to unravel the process of kleptoplasty

Felisa Rey, Paulo Cartaxana, Ricardo Calado, M. Rosário Domingues and Sonia Cruz

Marine environments are a surprise box where it is possible to find astonishing associations, such as solar-powered animals. Although photosynthesis and animals are two apparently incompatible terms, both merge in sacoglossan sea slugs. This animal group is the only metazoan known to retain functional chloroplasts from its macroalgal food (Rey et al., 2017). These sea slugs are very selective feeders, choosing their food items from a small group of macroalgae. During feeding, these sea slugs use their radula to penetrate the cell wall of si-

phonaceous algae and suck the entire cytosolic content. The whole algal content is digested except for chloroplasts, which are phagocytized into the digestive epithelium. These stolen chloroplasts (kleptoplasts) remain intact and photosynthetically active inside the animal cells for different periods of time, from only a few hours to several months, depending on the sea slug species. While the mechanisms and functions of kleptoplasty are still poorly understood, there are growing experimental evidences that kleptoplasts provide an additional source of energy to sacoglossan sea slugs (Cartaxana et al., 2017). Chloroplast envelop membranes are lipid-rich structures, where glycerolipids (galactolipids, phospholipids, sulfolipids) represent the bulk matrix maintaining membrane architecture and, together with pigments, represent the essential elements to perform photosynthesis (Block et al., 2007). The lipidome profile of chloroplast membranes includes glycolipids (e.g. galactolipids, sulfolipids), exclusive lipids of chloroplast membranes. The objective of the present study was to identify potential shifts in the lipidome of macroalgal chloroplasts sequestered by sacoglossan sea slug species with different retention times of kleptoplasty. *Elysia viridis* and *Placida dendritica* are two autochthonous species of the Portuguese coast, with high and low retention time of kleptoplasty, respectively. The natural habitat of both species is the green macroalga *Codium tomentosum*, where both species inhabit. Sea slug species were surveyed at two different periods: immediately after being collected from the field and after one-week of starvation in captivity. A lipidomic mass spectrometry-based analysis was performed to study the exclusive lipids from chloroplast membranes in *C. tomentosum*, *E. viridis* and *P. dendritica*, using hydrophilic interaction liquid chromatography-mass spectrometry (HILIC-LC-MS) and MS/MS. The lipidomic analysis of sea slugs collected in the field revealed that the most abundant molecular species of galactolipids (MGDG, MGMG, DGDG, DGMG) identified in *C. tomentosum* were also present in both sea slug species, although in lower concentrations. The total number of galactolipid molecular species identified in *P. dendritica* was lower than in *E. viridis*, which was lower than in *C. tomentosum*. After one-week of starvation, there was a general decrease in the relative abundance of several molecular species. In *P. dendritica* all molecular species presented a significant lower relative abundance, or even disappeared during starvation. This finding confirmed the loss of photosynthetic activity by stolen plastids. On the other side, in *E. viridis*, this reduction was less marked, with the lyso-forms of galactolipids (MGMG, DGMG) increasing under starvation (likely related to the activity of galactolipases during this stressful period). Regarding sulfolipids (SQDG, SQMG), the lipidome of *E. viridis* was very similar to that of *C. tomentosum*, indicating a preservation of photosynthetic activity in kleptoplasts. These results confirm that an earlier degradation occurs in the chloroplast membranes of *P. dendritica*, when compared to *E. viridis*. Moreover, a remodeling of kleptoplast membranes during starvation in *E. viridis* is also likely to occur. Figure caption Figure 1 Schematic representation of the methodology used in the lipidomic mass- spectrometry analysis of the macroalga *Codium tomentosum* and the sacoglossan sea slugs *Elysia viridis* and *Placida dendritica*. Figure 2 a) Number of molecular species of galactolipids (MGDG, MGMG, DGDG, DGMG) and sulfolipids (SQDG, SQMG) identified in the macroalga *Codium tomentosum* and the sacoglossan sea slugs *Elysia viridis* and *Placida dendritica*. Both sacoglossan sea slug species were surveyed immediately after being collected from the field and after one-week of starvation in captivity. b) Relative abundance of MGDG, DGDG and SQDG molecular species identified in *E. viridis* and *P. dendritica* immediately after being collected from the field and after one-week of starvation in captivity.

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Keywords: Chloroplasts, *Codium tomentosum*, Glycolipids, Mass Spectrometry, Sacoglossan sea slugs, Galactolipids, Sulfolipids

Health status-associated microbiota fingerprints within the oral cavity of cetaceans

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Human impacts affect directly ocean life, often leading marine species to an endangered status. Cetaceans, particularly the Odontoceti, occupy high trophic levels (predators, primary or secondary consumers) and, consequently, their condition and wellbeing reflect the health and status of lower trophic levels. Moreover, the composition of mammalian microbiota has been related with either health or disease statuses of the host. Recently, a metabarcoding 16S rRNA-based approach was successfully implemented to investigate microbial communities present in a striped dolphin [1] stranded in Continental Portugal. In this study, we implemented the methodology validated by Godoy-Vitorino et al. 2017 [1] to assess the oral microbiome of 3 cetacean species most commonly found stranded in Iberian Atlantic waters (the common dolphin *Delphinus delphis*, the striped dolphin *Stenella coeruleoalba* and the harbour porpoise *Phocoena phocoena*), using 16S rDNA-amplicon metabarcoding. A Constrained Canonical Analysis approach (CCA) showed that the major factors shaping the composition of 38 oral microbiomes (p-value<0.05) were i) animal species and ii) age class, segregating adults and juveniles. The correlation analysis also grouped the microbiomes by animal stranding location (northern or western Iberian coast) and separated samples from dolphins that died as a result of fisheries bycatch from those that showed signs of disease. Similar discriminatory patterns were detected using the data from a previous study on healthy bottlenose dolphins (*Tursiops truncatus*) [2], indicating that this correlation approach may facilitate data comparisons between different studies on several cetacean species. A total of 15 bacterial genera and 27 OTUs comprised the main taxa discriminating between the observed CCA groups (p-value<0.05). The bacterial genera *Dethiosulfovibrio* (OTU141, with a 94% nucleotide identity corresponding to the anaerobic *Dethiosulfovibrio marinus* strain WS100) and *Marinicella* (OTU255, with a 96% nucleotide identity corresponding to the marine *Marinicella pacifica* strain sw153) discriminated harbour porpoise oral microbiomes from common dolphin and striped dolphin oral microbiomes. Regarding the age class and sexual maturity of the sampled animals, the genera *Arcobacter* (OTU334 with a 98% nucleotide homology to *Arcobacter venerupis* strain CECT7836T and OTU852 with a 97% homology to *Arcobacter butzleri* strain NCTC12481), *Dielma* (OTU153 with a 88% homology to *Dielma fastidiosa* strain JC13) and *Peptostreptococcus* (OTU104 with a 99% homology to *Peptostreptococcus russellii* strain ING2-D1D) were associated with adult/mature animals, whereas potential signature bacterial taxa were not detected as statistically significant for juveniles. Moreover, the separation according to animal stranding location registered a significant over-representation of the genera *Campylobacter* (OTU663 and OTU1279 with <90% homology to known species), *Marinifilum* (OTU107 with 93% homology to *Marinifilum fragile* strain JC2469), *Microbacter* (OTU114 with 86% homology to *Microbacter margulisiae* strain ADRI) in animals stranded on the western Atlantic Iberian coast, whereas members of *Clostridium sensu stricto* cluster 1 (OTU885 with 99% homology to *Clostridium moniliforme* strain HYN0057) and *Peptoclostridium* (OTU1385 with 90% homology to *Peptoclostridium acidaminophilum* strain a1-2) were associated to the animals of the northern Atlantic Iberian coast. In this study, the abundance of the genus *Gemella*, namely a *Gemella* OTU with a 97% nucleotide homology to *Gemella palaticanis*, was associated with the group of diseased animals. By integrating the data from Bik et al. 2016 [2], the analysis of potential indicator bacterial signatures suggested that healthy cetaceans were also associated to the predominance of *Desulfobacterium* spp., *Marinifilum* spp., *Maritimimonas* spp., *Microbacter* spp., *Sphaerochaeta* spp., *Tannerella* spp., *Thalassobius* spp. and *Thiothrix* spp. in the oral cavity. The bacterial taxa underlying the CCA profiles observed in this preliminary study may thus be explored as microbiota fingerprints to develop bio-monitoring approaches to assess the health of marine ecosystems from the Iberian Atlantic basin, using cetaceans as sentinels. This work was supported by the project RECI/AAGGLO/0470/2012 (FCOMP-01-0124-FEDER-027472), by the strategic program UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569), by the GenomePT project (POCI-01-0145-FEDER-022184), supported by COMPETE 2020 - Operational Programme for Competitiveness and Internationalisation (POCI), Lisboa Portugal Regional Operational Programme (Lisboa2020), Algarve Portugal Regional Operational Programme (CRESC Algarve2020), under the PORTUGAL 2020 Partnership Agreement, funded by national funds through the Fundação para a Ciência e a Tecnologia (FCT) I.P. and the European Regional Development Fund (ERDF), through the COMPETE2020-Programa Operacional Competitividade e Internacionalização (POCI). The work was supported as well through CESAM UID/AMB/50017/2013 co-funded by the FCT/MEC and FEDER within PT2020 and Compete 2020 (C.E.) and grants SFRH/BD/30240/2006 (M.F.) and SFRH/BPD/82407/2011 (A.L.).

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Keywords: oral cavity, Host-Microbiome Interactions, 16S rRNA gene metabarcoding, common dolphin, *Delphinus delphis*, Harbour porpoise, *Phocoena phocoena*, Striped dolphin, *Stenella coeruleoalba*

Occurrence of the toxic dinoflagellate *Alexandrium ostenfeldii* in the coastal waters of the southeastern Bay of Biscay

Luis Ferrer, Marta Revilla, Aitor Laza-Martínez, Yolanda Sagarmínaga, Almudena Fontán, Joana Larreta, Izaskun Zorita, Oihana Solaun, José Germán Rodríguez, Leire Arantzamendi, Manuel González and Andrea del Campo

Marine phytoplankton, as primary producers, contribute to the sustenance of bivalve aquaculture. However, some phytoplankton species can synthesize potent toxins, which are ingested by filter-feeding organisms and pose a threat to human health. These toxic species can also cause serious impacts to marine environment and economic activities at coastal sites. In temperate areas, phytoplankton abundance and composition show large spatio-temporal fluctuations that involve seasonal cycles, species succession and sporadic blooms. This variability is controlled by both top-down factors (e.g., grazing) and bottom-up factors (e.g., nutrients and light availability), being all of them ultimately driven by oceanic and meteorological processes, and anthropogenic pressure on coastal zones. Among those phytoplankton organisms, some species of the genus *Alexandrium* produce paralytic shellfish poisoning (PSP) toxins. It is known that the responses to the environment differ among toxic phytoplankton taxa, even within the same genus. Here we address how physical drivers and bottom-up factors control toxic microalgae in the coastal waters of the southeastern Bay of Biscay. In these waters, a recent development of bivalve aquaculture at a pilot plant (called “Mendexa”) has led to monitor phytoplankton, toxins and hydrographic variables since 2014. Mendexa is located 2 km off the coast of the Basque Country (northern Spain), at a depth of approximately 45 m (Figure 1). The Basque coast is influenced by 12 short rivers with big slopes, which are torrential in character and provide, annually, 150 cubic metres per second of freshwater. This freshwater supply alters the physico-chemical composition of the shallow waters and often leads to an increase of nutrient concentration in the inner shelf waters (Valencia et al., 2004). The pilot plant uses a longline system for the culture of mussels (*Mytilus galloprovincialis* Lmk.) and occupies a total area of 1 ha. For more detailed information, the reader is referred to Azpeitia et al. (2016) and Muñiz et al. (2019). At Mendexa, toxins are analysed monthly in the mussels collected in the upper metres of the water column. When any toxin exceeds the regulatory limit, samples are taken at a higher frequency (twice a month). According to the European legislation, the toxins analysed are the following: domoic acid, saxitoxins and lipophilic toxins (i.e., okadaic acid, dinophysistoxins, pectenotoxins, azaspiracids and yessotoxins). Saxitoxins, which are PSP toxins, are analysed by means of the mouse bioassay. For phytoplankton, the hose sampling method and a plankton net (20 µm) are used to take integrated samples through the water column, as dinoflagellates can present heterogenous vertical distribution. These samples are fixed with acidic Lugol’s solution and analysed following the Utermöhl sedimentation method. Occasionally, the identification of dinoflagellates is carried out using fluorescence microscopy, after staining with calcofluor. The physico-chemical conditions are characterized using a CTD (Conductivity, Temperature and Depth sensors). Also, several water samples are collected to quantify dissolved inorganic nutrients by colorimetric methods. Specifically, here we study the events of high PSP toxin concentration in mussels (growing on longlines offshore) which occurred in autumn 2018, when the only PSP producer that could be found was *Alexandrium ostenfeldii* (Paulsen) Balech & Tangen. Previously, *Alexandrium* spp. had been reported along the Basque coast (e.g., Aylagas et al., 2014). In order to assess the influence of both local and regional processes on these events, meteorological and hy-

drographic variables together with satellite chlorophyll-a data were used. Also, particle tracking models and machine learning techniques were considered. Our hypothesis is that the occurrence of the dinoflagellate *A. ostenfeldii* and the high PSP toxin concentration were caused by the meteorological and oceanic conditions existing in autumn 2018. The month of September was characterized by warm and very dry conditions. In contrast, October was very rainy (i.e., high river runoff), which raised the concentration of inorganic nitrogen in the surface waters of the southeastern Bay of Biscay at mid-autumn. This together with stable atmospheric and oceanic conditions triggered the PSP toxins to exceed the regulatory limit in November and December, coinciding with the presence of 80 cells per litre of *A. ostenfeldii* (Figure 2). We anticipate our results to be a starting point for the development of predictive models that will play a useful role in the study of the evolution of Harmful Algal Blooms (HABs) in the southeastern Bay of Biscay.

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Keywords: *Alexandrium ostenfeldii*, Dinoflagellate, HABs, Phytoplankton, toxic

Presence of *Vibrio mediterranei* associated to major mortality in stabled individuals of *Pinna nobilis* L.

Patricia Prado, Gaetano Catanese, Amalia G. Jofre, Karl B. Andree, Jose Rafael Garcia March, Pep Cabanes, Francesca Carella, Jose Tena and Noèlia Carrasco

A large number of works have established bacterial implications in bivalve disease outbreaks within maintenance tanks. Usual stabling conditions linked to animal density, high temperatures and addition of food, can favor the growth of high concentrations of bacteria belonging to *Vibrio* sp. A rescue project of 221 adult individuals of the endangered pen shell, *Pinna nobilis* L. was conducted in November 2017 in two areas of the Spanish coast where the species was still abundant and apparently free from the parasitic disease caused by *Haplosporidium pinnae*: Port Lligat in the Costa Brava, and the Alfacs Bay in the Ebro Delta. For biosecurity reasons, the 106 individuals from the Ebro Delta were stabled at the IRTA facilities located next to the Alfacs Bay, whereas the 115 individuals from Port Lligat were stabled in different institutions throughout the Spanish territory. Initial biopsies showed that individuals from the Ebro Delta were free of the parasite whereas most individuals from Port Lligat already were already parasitized and died in the following months. Individuals at IRTA were stabled in five tanks and fed ca. 4% of their DW with a mix of three species of phytoplankton and fine riverine sediments rich in OM (13%). Seawater was filtered through 1, 5 and 10 µm to ensure the absence of the parasite, and then disinfected and kept free of bacteria with UV light. No losses occurred during the 4 initial stabling months, but individuals started to die in April-May 2018 at temperatures from 16 to 19 °C. A peak of mortality occurred during the summer months and early fall (53%) of with maximums coinciding with temperatures above 25 °C and decreased during the winter period. Individuals were analyzed with PCR and histology for the presence of *H. pinnae*, *Mycobacteria* sp., *Vibrio splendidus*, *V. aestuarianus* and Herpesvirus microvar, considered important pathogens of pen shells or commercial bivalves also present in the Alfacs Bay, but results were all negative. Microbiological culture and isolation of bacteria from three ailing individuals

sacrificed for study purposes demonstrated *V. mediterranei* as the dominant species, and further PCR analyses in deceased individuals also identified the presence of the bacteria. Overall, our results suggest that *V. mediterranei* is an important opportunistic pathogen of stabled individuals possibly subjected to captivity stress, and that antibiotic treatment (Florfenicol) combined with vitamins and controlled water temperature (18°C) can be used to control the disease. Further research is needed to develop specific PCR and qPCR techniques that could allow a more adequate follow-up and pathogenesis of this emerging disease.

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Keywords: Captivity stress, pen shell disease, antibiotic treatment, temperature, Vitamins

Comparing toxicity and bioactivity of gold nanoparticles synthesized using two brown algae *Cystoseira* sp

Sofia Machado, Anabela Gonçalves, Noelia González-Ballesteros, Luana Magalhães, Marisa P. Sárria, Maria Carmen Rodríguez-Argüelles and Andreia C. C. Gomes

The marine ecosystem has captured a major attention in recent years since various biologically active compounds have been isolated and screened for pharmacological activity from dissimilar marine provenience. Marine biological resources can be considered an essential for nanotechnology, researchers had been interested to synthesize metallic nanoparticles from marine source because it is thought to be ecofriendly, non-toxic, environmentally acceptable "green procedures", reduces the down-streaming process making it very cost effective and the availability of the source from the diverse marine ecosystem becomes a much easier task. The biosynthesized nanoparticles from marine compound offer stabilized nanoparticles through compounds present in the marine source that make them more efficient for both biomedical and industrial applications [1]. In this regard, we have focus our attention in two algae of *Cystoseira*. It is a genus of marine brown macroalgae composed of about 40 species. It is widely distributed in the North East Atlantic, the Baltic Sea and the Mediterranean. This genus contains a wide variety of secondary metabolites (e.g. terpenoids, steroids, phlorotannins, phenolic compounds, carbohydrates, triacylglycerols/fatty acids, pigments, vitamins) that are associated with pharmacological properties, such as antioxidant, anti-inflammatory, cytotoxicity, anticancer, cholinesterase inhibition and anti-diabetic activities but also antibacterial, antifungal and anti-parasitic activities [2]. *Cystoseira baccata* (CB) and *Cystoseira tamariscifolia* (CT) are two species of this genus that differ in geographic distribution, morphology and possibly content of secondary metabolites. Gold nanoparticles are presently under intensive study since they can be the remarkable scaffold for novel biological and chemical applications owing to their unique electrical, chemical and optical properties. Such as their easy synthesis and good biocompatibility due to the low reactivity of the golden core. Therefore presenting potential applications in the development of new technologies in different fields such as in the food, cosmetic and pharmaceutical industry [3,4]. Previously, González-Ballesteros et al have reported the synthesis of gold nanoparticles with antitumor activity led by the macroalga CB [5]. In the present study, we have compared the ability of *C. baccata* and *C. tamariscifolia* (CT) in the synthesis of gold nanoparticles. With this aim, aqueous extracts of the two macroalgae were prepared and their reducing activity, total phenolic content and DPPH scavenging activity were determined before and after the synthesis of nanoparticles. Results showed that CT possess three times more reducing power, almost 4 times more phenolic content and 4 times more DPPH scavenging activity than CB. The nanoparticles obtained were characterized by UV-Vis spectroscopy and Transmission electron microscopy confirming the formation of spherical nanoparticles with a mean diameter of 8.4 ± 2.2 nm in the case of Au@CB and 7.6 ± 2.2 nm for Au@CT. In order to compare the biological potential of both CT and CB extracts and their respective gold nanoparticles (Au@CT and Au@CB), it was investigated whether they affect the viability in mouse (L929 cell line) and human (BJ5-ta cell line) fibroblast cells as in vitro models, by evaluating cellular metabolism by tetrazolium-based colorimetric cellular assay (MTT); cell membrane integrity by lactate dehydrogenase activity (LDH); wound-healing assay to assess effects on cell proliferation and migration capacity. Both algae extracts and derived nanoparticles present a non-cytotoxic profile in lower concentrations, which are efficient in cell regeneration, although with some differences between both species. For a correlation between in vitro and in vivo toxicity, the zebrafish embryo toxicity (ZET) assay, recommended by

OECD to evaluate acute and chronic toxicity, was performed. This model organism has a translucent body, allowing real time observation, and allows the evaluation of whole organism responses, from mortality to more specific parameters such as neurotoxicity. The results show that toxicity is evident only at very high concentrations. These promising results thus support that green synthesis in CB and CT extracts of non-toxic, bioactive nanoparticles have an interesting potential for biomedical applications.

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Keywords: Green synthesis and nanoparticles, algae extracts, Zebrafish embryo acute toxicity test, cell regeneration, gold nanoparticles

Cephalopods from the Canary Current upwelling ecosystem (Northwest Africa)

Amanda Luna, Ana Ramos, Francisco J. Rocha and Catalina Perales-Raya

The Canary Current Large Marine Ecosystem (CCLME) constitutes one of the four great marine systems of upwelling in the world, being the third in primary productivity and supporting the largest fisheries of the African coast, with an annual production of around 2-3 million tons (Valdés and Déniz-González, 2015). The cephalopod's fauna of Northwest African region is diverse and abundant and includes species of wide distribution and elevated commercial value, among which are found squids, cuttlefish, and octopuses, whose catches in the area reach 80,000 - 120,000 tons per year. Although most of the fishery target species have been well studied, the knowledge of the unexploited species is very scarce (Rocha et al., 2017). Between 2002 and 2012 the Spanish Institute of Oceanography (IEO) and the FAO carried out eleven multidisciplinary surveys in northwest Africa, covering the shelf and continental slope from Morocco to Guinea. Quantitative data by species and important collections of benthic and pelagic cephalopods were gathered in 1334 trawl stations over the soft bottoms between 20 and 2000 m depth. Besides, oceanographic data were recorded in 1180 stations by different CTD devices. Almost 140 species have been currently identified. Among them, Sepiidae, Lolliginidae and shallow-water octopus dominate the coastal assemblages, while at the slope waters Ommastrephidae and deep-water octopuses are more abundant. The collections and databases taken during these 11 trawl surveys represent an exceptional source of regional information on the biodiversity, composition, and distribution of cephalopod's assemblages inhabiting North-west African continental margin.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Keywords: cephalopods, Canary current, upwelling, Northwest Africa, Biodiversity

A glance into the global phylogeographic history of hydroids of the superfamily Plumularioidea (Cnidaria, Hydrozoa)

Carlos J. Moura, Allen G. Collins, Ricardo S. Santos and Harilaos Lessios

Marine benthic hydroids are common worldwide and often abundant in subtidal rocky communities. Amidst the great hydrozoan diversity, an extremely speciose taxon, including some of the largest and frequently encountered hydroids, is the superfamily Plumularioidea (including the families Aglaopheniidae, Plumulariidae, Halopterididae and Kirchenpaueriidae) – the taxon of focus. The Plumularioidea are ubiquitous, ecologically important and relatively well documented in taxonomic studies. Many Plumularioidea are believed to have large geographic distributions. However, only few diagnostic morphological characters are used to categorize its taxa, a consequence of their structural simplicity, and the recent use of molecular characters has uncovered high levels of cryptic diversity (Moura et al. 2018). The phylogeographic history of the Plumularioidea has remained little explored. We focused sampling effort across the NE Pacific and throughout the N Atlantic (in both shallow and deep waters), but we also generated molecular data for other marine areas worldwide. After generating 659 DNA Barcodes of the 16S marker, and after investigating taxonomic relationships of Plumularioidea hydroids with the addition of Genbank sequences (Moura et al 2018), we now investigate phylogeographic and evolutionary patterns with 1114 16S sequences of 199 putative species of Plumularioidea. We calibrated the ‘molecular clock’ of these hydrozoans with sister clades separated by the Isthmus of Panama, with a Bayesian relaxed dating, to estimate divergence times. We also used ancestral state reconstructions to infer direction of colonization across depths and oceanic regions, and relate these to major changes in climate and ocean circulation along the Cenozoic Era. The phylogeographic patterns highlighted were contrasted, and revealed generally concordant with those observed in other marine taxa. Presently, few species effectively display wide bathymetrical and/or biogeographic ranges. Contemporary Trans-Atlantic genetic interconnections are unlikely for the majority of the tropical-temperate species, but were probably more frequent until around the Miocene-Pliocene transition, and were predominantly directed westwards. The Azores were colonized multiple times and through different routes, predominantly from the East Atlantic, since the Pliocene. Contemporary geminate clades separated by the Isthmus of Panama have predominantly Atlantic origin. Various ancient migrations mainly directed from the Indian Ocean to the Atlantic, occurred through the Tethys Sea and around South Africa. Shallow-water taxa have transited to deeper depths frequently since around the middle Miocene. Some transitions from deep to shallow waters occurred recently, related with particular oceanic currents and/or post-glacial epochs. Thermal tolerance and oceanic currents seem highly relevant for dispersal and speciation.

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Keywords: Hydroids, evolution, molecular clock, Glaciation and Sealevel fluctuations, Global Warming, Oceanic currents, deep sea, coastal, biogeography, Oceanic barriers



Poster Presentations

Poster session 1

Assessment of long-term interaction between an opportunistic macroalga and a native seagrass in a Mediterranean coastal lagoon

Maria Dolores Belando, Jaime Bernardeau-Esteller, Iosu Paradinas, Aranzazu Ramos-Segura, Rocio Garcia-Muñoz, Pedro Garcia-Moreno, Lazaro Marin-Guirao and Juan M. Ruiz

Algal overgrowth has been recognized as an important potential threat to seagrass habitats in many coastal areas. Opportunistic seaweed species can quickly develop large and dense populations able to replace seagrass species due to their higher ability to compete for resources (e.g. light, nutrients). They can also modify sediment properties, generating adverse conditions for seagrasses (e.g. sediment anoxification) and indirectly favoring their substitution. The replacement of seagrasses by seaweeds usually triggers deep ecosystem transformations with the potential for generating alternative states and the loss of the valuable ecosystem functions and services that seagrass habitats provide. During the 1970s, the Mar Menor coastal lagoon (SE Spain) experienced a deep transformation due to human-induced changes in the hydrographic conditions of the ecosystem. The widening of a navigational channel reduced salinity levels of lagoon waters allowing the introduction and massive spread of the seaweed *Caulerpa prolifera*. Subsequent studies in this coastal lagoon have assumed that the introduced seaweed had replaced *Cymodocea nodosa* populations living in deep bottoms (Lloret et al., 2005; Ruzafa et al., 2012). The modification of sediment properties (e.g. increase in silt and organic matter content) provoked by the high abundance of *C. prolifera*, in combination with reduced light levels, have been argued as the major causes of seagrass decline in deep bottoms of the Mar Menor lagoon. This hypothesis, however, has been derived from studies using inappropriate sampling techniques for estimating seagrass abundance (e.g. Van Veen drag) and with very low spatial coverage (i.e. a few sampling points). Moreover, there is no evidence to support the existence of competitive interaction between both benthic macrophytes so far. In this study, the competitive interaction between *C. nodosa* and *C. prolifera* has been evaluated in the Mar Menor lagoon by (i) a high resolution and precision mapping of benthic communities and (ii) exploring relationships between seagrass abundance and macroalga abundance and abiotic factors (e.g. depth and sediment properties) using Bayesian linear regression models. Benthic cartography was obtained based on direct visual observations and recordings from trawled underwater videocamera. Abundance of both macrophytes was measured by divers at different spatial scales in 49 sampling points distributed throughout the lagoon. Macrophytes abundance was characterized at the landscape level (macrocover) and within meadow patches (mesocover and biomass). For *C. nodosa*, shoot density was also measured within patches. Granulometry and organic matter content were used to describe sediments properties. Our results showed that the dominant benthic macrophytes in the Mar Menor were *C. nodosa* and *C. prolifera*. The seaweed occurred over most lagoon areas (90% of the surface), whereas the seagrass was present in 60% of the lagoon bottoms, forming mixed meadows with *C. prolifera* in deep bottoms (>3 m, 55% of the lagoon area) and relatively continuous monospecific meadows in shallow areas. These results contrasted with the absence of *C. nodosa* in deep bottoms of the lagoon described in previous studies (Lloret et al., 2005; Ruzafa et al., 2012). Figure 1. Macrophyte species distribution in the Mar Menor lagoon. Linear models did not recognize *C. prolifera* abundance as a relevant factor for most *C. nodosa* descriptors (macrocover, mesocover and biomass). However, the interaction between alga mesocover and depth or organic matter showed a positive effect on seagrass abundance at the meadow level. This finding evidenced that the greatest seagrass abundance in deep bottoms of the lagoon occurred simultaneously with a strong presence of *C. prolifera*, which supported the absence of a competitive interaction between both macrophytes. Figure 2. Relationships between *C. nodosa* mesocover, depth and *C. prolifera* mesocover based on the 49 sampling points distributed throughout the Mar Menor lagoon. Our findings confirm that *C. nodosa* has not been replaced by the seaweed *C. prolifera* in deep areas of the lagoon, where both species live together developing high abundances. Curiously, this pattern of distribution suggested the existence of potential positive interaction between both species, although experimental manipulative works would be necessary to obtain a thorough understanding of this interaction.

Acknowledgements

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Assessment of spatial and seasonal biomass variation of intertidal seaweed species with commercial potential use as basis for future tailored standing stocks management

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Seaweeds are key components of coastal ecosystems contributing considerably to the global primary production and providing a variety of ecosystem services to coastal communities, such as coastal protection from waves, removal of dissolved nutrients from coastal waters, Carbon sequestration and CO₂ mitigation. Many of them are key structuring species with an important role as providers of habitat, food and refuge for the associated biota. Seaweeds are important resources increasingly used in diverse applications such as food and nutraceuticals, in biotechnological and pharmaceutical applications, cosmetics and well-being, as fertilizers and animal feed, and in bioremediation. Therefore, the increasing global demand for seaweed biomass must be compatible with a sustainable management of these resources. Portugal is no exception, and the interest in intertidal seaweeds is growing although regulation of its harvesting has not yet followed such trend. Before establishing management plans, including harvesting regulations there is the need for understanding the ecology and biology of the target species, including patterns of spatial and seasonal abundance. Such knowledge can be complemented by snapshots of local standing stocks. The overall goal of this study was to provide background data for the management of seaweed beds with potential commercial use in northern Portugal. To achieve this goal, the spatial and seasonal variation in abundance of five target species was evaluated and the standing stocks of those target species were assessed in northern Portugal. The study was carried out between Moledo (41.4195°N, -7.549°W) and Aguda rocky shores (41.04425°N, -6.5335°W). Within this area, three shores, Vila Chã (41.29554°N, -7.3701°W), Belinho (41.59171°N, -6.563°W) and Viana (41.69620°N, -6.534°W) were used to evaluate the spatial and seasonal biomass variation from spring 2017 to winter 2017/1. For assessing the biomass seasonal variability, sampling was performed at three different months within each of the spring/summer and autumn/winter periods, at each of the three rocky shores. The following target species were selected by their potential commercial interest: *Chondrus crispus*, *Codium* spp., *Osmundea pinnatifida*, *Mastocarpus stellatus* and *Chondracanthus acicularis*. Data on percentage cover were collected, for each target-species, using sampling quadrates of 50x50 cm (0.25 m²). Percentage cover was converted to biomass (gdw.0.25m⁻²) using a predictive equation developed for each species relating the field percentage cover and the respective biomass. For that purpose, seaweed samples corresponding to a wide range of percentage cover were collected and subsequently dried at 60°C for 4 h for determination of the dry weight (dw). Spatial and seasonal variation were analysed by a two-factor model. The tested hypothesis was that shore and season would influence the abundance of the target-species. Stock assessment of the target species was performed within the study area, by surveying 19 shores during summer and early autumn 2017. In each shore, the area covered by seaweed beds in the low intertidal was delimited by foot, using a handheld GPS device, and their area was calculated in QGIS software. When seaweed beds were too narrow for GPS readings, areas were estimated by measuring their length and mean width (measured every 3 meters of length), using a tape-measure. Each species standing stock at each site was estimated based on mean biomass density and mapped bed area. The obtained predictive equations relating percentage cover (pc) and dry biomass (dw) for each target-species were as follows, *C. crispus*: $\ln(dw) = 1.0945 \times \ln(pc) + 0.1799$ (n=61, r=0.91);

Codium spp.: $\ln(dw) = 1.0765 \times \ln(pc) + 0.3749$ ($n=41$, $r=0.90$); *O. pinnatifida*: $\ln(dw) = 1.1577 \times \ln(pc) - 0.366$ ($r=0.65$, $r=0.5$); *M. stellatus*: $\ln(dw) = 0.9943 \times \ln(pc) + 0.4012$ ($n=44$, $r=0.75$); *C. acicularis*: $\ln(dw) = -0.34 \times \ln(pc) + 2.26 \times \ln(pc) - 0.1165$ ($n=49$, $r=0.6$). Results from the spatial and seasonal variation analyses indicated that no significant differences were detected on biomass abundance between seasons for any of the target species (Figure 1). In what concerns the spatial biomass variation, significant differences between shores were detected for *C. crispus*, and *C. acicularis* (Figure 1: $F_{2, 12} = 10.4$, $p < 0.05$; $F_{2, 12} = 15.56$, $p < 0.05$; respectively). Since shores were considered a random factor in the ANOVA no further comparisons will be made between them. However, this result indicates that despite the absence of significant differences between seasons for this species, the shore factor has significant influence on their abundance. Although demanding further studies, there might be physical and biological idiosyncrasies determining those abundance variations that should be considered for future management regulation. The present study assessed seaweed beds of 19 shores in northern Portugal encompassing a total area of approximately 64,9000 m² corresponding to a standing stock of 6,256.35 kgdw of all combined five target species. Globally, for the entire area surveyed, despite high between-shores variation, *C. acicularis* attained the higher standing stock (2,357.46 Kgdw) and *C. crispus* and *O. pinnatifida* presented the lowest standing stock values (401.2 Kgdw and 425.64 Kgdw, respectively). Although in general higher seaweed beds corresponded to higher standing stocks per shore there was considerable variability between-shores on the target species abundances (gdw.m⁻²): shores with similar areas (e.g. São Bartolomeu and Amorosa) presented different abundances across the target species. Results obtained in this study highlight the between-shores variation on seaweed abundance and standing stock for the five target species, indicating that species-specific management regulation should be tailored to encompass variation at such spatial scale. The definition of future species-specific quotas of harvesting, per site, should also certify that after harvesting, the remained biomass will allow the population recovery between harvesting periods. In that sense it is essential to apply harvesting methods that ensure such biomass recovery thereby promoting the sustainable use of these resources.

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Keywords: Seaweed standing stock assessment, Seasonal variation, Seaweed beds delimitation, Between-shores variability, Biomass estimation

Photosynthesis and carbon balance in deep *Posidonia oceanica* meadows under the influence of diffuse anthropogenic pressures

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Cultural eutrophication has been identified as one of the main causes of seagrass decline worldwide. The discharge of nutrients and particulate materials into coastal waters by human activities has the potential to trigger direct and indirect interactions in seagrass communities, with the potential to impact seagrass fitness and survival, mainly through light limitation (Burkholder et al., 2007). Slow-growing species, such as the Mediterranean endemic species *Posidonia oceanica*, can be particularly sensitive to light reductions due to its high light requirements (Ruiz and Romero, 2001). This sensitivity is expected to be critical in deep *P. oceanica* meadows, where light levels are close to the minimum light requirements of the species (Ralph et al., 2007, Beer et al. 2014). In order to protect these valuable habitats from eutrophication, European and National environmental agencies are moving those activities responsible of nutrient enrichment impacts (e.g. urban sewages and aquaculture) away from the shoreline and seagrass habitats. However, there is no empirical evidence about "safety distances" and, the relocation of such activities might not be sufficient to warrant the absence of impacts on seagrass meadows. The high potential of these pressures to exert a remote influence on nearby deep seagrass meadow limits have, indeed, already been evidenced (Ruiz et al. 2010). However, most studies evaluating the effects of nutrient enrichment on these habitats have been conducted at depths where light availability is not as limiting as in deep meadow margins. Consequently, there is a lack of scientific knowledge on how offshore

discharges can impact the integrity and survival of deep *P. oceanica* meadows. The ability of deep meadows to cope with such impacts will largely depend on the physiological and metabolic plasticity of the species to minimize metabolic carbon unbalances. The aim of this study was to characterize photoacclimative and metabolic responses of deep *P. oceanica* plants under the remote influence of offshore anthropogenic discharges. Five stations were distributed along the deep limit (26-2 m) of an extensive meadow (from north to south, A, B, C, D and E stations) following a gradient of anthropogenic pressure caused by the discharge of organic wastes (i.e. urban sewage and aquaculture facilities; Figure 1). Photosynthesis-Irradiance curves and daily light curves were characterized at each site to estimate the metabolic carbon balance of plants through the integration of photosynthetic variables and light regimes. The metabolic status of plants was also evaluated on the basis of carbohydrate concentrations in plant tissues. Determinations were conducted in summer and autumn, two different seasons of the annual growth cycle of the species, during which photosynthesis and carbon storage are at their maximum. Figure 1. Map of the study area showing the location of sampling stations (A-E) and the main sources of nutrient enrichment: the aquaculture facility (about 7.000 tons year⁻¹) and the pipeline through which urban wastes are delivered to offshore waters. Results showed that light levels in sites close to the pressure sources were significantly lower (20% in station A and 34% in station B) than in sites away from the influence of the discharges (control stations D and E). In summer, light availability in all stations was above the minimum light requirement of this seagrass species (10-16% relative to the surficial irradiance I_0 ; Ruiz and Romero, 2001) but during autumn light levels in stations A and B were below such threshold (9.07-6.2% I_0). This limited availability of light caused some modest but negative effects on the photosynthetic performance of plants (e.g. reduced net photosynthetic rates, effective quantum yield and relative ETR). Station B was the only site where some photo-acclimatory effort was observed in autumn plants, as reflected the significant increment in compensation irradiance (I_c). Carbon balance of summer plants was highly positive in all stations but contrarily, it was significantly reduced in autumn plants, whose respiration rates almost doubled those of summer plants. In particular, the carbon balance of autumn plants from stations A and B was significantly lower than in control stations (from 54 to 173%). It was negative in station A but slightly positive in station B, despite the fact that light reduction was more pronounced in this latter station. This result could be attributed to the photoacclimative mechanisms activated by plants from station B during autumn. As a result of the light reduction caused by organic discharges, the energy status (i.e. carbohydrates accumulation in rhizomes) was lower in plants from stations A and B during summer and only in station B during the autumn season. Figure 2. Mean (standard error) of compensation irradiance (I_c) and metabolic carbon balance (CB) in sampling stations A-E in summer (grey bars) and autumn (black bars). Different letters indicate groups of homogeneous means obtained in the post-hoc test SNK ($p < 0.05$). In summary, our results suggested the influence of remote pressures on stations A and B. Alteration of light regimes in these sites is consistent with some photosynthetic responses and with the weakening of carbohydrate reserves of affected plants, although such responses also evidenced the low plasticity of the species to cope with reduced light conditions. The alteration of the carbon metabolism had no impact on the carbon balance of summer plants, but it affected autumn plants mainly due to their high respiratory rates. All these effects pointed to a reduced resilience of deep seagrass meadows to overcome subsequent light reductions during the winter, which is consistent with the general plant decline observed in the last years in stations A and B. In conclusion, this work evidenced the high vulnerability of deep *P. oceanica* meadows to remote, diffuse pressures and hence the urgent need to determine and to implement safety distances between organic wastes discharges and these valuable and vulnerable habitats.

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Physiological tipping points to light reduction underlie seagrass population collapse and abrupt shift in a shallow coastal lagoon

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Tipping points are events where small changes in environmental conditions cause rapid and extensive ecological change. These ecological phase-shifts generally occur when environmental control variables exceed some thresholds, but the actual mechanisms driving this abrupt changes are often unknown (Scheffer et al., 2001). At the ecosystem level, feedback mechanisms play a major role in nonlinear responses of natural systems to environmental changes. Positive and negative feedback mechanisms can stabilize and destabilize ecosystems, which create potential for alternative and potentially stable states. At the organismal level, phenotypic plasticity could also be important for buffering ecological tipping points, although current discussions of the concept of tipping points seldom incorporate the physiological performance of the constituent organisms (Harley et al., 2017). These physiological approaches are particularly relevant in ecosystems dominated by habitat-forming species, since the extinction of a single species has an overall impact on the functioning of the ecosystem and its integrity. The Mar Menor is one of the biggest coastal lagoons in the Mediterranean area. The lagoon underwent a recent phase-shift, where a clear-water, macrophyte-dominated state gave way to a turbid-water, plankton-dominated state via eutrophication (Pérez-Ruzafa et al., 2019). In the spring 2016, an abrupt increase in nutrients and chlorophyll a concentration was detected, producing a dramatic loss of water transparency. These abnormal turbidity conditions remained for at least one year, resulting, after several months, in the massive die-off of the benthic vegetation dominated by the macrophytes *Cymodocea nodosa* and *Caulerpa prolifera* (Belando et al. 2017). In this study, we aimed to explore the physiological basis underlying the ecological regime shift in the Mar Menor by studying the phenotypic plasticity of the dominant species *C. nodosa* to the loss of water transparency. The hypothesis is that the observed ecological tipping point in the lagoon is explained by nonlinearities in how light reduction is translated into physiological responses, and how those drive population dynamics that affect ecosystem-level patterns. We used a field gradient design experiment to capture nonlinearities of the physiological responses in the species (Kreyling et al., 201). Nine shading treatments were applied to a shallow (1 m depth) *C. nodosa* meadow by using neutral density screens. The selected light treatments encompassed a broad range of light availabilities: from 26% of subsurface irradiance (I_0 ; 29.9 mol quanta $m^{-2} d^{-1}$) in controls to 96% of I_0 (2.3 mol quanta $m^{-2} d^{-1}$) in the most intense treatment. Changes in meadow structure (shoot density, meadow cover and plant biomass) were followed for the six-month duration of the experiment to characterize nonlinear population responses and the level of light reduction at which *C. nodosa* population start to decline. Morphological and (photo-)physiological plant responses were analyzed after two months of experimental shading for detecting and quantifying nonlinear trait responses that may underlie population collapse and ecological abrupt phase transition. Our results revealed that *Cymodocea nodosa* is able to maintain high plant density and biomass up to a light reduction of about 75% of subsurface irradiance (Figure 1), thanks to the activation of physiological mechanisms for plant acclimation to low light. Plastic plant traits contributing to this “homeostatic” population response included changes in canopy height, leaf concentration of photosynthetic pigments and reduction in the saturation irradiance (E_k) of shaded plants, among others. The integration of such complex responses resulted in an almost unaltered daily carbon balance of shaded plants, which favored the preservation of their energy reserves (i.e. total non-structural carbohydrates concentration in rhizomes; Figure 2). Figure 1. Shoot density (B) and total plant biomass (C) in each of the nine light reduction treatments at the end of the experiment. Further light reductions (>75% I_0), however, drastically reduced the standing biomass of the population and significantly increased plant mortality rates (Figure 1), most likely due to the nonlinearity of some physiological responses. Gross-photosynthesis slowly decreased with decreasing irradiance, but this trend was four times stronger when light reduction exceeded this light threshold. Respiration was another key physiological trait, and showed a strong reduction at the same light threshold, which allowed plants to maintain their daily carbon balance unaltered (Figure 2). However, respiration cannot be further reduced under more intense shading, with direct consequences on the plant carbon balance and hence, on the energetic status of plants (Figure 2). Figure 2. Respiratory rates (B) and total non-structural carbohydrates concentration in rhizomes of *C. nodosa* from the nine light reduction treatments of the experiment. Our findings evidenced that the nonlinear physiological responses of *C. nodosa* to turbidity may be underlying the regime shift in this coastal ecosystem. The high phenotypic plasticity of *C. nodosa* to light reduction is one of the main mechanisms that would explain the high resilience of the coastal lagoon to decades of eutrophication. Plants are able to activate successful acclimative (homeostatic) mechanisms to buffer population responses (e.g. shoot

density, plant biomass) to light reductions up to 75% of the natural/undisturbed levels. However, higher light reductions exceed the limits of physiological plasticity in the species, when plants revealed a marked steeping in their responses and where small increments in light reduction provoked profound changes in the population structure. We concluded that, understanding the mechanisms underlying the complex interaction between changes in the environment (e.g. light reduction) and organismal (physiological) and ecological (community) responses is critical if we are to predict ecosystem tipping points, mainly in those systems dominated by foundation species.

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Keywords: Critical transition, Nonlinear responses, turbidity, foundation species, *Cymodocea nodosa*

Spatio-temporal variability of wrack abundance and composition along the north Portuguese sandy beaches

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Many detached macroalgae and seagrasses leads in amounts of detritus on the shorelines forming accumulations commonly named as “wrack”. Wrack is exported from highly productive rocky shores to low-productive sandy beaches. This accumulation of marine macrophytes also plays key roles providing nutrients to primary producers and food for decomposers. So high is the value of wrack as a substitute for nutrients that humans have used this vegetal detritus as fertilizer for centuries. However, to the date, no study has proved the value of wrack in the sandy Portuguese coasts. The interest of the sandy beaches as spaces for leisure, tourism and recreational activities makes authorities to remove wrack and other debris in order to improve their aesthetics, amenity and utility for the user demand. This removal of debris is usually carried out in summer and, in most of the cases, the wrack is treated as residues without any additional valorisation. The project Val-Wrack was created with the aim of exploring the ecological drivers that control the amount of wrack that arrives to Portuguese sandy beaches testing its potential use as economical resource. One of the aims of this project is to explore the natural variability of wrack abundance along the north Portuguese sandy beaches. For this aim, several beaches were visited in order to estimate the species composition and total biomass of wrack. Sampling was carried out during low spring tides. Samples were randomly collected from the base of the dune to the lowest swash level along transects of 100 m, deploying randomly 10 quadrats of 100 x 100 cm and collecting macrophytes within them. To explore species composition, the wrack of each quadrat was stored separately in bags and frozen. Subsequently, seaweed and seagrass species were separated and total biomass of each one was calculated by drying samples at 60°C. The spatial and temporal variability of biomass and number of

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species was explored by analysis of variance (ANOVA). Spatial and temporal changes on the structure of wrack assemblages was explored by permutational multivariate analysis of variance (PERMANOVA). Until now, we collected the wrack supply of four sandy beaches, two at the north (Cabedelo and Âncora) and other two at the south in the Esmoriz area. Results showed that in northern beaches, wrack was predominately composed by the fucoids *Fucus* spp., *Ascophyllum nodosum*, *Pelvetia canaliculata* and the invasive *Sargassum muticum*. However, in the southern beaches wrack was composed by *Fucus* spp., *S. muticum* and the seagrass *Zostera noltei*. As in the north of Portugal, *Fucus* sp. and *A. nodosum* reach their southern distribution limit, in a future scenario of global change, the exportation to sandy beaches of these two macroalgae could disappear, with negative consequences because our results showed that these are the main species present in wrack. On the other hand, the presence of the seagrass *Z. noltei* as member of wrack in the southern most beaches indicates an important contribution from Ria de Aveiro, where this species is widely distributed. Therefore, our results highlight the importance of the conservation of *Zoostera* beds in the Ria de Aveiro. The knowledge provides in this study, could help us to understand the consequences that climate change could have on the coasts of northern Portugal. In addition, this study provides us valuable information about the seasonal and spatial variability in the abundance and composition of our resource (wrack). This is a critical point to plan its exploitation and therefore, its viability as resource.

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Keywords: Wrack, North of Portugal, sandy beaches, Fucoids, seagrass

Biobanking of local seaweed species to support conservation and commercial aquaculture: *Saccharina latissima* at its southern distribution limit.

Isabel C. Azevedo, Rosa Melo and Isabel S. Pinto

Interest in seaweed has been rising due to the multiple high value applications of their biomass. They are considered a healthy food, due to a balanced nutritional composition, and a valuable source of bioactive compounds for the food, feed, cosmetic and pharmaceutical industries. Additionally, seaweed is used for nutrient bioremediation and is also considered a good candidate for biofuels production. Since at present seaweed production in Europe is originated mainly from harvest, an increased demand may cause the overexploitation of natural populations that are already under stress by global climate change and anthropogenic pressures. In order to fulfil this increasing biomass demand, sustainable seaweed aquaculture methods are needed to avoid the risk of overexploitation of wild stocks. These methods include the implementation of breeding strategies based on strain selection and improvement for cultivation. Natural populations are a repository of genetic diversity which plays a fundamental role in their adaptation to changing environmental conditions. This genetic diversity may also be an important source of genetic material for the selection of strains that present interesting traits for commercial or conservation aquaculture. Traits useful for commercial aquaculture include high growth rates, resistance to herbivory and adaptation to the environmental variability of the cultivation site, whereas traits such as reproductive output are important for conservation aquaculture. Natural populations at the rear edges - the low-latitude limit of species ranges - are recognized as particularly vulnerable areas of diversification and endemism. They often have unique genetic diversity which may hold valuable genetic traits important for conservation and sustainable seaweed aquaculture development. In the framework of the GENIALG project, strains of *Saccharina latissima*, a kelp with high commercial potential, were collected from populations of northern Portugal and Galicia (northern Spain) for the establishment of a local biobank. Biometric data corresponding to each strain were also collected. These populations are at the southern distribution limit of the species in the western Atlantic coast. The site in Portugal is Amorosa (41.64167°N; -2339°W), the southernmost site where *S. latissima* can be found attached to pebbles that appear in the intertidal. This population is very dispersed and dynamic, since all the individuals found are attached to pebbles that are mobile with the currents and wave action. In Galicia, the site is located in the Ría de Muros y Noya, near Esteiros (42.76667N; -9.66111W), characterised by more sheltered conditions, compared to the Amorosa shore which is a very exposed area. Strains were collected at Amorosa in several sampling periods (between March 2017 and March 2019), whereas the population in Galicia was sampled in February 2019. For each individual, the

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biometric data collected were: total length, stipe length, blade and stipe width, meristem and blade thickness, blade area and sori area. For the fertile ones, samples from the reproductive tissue were collected for spore release, which were placed in Petri dishes at 15 °C under red light to avoid fertility. Tissue samples were also collected for genotyping. Presently, the biobank includes 44 strains of the Amorosa population and 25 of the Esteiros population. Considering the Amorosa population, fertile individuals were found from November to March, the typical reproductive season of cold water kelp species. The largest individuals, reaching 133 cm, were found in September, decreasing in the following sampling dates. These data, although limited, indicate that spores are released during the winter, growth occurs during the spring and summer, and in autumn size decreases due to blade erosion when they become reproductive. In March some mature individuals presented a constriction separating the old from a new blade, indicating that the growing season starts in early spring. Considering the reproductive effort, measured by the percent sori to blade area and number of spores per cm², no obvious trend was observed although the highest values were found in February. Comparing the Esteiros population biometric data with data from the Amorosa population of the same period, it is apparent that sporophytes from the Galician population are larger, with longer and wider blades. This may be related to adaptation to environmental conditions, especially hydrodynamics, since the Galician site presents more sheltered conditions than the Portuguese site, influencing blade morphology. The on-going genotyping of the strains included in this biobank will allow assessment of the genetic diversity in these range-edge populations. Future work will include the study of the influence of environmental conditions on phenotypic traits and their relationship with genotypes. The establishment and development of this local biobank for *S. latissima* will be useful for development of sustainable aquaculture methods with improved strains, better adapted to local conditions and also for supporting conservation actions in local kelp beds.

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Keywords: *Saccharina latissima*, biobanking, Distribution limits, Aquaculture, kelp forests

A general, easy and practical model to evaluate a saltmarsh space capacity as CO₂ sink and role as an Ecological Service Provider

Christian Requena, F. Xavier Niell

The main objective of this study is to develop and test a model to estimate the capacity of saltmarsh assemblages of plants as CO₂ sinks. A previously widely and well-studied saltmarsh has been chosen for this test. The saltmarsh is a 1.53 ha space in the Estuary of Palmones River (Southern Spain)

The basic data to implement the model has been: 1) The values of Photosynthetic Active Irradiance (PAI) obtained in the field and checked in laboratory for each species with conspicuous biomass in the saltmarsh; 2) the averaged values of real daily light irradiance recorded during the year. The instantaneous values of photosynthetic rate are obtained by the product of these 2 variables. By integration of all the daily data, it was calculated the amount of net assimilated carbon during a given period of time.

The calculated photosynthesis in this way, is an estimation for a monolayered photosynthetic biomass, and must be corrected by the real Leaf Area Index (LAI) deep in the canopy. This value multiplied by the surface of the studied area gives the total sink of C. In this study, the average total C sink was estimated as 1994±453 g C m⁻²yr⁻¹. The sinking values must be corrected by the net reemission from the soil respiration, measured by Infrared Gas Analysis (IRGA), and be discounted from the net sink. The soil reemission of CO₂ averaged 1174±365 g C m⁻²yr⁻¹. The turnover rate calculated for C circulating in the plant structures and soil, both together as a system, amounted to 22% of biomass per unit of time.

The net sink of carbon computed was 529 ±122 g C m⁻²yr⁻¹. The whole weighted balance of C sequestered was 10±113 tones/y in all the area of study. The estimated value of this sector of the saltmarsh of Palmones as Ecological Service Provider was 5137±719 € per year (estimated error, 13,9 %).

The application of this model leads to realize the high turnover of the C cycle in this type of communities. The main problem detected for this method is the excessive error in the direct measurements because the averages are estimated always with a high standard deviation

Keywords: Ecology, saltmarsh, Ecological Service Provider

Diversity Assessment of Benthic Marine Macroinvertebrates Using Artificial Substrates

Regina C. Neto, Hugo R. Ferreira, Diego CArreira-Flores and Pedro T. Gomes

Marine macroalgae are dominant and abundant organisms that act as a biological structural substrate with positive impacts on the survival, richness, and abundance of local species. Through their physical structure, these organisms alter the physical conditions of the habitat and influence, directly and indirectly, the biological interactions, playing an important role in the structuring of the macrobenthic communities. In the nearshore temperate zones, macroalgae are amongst the major players in habitat formation and important elements of many organisms in several stages of their life cycle. However, the sampling of these kinds of habitats has some drawbacks, namely the need to act in a destructive way in order to access the resident fauna and the difficulty in standardizing procedures. In the long term, with certain types of biotopes, the sampling activity may also compromise the habitat which is not desirable. Therefore, there is a need for an alternative methodology's that can give equivalent results without destroying the habitat and that can be easily replicable. Artificial substrates (AS) may constitute a viable alternative, as they can be easily standardized, fundamental for statistical quantitative studies and, eliminate as well the need for destructive sampling of natural substrates. Through those AS we intended to mimic the structure of 2 kinds of natural environments: a leafy complex alga (*Cystoseira* sp and other structural similar algae), designated as AS1, and the space provided by the rhizoids of some Laminariales (*Saccorhiza polyschides*, for instance), designated AS2. We placed in the Ria de Ferrol (Spain), a set of 6 replicates at 2 meters depth (Torpedera) and another at 9 meters depth (San Cristovo) during a period of 3 months (May to August). After sorting, a total of 9710 organisms was collected and identified to the maximum taxonomic detail possible (species when possible; otherwise the genus or family), amounting to 194 different taxonomic entities. The biological metrics (richness, abundance, diversity and equitability) showed a significant difference between the substrates placed at 2 meters depth, whereas at 9m depth only differences in the abundance of organisms were found. We believe that at Torpedera, the exposure to currents, plus the water flow led to the provision of a larger niche for filtering and tube builder's organisms in the substrate AS1. Additionally, it also led to a greater settlement of particles and sediments in the substrate AS2, allowing for a greater number of detritivores. At higher depths there was preference of certain species for the leafy substrate AS2, namely by the presence of larger organisms.

The patterns of abundance and richness of macroinvertebrates suggest that habitat preferences may be related to different feeding modes, reproductive strategies, morphologies, and mobility, besides confirming the dependence of several groups of organisms on the habitat architecture as well as the ability of AS to support diverse communities.

Keywords: Artificial substrate, Structural complexity, macrofauna, Taxonomic diversity, colonization

Cryptic or cosmopolitan? Unveiling the *Laeonereis culveri* complex along South American estuaries through DNA barcoding

Bruno R. Sampieri, Pedro E. Vieira, Marcos A. Teixeira, Antonia C. Amaral¹ and Filipe O. Costa

DNA barcode-based studies on the diversity of polychaete species occurring in Brazilian coasts have received little to no attention. Nevertheless, it has been suspected that several polychaete taxa have hidden diversity, including cryptic and pseudocryptic species. *Laeonereis culveri* (Nereididae) represents an old case of taxonomic ambiguity, which since 1971 is assumed as a single species occurring from north to south along the Atlantic Coast of the Americas. In 2009, a taxonomic revision based exclusively on morphology corroborated the previous status. The current study aimed to review *L. culveri* taxonomy, diversity and distribution through the morphological and DNA barcode-based approach. Herein, we sampled and examined 23 populations spread along the South American Atlantic coast from North Brazil to Mar del Plata, Argentina. All specimens were identified morphologically and processed for DNA extraction, amplification and sequencing of the mtDNA COI-5P and 16S rRNA 16S genes, as well as the nuclear 2S rRNA gene. Sequences were processed and edited in MEGA 7.0, where genetic distances were also calculated. Four molecular-based species delimitation methods were applied, the ABGD, bPTP, GMYC and BIN. Bayesian trees were estimated in Mr. Bayes and the haplotype network was determined using the reduced median algorithm in Network 5.0. Specimens from the sampled populations were confirmed as *L. culveri* based on external diagnostic characters. The COI barcodes split the morphospecies in seven completely sorted MOTUs, displaying deep genetic divergence (K2P ranging from 6.6% to 21.9%). Most of MOTUs were clearly sorted geographically, except for the overlap in the geographic boundaries between MOTUs 2 and 3 occurring in Amapá state, and MOTUs 6 and 7 occurring both in the states of Rio de Janeiro and São Paulo, Brazil. Sequences from 16S rRNA and 2S rRNA genes, support the same seven MOTUs and geographic sorting identical with the COI-based pattern. We have found morphological and multi-locus evidence for the existence of at least seven cryptic species within the largely distributed *L. culveri* morphotype. The role of *L. culveri* as an important bioindicator of organic enrichment in estuaries needs to be revised in light of these new findings, namely through detailed ecological characterization of the MOTUs here detected. The deep divergences and geographic sorting of these species suggests that their current distribution still reflects a diversification process that likely initiated millions of years ago. *L. culveri* may therefore provide an important case-study to bring light into the poorly known phylogeographic history of marine macrobenthic invertebrates along the Atlantic coasts of South America.

Keywords: DNA barcode, Polychaeta, Nereididae, Biodiversity, phylogeny

Genotyping impact of mixing samples from multiple non-model species in population genomic analyses

Carlos Carreras, Sergi Pous, Marc Vega, Enrique Macpherson and Marta Pascual

The rise of genomics during the last decade using high-throughput sequencing has allowed the genotyping of thousands of markers on a genome-wide approach, even in non-model organisms. This major advance in research enhances the resolution of population genetic analyses but also brings new research opportunities for non-model species, including the analysis of outlier loci to look for genomic signals of adaptation or genomic association to environmental cues and phenotypic variants. However, the call of usable loci for non-model species relies on 'de novo' assembly pipelines due to the lack of a reference genome. Consequently, the analysis could be very sensitive to the presence of non-target species within the sequenced samples, as available pipelines would not recognize them as samples from foreign species. In order to evaluate the impact of mixing samples from different species on the number of markers obtained we used two different datasets of samples of fish species sequenced by Genotyping by Sequencing (GBS). Firstly, we evaluated the effect of the phylogenetic distance between the mixed species by combining data from individuals from five

different species (4 of the genus *Symphodus* and 1 of the genus *Serranus*) collected in the Mediterranean and Black seas. We found that mixing samples from different species decreases the number of shared loci although increases the total number of loci and that this variation is related to the phylogenetic distance among the mixed samples. Therefore, the phylogeny of the analysed species could be reconstructed considering the percentage of loci lost when the species are pair-wise mixed. Furthermore, the number of different species within the sample set also has an incremental effect on the loss of shared loci. Secondly, we evaluated the effect of the proportion of mixed samples in the number of identified loci by using data from two species of the genus *Symphodus* collected in four western Mediterranean locations. We randomly mixed reads from individuals at different proportions and we found that the loss of shared loci was related to the proportion of samples from the non-target species in accordance to the threshold of missigness allowed during the filtering process. All analyses were performed using UNEAK and GibPSs pipelines for loci identification and individual genotyping, both designed to be used in non-model organisms. For the two pipelines we found the same trends described above when mixing samples from different species although UNEAK generally yielded less loci than GibPSs. Our results highlight the importance of detailed analysis to detect outlier samples that may interfere with the study when working with non-model organisms but also indicates the potential of this type of analysis to detect incorrectly classified samples or even new cryptic species.

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Keywords: Genotyping by sequencing (GBS), Bioinformactics, *Symphodus*, *Serranus*, Mediterranean, Black Sea

How cryptic is cryptic? Combining histology with DNA barcoding to elucidate taxonomic ambiguities in polychaete species

Bruno R. Sampieri, Marcos A. Teixeira, Pedro E. Vieira, Filipe O. Costa and Antonia C. Amaral

Polychaetes are common and abundant in marine environments worldwide and constitute one of the most diverse and complex groups of marine invertebrates. The morphology and physiology of the female reproductive system (FRS) can be understood by using histological tools to identify and differentiate polychaete species. However, this histology-based approach is rarely combined with molecular tools, such as DNA barcoding, which is known to accurately reflect species boundaries in most cases. Therefore, the present study aims to test a multi-tool approach to resolve taxonomic ambiguities in three common and abundant polychaete species on the South American coast (*Laeonereis culveri*, *Scolelepis goodbodyi* and *Capitella biota*). Moreover, this study also aims to demonstrate that the FRS corroborates with the molecular data and can be used to delimitate species/cryptic species. Specimens were collected in the northern coast of São Paulo state, Brazil, in sandy and muddy bottoms during low tides. Five specimens of each species were fixed in 10% glutaraldehyde solution in Phosphate Saline Buffer (PBS) with addition of 7% sucrose for histological techniques and five of each were fixed in ethanol 96% to perform DNA extraction. The histological data generated in the present study were compared to previous published papers of histological features regarding the FRS within the same species and/or congeneric ones. On the other hand, molecular data were analyzed based on sequences generated herein and deposited on DNA databases (GenBank and BOLD). The results demonstrate that the following histological characters of the FRS can be relevant for the separation of closely related species: a) oogenesis type, b) occurrence or absence of a true ovary, c) ovary tissue organization, d) type of accessory cells present, and e) oocyte morphology. Additionally, these histological features matched with the boundaries suggested by the genetic data. Within the *Laeonereis culveri* species, it differentiates between North and South American Atlantic populations (16.7% genetic distance) while in *Scolelepis goodbodyi* and *Capitella biota* it discriminates them from their congeneric species. Clearly, in *L. culveri* case, we are dealing with a cryptic species in process of being elucidated through DNA barcode-based approach, brought to light also by histological methods. These results highlight the importance of multi-tool approach and shows that both FRS histology and histophysiology, and DNA barcoding can be used to identify and discriminate cryptic species, which is

usually not possible when using external morphological characters. Besides, these characters may also be useful in differentiating related species, and/or geographically distinct populations among polychaetes.

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Keywords: Annelida, reproductive tract, Oogenesis, Integrative taxonomy approach, Microscopy

Medusozoa in Portugal: impact on the ecosystems and development of DNA-based tools for the early forecasting of mass occurrences

Tomás Rodrigues, Ana Matos, Dany Domínguez-Pérez, Joana Falcão, , Sónia C. Marques, , Sérgio Leandro, Daniela Almeida and Agostinho Antunes

Medusozoa is a subphylum of Cnidaria composed of aquatic animals generally toxic with complex life cycles that include the two main body forms of cnidarians: a benthic polyp and a pelagic medusa. Medusozoans are divided into four classes: Hydrozoa (Owen, 143) - hydras, hydroids, hydromedusae, and siphonophores; Scyphozoa (Goette, 17) - true jellyfishes; Cubozoa (Werner, 1973) - box jellyfishes or sea wasps; and Staurozoa (Collins & Marques, 2004) - stalked jellyfishes. Some of the species encompassing this group have the capability to fast increase in abundance leading to huge impacts in fisheries, public health, tourism, industry and aquaculture. Those impacts are produced by the free-living stage of cnidarians known as "jellyfish", influenced by some natural factors, but also induced by global warming, overfishing, eutrophication and widening of invasive species habitats. These events have been reported worldwide and the global panorama corresponds to an increase on the occurrence of this phenomena. Despite the common occurrence of these events, early warning tools are still lacking. Specifically, in Portugal, although jellyfish blooms are commonly reported by the Portuguese National Authorities, the information related to Medusozoa, their mass occurrence events and associated impacts is scarce. Thus, in order to increase the knowledge into this topic, the main aims of this work included: (i) a bibliographic review compiling for the first time a list of the Medusozoa reported in Portugal (mainland, Azores and Madeira); (ii) a collection of the genetic information available in public databases for the Class Scyphozoa representing the "true jellyfishes", organisms able to produce mass occurrence events; and (iii) increase the genetic data in Scyphozoa. The resulting information will improve the accuracy for the characterization at the genetic level of these species and thus, be pivotal for the development of early-detection DNA-based tools for mass occurrence events produced by jellyfishes. Overall, the compilation of all information available revealed that all the four groups of Medusozoa are represented in Portuguese waters, with a total of 26 different species, specifically: 250 hydrozoans; 15 scyphozoans; 1 cubozoan; and 2 staurozoans species. The analysis of the genetic information available at the nucleotide database of National Center for Biotechnology Information (NCBI), for the 15 scyphozoan species that are reported in Portugal, showed that only *Chrysaora quinquecirrha* and *Aurelia aurita* had the complete mitochondrial genome available. Remarkably, *Catostylus tagi*, which is one of the most common scyphozoan species in Portugal, has only three sequences available, corresponding to three genetic markers: 1S ribosomal RNA (1S), 2S ribosomal RNA (2S), and Internal Transcribed Spacer 1 (ITS1). Considering the low information at molecular level available from *C. tagi* and its capability to occur in mass, herein we used different experimental protocols for the amplification of nuclear and mitochondrial genetic markers. The specimens were sampled in Tejo River on October 4th, 201. The collected organisms were dissected and divided into triplicates of gonads and tentacles. Genomic DNA (gDNA) of those samples was extracted using the PureLink™ Genomic DNA Kit (Invitrogen, Carlsbad, CA, USA), following the protocol for Mammalian tissues. DNA was eluted in 50 µL of Elution Buffer. Genomic DNA was quantified in the spectrophotometer DeNovix® DS-11 FX and stored at -20°C for further analyses. We selected for amplification some of the most phylogenetic informative genes (COI: cytochrome c oxidase subunit I, COIII: cytochrome c oxidase subunit III, 1S, 2S, ITS1 and ND6: NADH-ubiquinone oxidoreductase chain 6). For that, we performed Polymerase Chain Reactions (PCR) using different sets of primers pairs, previously used for Cnidarian species, namely (gene - primer pairs): COI gene - HCO1490/LCO219 [1]; COIII gene - COIIIF/COIII3R [2]; 1S gene - 1S:primer A_F/1S:primer B_R [3] and 1SFb/1SRb [4]; 2S gene - 2S:primer F/2S:primer R [5] and 2S:LSUD1F_F/2S:D3Ca_R [6]; ITS1 - ITS1:jfITS1-5f_F/ITS1:jfITS1-3r_R [7]; ND6 gene - Nd6F/Nd6R (Frazão et al.,

in review). The PCR cycling conditions applied were the same described by the previously mentioned works for primer pairs sequence with the following exceptions: 2S - a pre-heating for 2 min at 95°C, followed by 30 cycles at 94°C (30 s), 56°C (30 min) and 72°C (1 min), with a final extension at 72°C for 10 min, using the set of primers 2S:LSD1F_F/2S:D3Ca_R, we followed the program by Osborn & Rouse, 2010 []; ITS1 - a pre-heating for min at 94°C, followed by 33 cycles at 94°C (45 s), 53.5°C (45 s) and 72°C (1 min), with a final extension at 72°C for 5 min. The results obtained originated the first submission to the NCBI of COI, COIII and ND6 sequences for *C. tagi*, as well as, new sequences of 1S, 2S and ITS1 genes. As future perspectives we aim to extend the sequencing of the aforementioned genetic markers to all the jellyfish species reported in Portugal. These sequences will be comprised in a database as a reference to detect/predict mass occurrences events based on molecular tools like metagenomic approaches.

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Keywords: Cnidaria, Medusozoa, Scyphozoa, jellyfish, *Catostylus tagi*, Early forecasting, Mass occurrences, Blooms

Polymerase chain reaction as a promising tool for DNA-based diet studies of crustaceans

Joana V. Campos, Cristiana Moreira, Sérgio C. Costa-Dias, Eunice Ferreira, Ana B. Matos, Vitor Vasconcelos and Agostinho Antunes

1. Introduction Feeding ecology studies provide relevant information to understand the ecosystem's trophic web and functioning. Diet investigations based on direct visual inspection of crustaceans' gut contents can be complicated due to partial ingestion and/or prey crushing, resulting in few identifiable food remains. In contrary, biochemical methods enable alternative, rapid and unambiguous prey identification. These include enzyme electrophoresis, immunological assays, and Polymerase Chain Reaction (PCR)-based methods in detecting prey DNA (Albaina et al.2010, King et al.200, Symondson 2002). The PCR technique is faster, more sensitive and specific in predator-prey studies than other methodologies, allowing a simple and rapid detection of the presence/absence of a potential prey by application of specific genetic markers. Here, we aimed to assess the utility of PCR as an alternative tool for studies on crustaceans' diet using the shore crab *Carcinus maenas* and the brown shrimp *Crangon crangon* as study models. 2. Material and methods 2.1. Sampling and stomach dissection Crustaceans and potential preys (common goby *Pomatoschistus microps*, ragworm *Hediste diversicolor*, flounder *Platichthys flesus*, peppery furrow shell *Scrobicularia plana*) were collected in the Minho Es-

tuary (Portugal). Size (mm; total length, TL, for shrimps; carapace width, CW, for crabs).The whole stomach was removed from about 100 shrimps and 25 crabs per campaign, weighted under sterile conditions and stored in Eppendorfs at -20oC. A stomach fullness index (SFI: 0=empty; 1=up to half full; 2=full) was recorded. Only SFI=2 stomachs were used for DNA extraction. 2.2. DNA extraction Shrimps full stomach was used for DNA extraction, since stomachs were all very small (mean stomach wet weight=0.0069g, sd=0.0052, n=664), while the large amount of the crabs' stomach content required subsampling by extracting a 1.5 to 25mg portion. Full stomachs and subsamples were homogenized prior to DNA extraction. DNA was extracted using the Pure-Link™ Genomic DNA Mini kit (Invitrogen, Carlsbad, California, USA) applying the protocol for mammalian tissues. Each month, up to ten stomachs (SFI=2) of shrimps and crabs were chosen for DNA extraction. The protocol was followed as recommended by the manufacturer, and the final DNA was eluted in 25 and 50 µL of elution buffer provided by the kit, respectively for shrimps and for crabs; 5µL of the extracted DNA were visualized by 1.5% agarose gel electrophoresis in 1X TAE buffer and stained with ethidium bromide to visualize the integrity and quality of the extracted DNA. 2.3. PCR specificity for the potential preys PCR amplification was performed using a muscle sample of each prey species applying specific primers previously described and tested for specificity, for *H. diversicolor* (Virgilio et al.2009), *P. flesus* (Espíñeira et al.200), *S. plana* (Santos et al.2012) and *P. microps* (Gysels et al.2004). The cytochrome subunit b (cytb) gene was used for the identification of *P. microps*, while the mitochondrial cytochrome oxidase c subunit I (COI) gene was used for the identification of the other prey species. All PCR reactions were performed in 20 µl containing 1x PCR Buffer, 2.5 µM of MgCl₂, 250 µM of each deoxynucleotide triphosphate, 10 pmol/µL of each primer, 0.5 units/µL of Taq polymerase (Bioline, Luckenwalde, Germany) and 5-10 ng of DNA. Bovine Serum Albumin (BSA) (10mg/mL) was also added to all the PCR reactions to remove any existing Taq inhibitors. All reactions were carried out in a Biometra thermocycler (Biometra GmbH, Goettingen, Germany). All potential prey were identified in the DNA extracted samples to determine their presence or absence in the stomachs. In shrimps, whole stomachs were tested for all preys, while in crabs the larger stomachs' content allowed to test the presence of more than one prey species per predator. For prey PCR identification primers sets and PCR conditions were applied according to those available in the literature (Espíñeira et al.200, Gysels et al.2004, Santos et al.2012, Virgilio et al.2009). In *H. diversicolor*, PCR conditions consisted of a denaturation at 94°C for 4 min, followed by 9 cycles at 94°C for 45 s, 55°C for 1 min and 72°C for 1 min, followed by a next 26 cycles at 93°C for 30 s, 45°C for 45 s and 72°C for 45 s in the end all samples were subjected to 72°C for 5 min (Virgilio et al.2009). In *S. plana*, PCR conditions consisted of an initial denaturation at 94°C for 5 min followed by 35 cycles at 94°C for 30 s, 40°C for 45 s and 72°C for 45 s, followed by a final extension at 72°C for 7 min (Santos et al.2012). In *P. microps* PCR conditions consisted in initial denaturation at 97°C for 3 min followed by 35 cycles at 95°C for 45s, 54°C for 45 s, 72°C for 45 s and final elongation at 72°C for 7 min (Gysels et al.2004). PCR conditions of *P. flesus* consisted in denaturation at 95°C for 3 min, followed by 35 cycles of 30 s at 95°C, 30 s at 54°C, and 30 s at 72°C, followed by a final extension at 72°C for 3 min (Espíñeira et al.200); 10 µL of each amplified DNA was visualized by 1.5% agarose gel electrophoresis in 1X TAE buffer. The gel was stained with ethidium bromide and photographed under an UV trans-illuminator. 3. Results A total of 699 shrimps (TL: 13.0 to 43.0 mm; 62% females, 33% males, 6% sex undetermined) and 142 crabs (CW: 23.71 to 64.40 mm; 39% females, 61% males; 57% green, 2% red, 15% undetermined) were collected and dissected. More than two thirds of the shrimps (71%) and crabs (5%) had food in the stomach. For both species, the highest percentage of full stomachs was found in February (23 and 56%, respectively, for shrimps and crabs, Figure 1). The primer sets were successfully tested to evaluate their ability to amplify the prey target from the stomach contents (Figure 2a and 2b). Only *S. plana* showed negative results in both predators, while the other prey species (*P. microps*, *H. diversicolor*, *P. flesus*) were present in the stomach of both crustaceans (Figure 2a and 2b). The stomach content of 29 crabs gave positive amplifications, four of them with two different prey species, while for shrimps, 11 positive amplifications were obtained corresponding to three prey species. In the positive amplifications, a single band was obtained at the corresponding size of the positive control (Figure 2a and 2b). In light of this data we found no need for the immediate DNA sequencing of these positive bands allowing this to be used in future studies. 4. Discussion Molecular techniques have recently been used to study predation by several taxa, but only a few studies have been dedicated to crustaceans. These included two shrimps *Crangon crangon* (Albaina et al.2010) and *Crangon affinis* (Asahida et al.1997), two crab species *Carcinus maenas* (Asahida et al.1997) and *Callinectes sapidus* (Collier et al.2014), the rock lobster *Jasus edwardsii* (Redd et al.2014), the amphipod *Themisto abyssorum* (Olsen et al.2014) and the calanoid copepod *Calanus finmarchicus* (Durbin et al.200, Nejstgaard et al.2003, Nejstgaard et al.200), as predators. Yet, such studies were focused on a single prey species – flatfish (Albaina et al.2010, Asahida et al.1997, Collier et al.2014), or species group – algae (Durbin et al.200, Nejstgaard et al.2003, Nejstgaard et al.200). Therefore, the present investigation enlarged the range of prey identified in crustacean stomachs through molecular methodologies. Moreover, these previous studies were mostly performed under controlled conditions, while in our study we investigated predation in the natural habitat. By adding a PCR inhibitor we obtained a more precise identification, diminishing any false negatives attributed to natural samples. Three additional improvements may be implemented to fully investigate crustaceans' diet through PCR-based methods: 1) a larger pool of primers; 2) the quantification of the relative importance of each prey spe-

cies; and 3) DNA sequencing of the positive amplicons in order to proceed to other type of studies (e.g. phylogenetic analysis by comparing these with DNA sequences from other geographical populations). Polymerase Chain Reaction (PCR)-based methods proved to be a very powerful alternative tool to study crustaceans' diet. The results suggest that these methods are reliable not only for detecting prey DNA in the stomach, but also for its identification to the lowest taxonomic level, i.e. the species level. Therefore, once potential prey and their availability in the system are known, this technique can be useful to overcome some of the limitations of traditional methods in the study of the crustaceans' diet, even when stomach contents include a great part of 'detritus'. List of figures Figure 1. Stomach fullness index (SFI, % of animals) results for *Carcinus maenas* (CM) and for *Crangon crangon* (CC) during the studied period. Figure 2. Electrophoresis of the positive amplifications obtained in the PCR with stomach contents and the prey species' controls. From left to right: 2a) DNA ladder, one *Pomatoschistus microps* (Pm), four *Platichthys flesus*, and three *Hediste diversicolor* positive results from crabs; 2b) DNA ladder, one P. microps, eight P. flesus and one H. diversicolor (Hd) positive results from shrimps. '+' is the positive control amplification for the prey species immediately on its left side.

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Keywords: Shore crab, *carcinus maenas*, Brown shrimp, *Crangon crangon*, Stomach content, Prey identification, PCR

Review of morphological characters, dermal denticle size and molecular analysis to help in the identification of *Deania* species

Cristina R. Ródenas, Montse Pérez and Irene G. Vazquez

The family Centrophoridae Bleeker, 159 which includes two genera: *Centrophorus* and *Deania* is one of the most taxonomically complex and confusing taxon of species currently under review. The high morphological similarity among some species, the scarcity of records, and few taxonomical analyses contribute to the great uncertainty concerning the taxonomy of some species. Three elasmobranch species of the genus *Deania* are currently reported in NE Atlantic waters: *D. calcea*, *D. hystricosa* and *D. profundorum*. Whereas *D. profundorum* is easily recognized by the presence of a sub-caudal keel on the lower caudal peduncle, the other two species are mainly discriminated by the size of the dermal denticles and body color. In this study we examined several individuals of *Deania calcea* and *D. profundorum* of diverse size and sex caught in north Spanish waters (Galicia and Cantabrian Sea, NE Atlantic) to check morphological and genetic differences. A review of historical descriptions and data is also considered. Multivariate analysis based on morphological characters was used to check differences between the two *Deania* species. The hierarchical analysis clearly identified three groups. Two corresponded to each species whereas a third one discriminated between small and large individuals of *D. profundorum*. The morphometric characters that contributed most to the divergence between both species were the length and base of the first dorsal fin (D1L, D1B), the distance or space between dorsal fins (IDS, 1Dto2D, IDS*) and additionally the distances from the snout to the origin of 1st dorsal fin (PD1) and pelvic fins (PP2). Not sexual dimorphism, based on morphological characters, was observed according to the non-metric multidimensional analysis (MDS). A positive significant correlation was found between shark total length (TL) and dermal denticle length (CL). Dermal denticles also varied in size and shape with placement on the shark body being larger those from the anterior area (close to head) and smaller those close to the caudal region. In *D. calcea*, CL ranged from 340 μ to 1400 μ considering all individuals sampled and body areas whereas in *D. profundorum* CL ranged from 195 μ to 650 μ . The phylogenetic tree reconstructed with COI sequences available on BOLD and those obtained in this study clearly separated molecularly the species *Deania profundorum* and *D. quadrispinosa* whereas *D. calcea* and *D. hystricosa* could not be discriminated.

Keywords: *Deania calcea*, *Deania profundorum*, morphometric, dermal denticles, DNA

Combining RNA-seq and ddRAD-seq to SNP discovery of a highly migratory and apex predator

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The shortfin mako, *Isurus oxyrinchus* is an oceanic pelagic shark species found worldwide in tropical and subtropical waters. It is one of the most frequently shark caught by pelagic longline fisheries, and despite its commercial importance and ecological significance, the understanding of its biology and ecology at molecular level is still incipient. Therefore, we combined two massive parallel sequencing approaches, RNA sequencing (RNA-seq) and double digest restriction site-associated DNA sequencing (ddRAD-seq), for single nucleotide polymorphisms (SNPs) discovery in the shortfin mako. A total of four individuals of the shortfin mako (four liver tissue and three eye tissue) were used to RNA-seq library preparation, whereas 34 individuals from five location of the Atlantic Ocean were used to ddRAD-seq library preparation. Both libraries were sequenced by Illumina paired-end sequencing technology (Hiseq 4000). A total of 9,95 Gb raw reads were obtained from RNA-seq. De novo transcriptome from eye and liver yielded a total of 506,66,525 reads and 336,210 contigs. A total of 274,21 and 310,62 contigs were mapped for eye and liver, respectively, and 37,227 (11.04%) sequences were successfully annotated based on sequence similarities against the UniProt, the Kyoto Encyclopedia of Genes and Genomes (KEGG), and the NCBI non-redundant (NR) protein database. In total, 119,121,972 raw paired-end reads were generated from ddRAD-seq sequencing approach. After demultiplex and trimming process, ten individuals had low read numbers and were removed from further bioinformatics analyses. Twen-

ty-four samples obtained high quality to further analysis and yielded a total of ,699,53 paired-end reads. For RNA-seq a total of 129,663 putative single nucleotide polymorphisms (SNPs) were found in shortfin mako shark. Among them, 7,59 were putative annotated SNPs, with 14,42 (19%) in coding regions and 63,756 (1%) in non-coding regions. After filtering procedure 1,166 annotated SNPs were selected. For ddRAD-seq, after filtering process, taking into account missing data rate, minor allele frequency, quality and deep sequencing, a total of 405 SNPs remained. Our results provide valuable sequence resources for future functional and population studies of the globally vulnerable shortfin mako shark.

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Keywords: *Isurus oxyrinchus*, next generation sequencing, Single nucleotide polymorphism, conservation, Sharks

Shotgun proteomics of ascidians tunic shed light into the interaction between host and their associated bacteria

Ana Matos, Dany Domínguez-Pérez, Daniela Almeida, Alexandre Campos, Hugo Osório, Vitor Vasconcelos and Agostinho Antunes

Marine ecosystems comprise a huge diversity of organisms and unique environments that allow the establishment of multiple taxa associations considerably increasing biodiversity and resources that are yet to be fully understood and explored. Tunicates are marine invertebrate organisms, belonging to a subphylum of the phylum Chordata, along with cephalochordates and vertebrates. These filter feeders are associated to microbiome communities that can provide them nourishment and protection through complex host-associated microbial communities interactions. Within tunicates, Ascidiacea members are the target of intense research since it has been reported the production of diverse bioactive compounds with biotechnological relevance by the embedded symbionts in their tunics. These features associated with the close phylogenetic proximity to vertebrates arise special interest in those organisms to be applied as models in evolutionary and developmental studies. To understand the bioactive potential of ascidians, firstly it is necessary to study the community of microorganisms associated to them. In this sense, next-generation sequencing tools, transcriptomic and metabolomic-based approaches are increasing the knowledge of ascidians and their symbiont products, but proteomic studies are still scarce. Hence, we used a shotgun proteomic approach to explore the tunic composition from three ascidian species belonging to *Molgula*, *Ciona* and *Halocynthia* genera from Portugal. Samples were dissected and 0.5 g from each tunic was homogenized in a proper buffer (SDT) using the Speed-Mill PLUS homogenizer (Analytik Jena AG, Germany). The extracted proteins from two technical replicates were processed on the Filter Aided Sample Preparation (FASP) protocol, using a filter cut-off of 10kDa. FASP protein digests were desalted using C1 Tips and processed using a nano LC-MS/MS, composed by an Ultimate 3000 liquid chromatography system coupled to a Q-Exactive Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany). The raw data was analysed using the Proteome Discoverer 2.2.0.3 software (Thermo Scientific) and searched against the UniProt database for the Metazoa and Bacteria taxonomic selection (201_07 release). Overall, 337 unique proteins were identified in the metazoan section with a total of 0 unique proteins belonging to *Halocynthia* sp., 135 to *Molgula* sp., while 149 proteins were identified in *Ciona* sp. tunic, with only two proteins being shared among the three species. On the other hand, 106 unique proteins corresponded to Bacteria having a total of 39 unique proteins belonging to *Halocynthia* sp., 43 to *Molgula* sp., while 33 proteins were identified in *Ciona* sp. tunic, but none of these proteins were shared among the three species. The proteins identified are involved in several biological processes, associated to molecular functions and as cellular components. In the Bacteria section, proteins as Ca²⁺-binding RTX toxin-like and spermidine/putrescine ABC transporter substrate-binding were found. Interestingly, serine-type endopeptidase activity in the Bacteria section was detected contrasting with the serine-type endopeptidase inhibitory activity in the Metazoa section. Some endopeptidases inhibitors were also identified. The presence of such protease inhibitors, even within the few shared proteins among the three species, may be related with a putative degradative prevention by the host against secretion of microbial proteases or catalytic enzymes. Our work reveals the proteomic composition of three ascidians outer tunics. The applied methodology may provide insights into the interactions between hosts and their associated microbiome and products. Moreover, this approach can be useful in unraveling the main pathways of host-microbiome interaction and to uncover the process of bacterial colonization.

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Keywords: ascidians, microbial association, Tunic, Filter aided sample preparation (FASP), Shotgun proteomics analysis

Current status of the DNA barcode reference library of non-indigenous marine species occurring in European coastal regions

Sofia Duarte, Pedro E. Vieira and Filipe O. Costa

Coastal habitats are among the most important, but also the most threatened ecosystems in the world, which may pose at risk many important services for human well-being (Solan et al. 2004). Along with global climate change, overexploitation, pollution and habitat destruction; the spread of invasive species is among the major threats to coastal ecosystems (Rilov 2009). Non-indigenous species (NIS), which can establish in areas outside their natural range and become invasive, can spread rapidly across the natural environment and displace and out-compete native species, driving to severe ecological changes that threaten ecosystem integrity (Rilov 2009). While morphology-based identification of taxa has largely contributed to appreciate the current status of NIS occurrence in coastal regions and marine environments (e.g. Chainho et al. 2015), this process is expertise-demanding, laborious and time consuming. Because of these limitations, most studies reporting bioinvasions and relying on morphotaxonomy are limited in time and space and only focus in the ecology of specific taxa or smaller groups at a time. Due to the above-mentioned reasons, it becomes a priority to develop and optimize novel detection methods capable to overcome some of these challenges and that would allow an early detection and ease the monitoring of NIS in coastal ecosystems. Particularly, the combination of DNA barcoding with high-throughput sequencing (HTS) renders metabarcoding the capacity to bolster current biodiversity assessments techniques (Cristescu 2014). Within this approach, genetic information can be obtained through the use of standardized DNA barcode markers targeting a wide taxonomic range of organisms in mixed specimens or environmental samples (Cristescu 2014). DNA-based tools promise a number of potential benefits over traditional methods, including increased sensitivity and specificity as well as greater throughput and cost effectiveness. However, the success of DNA-based methods is greatly dependent on the availability, taxonomic coverage and reliability of reference sequence databases, whose deficiencies can potentially compromise species identifications through HTS (Briski et al. 2016). In this study, we conducted a species-gap analysis on the availability of accessible DNA barcode sequences for marine NIS occurring in European coastal regions using the Barcode of Life Data System V4 (BOLD V4) (Ratnasingham and Hebert 2007). The checklist of NIS occurring in European seas was compiled by using two online databases: i) European Alien Species Information Network (EASIN) and ii) Information System on Aquatic Non-Indigenous and Cryptogenic Species (AquaNIS). The taxonomic classification of the NIS compiled in the checklist followed the World Register of Marine Species (WoRMS). All records without taxonomic assignments at the species level were removed from the list and only taxa with accepted scientific species names were maintained. The species list was submitted to BOLD v4 and the checklist progress report option implemented in BOLD was used to conduct our gap analysis (Ratnasingham and Hebert 2007). Barcoded species were considered to be those with sequences that meet the following requirements: i) minimum sequence length of 500 bp; ii) < 1% ambiguous bases; iii) presence of forward and reverse trace files; iv) minimum quality of trace files status and v) presence of a country specification, either with co-ordinates or country name. Barcode compliance flags were applied to the following markers if the sequences meet the above requirements: the mitochondrial cytochrome C oxidase gene (COI), for animals and the maturase K gene (matK) or the rubisco large subunit gene (rbcL), for plants. For Chromista, due to the high diversity within this kingdom, barcode compliance flags were applied to the following markers: COI for all Chromista groups, and, in addition to COI, matK and rbcL for Cryptophyta, Haptophyta and Ochrophyta. A list of 1,915 NIS occurring in European coastal regions was generated and uploaded to BOLD v4 (checklist: CL-NISEU). The species in the list were distributed by three kingdoms: i) Animalia (1,411 species), ii) Chromista (276 species) and iii) Plantae (22 species), comprehending 2 phyla. The most well represented phyla with NIS were Arthropoda (331 species), Mollusca (324 species) and Chordata (25 species) within Animalia; Ochrophyta (105 species), Myzozoa (79 species) and Foraminifera (70 species), within Chromista and Rhodophyta (171 species) and Chlorophyta (40 species), within Plantae. From the total species in the list, 1,109 were still missing a DNA barcode on BOLD (including private and public records), which corresponded to ca. 60% of the total NIS. In addition, within barcoded species ca. 13% were singletons (i.e. only one barcode sequence

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available). However, when only high-impact species were taken into account (146 species with the status of established or invasive), the gap was much lower (ca. 22% of the species). Animalia NIS were the most well represented with DNA barcodes, but still missing for ca. 49% of the species. Only 5 out of the 16 represented phyla have a % of barcoded species higher than 50% (Acanthocephala, Arthropoda, Chordata, Echinodermata and Nemertea). For Chromista and Plantae the gap of missing species was higher than 0%. Among Chromista, only 2 out of phyla (Myzozoa and Ochrophyta) were represented with DNA barcodes, but the % of covered species was lower than 30%. Among Plantae phyla, the % of barcoded species varied between 50 and 100%, but for Rodophyta NIS no DNA barcodes were yet deposited on BOLD. The current study allowed us to gauge the gaps of DNA barcodes for NIS occurring in European coastal ecosystems. The few marine NIS eradications attempts that proved successful in the past owe their success to an early detection, soon after introduction. DNA-based tools would allow the detection of early developmental stages or smaller organisms, reducing the time from introduction to detection and increasing the success of NIS control and/or eradication (Holman et al. 201). However, the gaps found in reference libraries can have strong implications for an accurate species identification through DNA-based tools. Prioritization efforts should be conducted in order to fulfil these gaps, in particular for high impact species still missing DNA barcodes.

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Keywords: Coastal ecosystems, Europe, Marine non-indigenous species, DNA barcode reference libraries, Gap-analysis

Founding in action: genetic assessment of new populations from the same reintroduction program

Anna Barbanti, Maria Turmo, Janice Blumenthal, Jack Boyle, Annette C. Broderick, Lucy Collyer, Gina Ebanks-Petrie, Brendan J. Godley, Marta Pascual and Carlos Carreras

Changes in species distribution can be favoured by variations in environmental conditions. Anthropogenic actions such as global warming or human mediated dispersal are two of the main causes of the current founding of new populations worldwide. The establishment of new populations can be challenging to study in long living and highly migratory species. For this reason, reintroduction processes represent unique opportunities for tracking these evolutionary processes. The green turtle (*Chelonia mydas*) populations of Grand and Little Cayman were considered extinct in the 0s. During the last 20 years the number of nests in these two sites have been increasing progressively. A previous study (Barbanti et al 2019) has shown that most of the wild females captured in Grand Cayman were related to a reintroduction program that started in 196 from captive breeding by the Cayman Turtle Center (CTC). This reintroduction offers the unique opportunity to study the rise of these new populations to have a better understanding on how the evolutionary forces determine diversity and differentiation on founding processes. We analysed 320 wild hatchlings sampled on Little and Grand Cayman in three consecutive nesting seasons using microsatellite and mtDNA D-Loop markers. We ran parentage and relatedness analysis against CTC breeding stock and wild females using previously genotyped data (Barbanti et al 2019). Both sites showed a high degree of relatedness to the CTC; 79% of Little Cayman hatchlings and 90% of Grand Cayman hatchlings were related to farm individuals. Despite this result, the two islands were found to be genetically different and both showed significant genetic differentiation from the CTC, when using nuclear markers. Only Little Cayman showed significant differentiation from the CTC using the mtDNA

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marker. Genetic drift, in fact, can be essential to determine genetic differentiation of population after only one generation. Moreover, we analysed nest site fidelity (NSF) of wild females by comparing geographic distances between each female's consecutive nests within a season. Wild females showed high degree of nest site fidelity, although we found few individuals reproducing on both islands. Finally, we used parentage analysis to identify or infer the parental individuals of each nest and calculate the sex ratio of breeding adults. The sex ratio did not show any significant shifting towards one sex, meaning that global warming did not affect the developmental ratio of these adult populations, considering the Temperature-dependent Sex Determination of this species.

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Keywords: Microsatellite (or short tandem repeat [STR]), reintroduction assessment, *Chelonia mydas*, breeding structure, Cayman Islands

Loving the warming: transcriptomic responses to acute thermal shifts in a recent colonizer in the Mediterranean, the sea urchin *Arbacia lixula*

Rocío Pérez-Portela, Ana Riesgo, Owen S. Wangensteen, Creu Palacín and Xavier Turon

Global warming is promoting demographic and spatial expansions of tropical and subtropical species towards temperate latitudes. The thermophilous sea urchin, *Arbacia lixula*, is an example of a successful subtropical Atlantic coloniser in the Mediterranean. Its density increased during the last decades in the Mediterranean, shifting macroalgal beds into barren grounds across high-populated sites due to its grazing activity. Recent studies suggested that the foreseen global warming can have a positive effect on larval survival, representing a potential increase of the species' impact on marine ecosystems. However, nothing is known about the warming effect on the general performance of adult individuals or phenotypic plasticity on the species. The aim of our study was to explore the short-term plasticity to acute thermal stress in *A. lixula*, and to identify the most important genes and molecular-physiological pathways involved in this plasticity; considering that the magnitude of its transcriptome-wide response is coherent with its physiological performance and stress response. To quantify rapid transcriptomic responses to thermal stress, we exposed specimens to three different treatments in controlled laboratory conditions for 20 hours: 13°C (control), 7°C (T7), and 22°C (T22). We set two different experiments: a "cold" experiment comparing T7 versus control conditions, and a "warm" experiment comparing T22 versus control. Coelomocytes from a total of 18 sea urchins (6 replicates per treatment) were collected, mRNA extracted and cDNA libraries constructed for each specimen. After filtering and trimming, reference transcriptomes were de novo assembled and differential expression analyses of the "cold" and "warm" experiments performed. Differentially expressed genes between treatments were identified, and their associated Gene Ontology terms annotated to understand the most important molecular, biological and cellular pathways involved in the cold and warm stress responses. While most experiments in marine organisms have revealed thermal stress to increased temperatures, our experiments showed a large response to the cold (7°C) treatment, with 1,181 differentially expressed transcripts between the 7°C and the control condition, as compared with only 179 in the 22°C treatment (Fig. 1). 87 differentially expressed transcripts (84 for the cold

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treatment and 3 for the warm one) could be linked to different Gene Ontology terms. Different heat shock and related proteins were up-regulated at 7°C, together with other genes related to apoptosis-suppressing activity and the immune response. No chaperone induction was measured at 22°C but some apoptotic-related activities were up-regulated for this treatment. All in all, our study shows that, in *A. lixula*, withstanding the stress associated with low temperatures requires a cost of energy expenditure. For high temperatures, no major metabolic changes were detected. Our results suggest that seawater warming will not reduce the biological efficiency of *A. lixula*, while it could actually favor the ongoing expansion of the species in the Mediterranean and their associated impacts on littoral communities.

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Keywords: thermal stress, Global Warming, Transcriptomics, Gene Expression, marine invertebrates

Species GAP analysis in DNA barcode reference libraries of marine non-indigenous species in the Azores archipelago

Sofia Duarte, Manuela I. Parente, Ana C. Costa and Filipe O. Costa

Coastal regions are widely recognised for their ecologic and socio-economic importance. The spread of invasive species, along with global climate change, overexploitation, pollution and habitat destruction, is a major threat to coastal ecosystems (Solan et al. 2004). Some of these species, in particular macroalgae and invertebrates, can spread quickly and become invasive, causing severe ecologic and economic impacts. When prevention fails, the early detection and surveillance of NIS could be essential for coordinating a timely and effective response to invasions. Marine ecosystems on oceanic islands, such as Azores, can be especially prone to invasions by non-indigenous species since they are characterized by a small number of native species and by a high availability of empty niches, and thus, the list of NIS is relatively long (Micael et al. 2014). There are no doubts that traditional morphology-based methods have highly contributed to the current knowledge about NIS communities inhabiting Portuguese coastal waters, including the Portuguese oceanic islands (Micael et al. 2014, Chainho et al. 2015). However, the hurdles associated with morphological methods may compromise the early detection and monitoring of the most risky species. On the other hand, DNA-based tools promise advantages over traditional ones, particularly the identification of individuals and life stages that cannot be recognized by using morphological approaches (Darling and Mahon 2011). Plus, most surveys typically target specific species or taxonomic groups, which may neither accurately represent overall ecosystems' condition nor allow an early detection of NIS. Due to the above-mentioned reasons, it becomes a priority to develop and optimize novel detection methods capable to overcome some of these challenges and that would allow an early detection and ease the monitoring of NIS in coastal ecosystems. Particularly, the combination of DNA barcoding with high-throughput sequencing (HTS) renders metabarcoding the capacity to bolster current biodiversity assessments techniques (Cristescu 2014). Within this approach, genetic information can be obtained through the use of standardized DNA barcode markers targeting a wide taxonomic range of organisms in mixed specimens or environmental samples (Cristescu 2014). DNA-based tools promise a number of potential benefits over traditional methods, including increased sensitivity and specificity as well as greater throughput and cost effectiveness. However, the success of DNA-based methods is greatly dependent on the availability, taxonomic coverage and reliability of reference sequence databases, whose deficiencies can potentially compromise species identifications through HTS (Briski et al. 2016). In this study we conducted a species-gap analysis of DNA barcode sequences available for marine NIS occurring in the Azores archipelago in the Barcode of Life Data System V4 (BOLD V4) (Ratnasingham and Hebert 2007) and in the GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>). The list of NIS occurring in Azores was compiled from a wide variety of sources, mainly from literature searches through scientific papers and reports, including recent field and taxonomic studies, and supplemented with existing databases and this is the list that was submitted to the 2019 interim ICES WGITMO report (International Council for the Exploration of the Sea; Working Group on Introductions and Transfers of Marine Organisms). The taxonomic classification of the compiled NIS followed

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the AlgaeBase and World Register of Marine Species (WoRMS) databases. A checklist of 0 marine NIS occurring in Azores archipelago was compiled and the species in the list were checked for the presence of public sequences on both BOLD v4 and GenBank databases. The species in the list were distributed by three kingdoms: i) Animalia (4 species), ii) Plantae (30 species) and iii) Chromista (2 species), comprehending 10 phyla. The most well represented phyla with NIS were Chordata (15 species), Mollusca and Bryozoa (both with species) within Animalia, and Rhodophyta within Plantae (26 species); while the only represented phyla within Chromista was Ochrophyta with 2 species. In total, 3,4 sequences belonging to 25 different markers were found for the 0 species, published in BOLD and GenBank. Among these published sequences, the COI-5P is the most well represented loci, with 3,224 records belonging to 44 species. However, from the total species in the list, 25 were still missing a DNA sequence, which corresponded to ca. 31% of the total NIS. In addition, within sequenced species ca. 14% were singletons (i.e. only one sequence available). Animalia NIS were the most well represented with sequences, missing only for ca. 27% of the species, while for Chromista and Plantae the gap of missing species was higher than 35%. The current study allowed us to characterize the gaps in available sequences in public repositories for marine NIS occurring in the Azores archipelago. Actions developing DNA-based tools should be a priority for detection and effective management of biological invasions. DNA-based tools would allow the detection of early developmental stages or smaller organisms, reducing the time from introduction to discovery and increasing the success of NIS control and/or eradication (Holman et al. 201). However, the gaps found in reference libraries can have strong implications for an accurate species identification through DNA-based tools. Prioritization efforts should be conducted in order to fulfil these gaps.

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Keywords: Azores archipelago, Coastal ecosystems, Marine non-indigenous species, DNA barcoding, Reference libraries

Genetic Studies Reveal the Presence of Unexpected Species in Commercial Fisheries

Judith Ollé, Laura Vilà, Núria Sanz, Rosa M. Araguas and Jordi Viñas

Conservation genetics has become a valuable resource for a proper stock management in wild fish populations. The analysis of the genetic variability permits a better identification of the species but it also allows assessing the status of the populations. Small tuna is a well characterised group of pelagic and migratory fish species usually exploited by traditional fisheries. In spite of that, morphological similarity between species of same genus can lead to incorrect species identification. Thus, the presence of multiple species within a single fishery can complicate its management hampering the viability of the populations. In addition, due to their life history traits -pelagic habitat, migratory behaviour, large population size, larval stage- and the apparent lack of natural barriers in their habitat, it has been postulated that these species are not prone to present population heterogeneity. However, several studies have shown population differentiation in some species. The aim of the present work, is to genetically validate the species of two sets of individuals from both the fishery of *Euthynnus alletteratus* and *Auxis rochei*, and later determine a putative population structure by using clas-

sic genetic markers. We analysed 175 individuals of *E. alletteratus* from four locations (Portugal, Tunisia, Ivory Coast and Senegal) and 43 individuals of *A. rochei* from eight populations (Portugal, Ceuta, Malaga, Cartagena, Valencia, Tarragona, Algeria and Tunisia). All specimens were sequenced for the mitochondrial DNA control region marker (mtDNA CR). Additionally, for *E. alletteratus* a subset of individuals was also tested for two unlinked nuclear genes, Rhodopsin and Calmodulin. For *A. rochei* a selection of individuals was sequenced for the Tmo4c4 nuclear gene. Sequences for all markers were analysed and aligned using Geneious 7.1.9 software. For both mtDNA CR alignments a list of unique haplotypes was created with DNAsp 6.12.03 and pairwise differences and FST were estimated in Arlequin 3.5.2.2. When analysing *E. alletteratus*, the BLAST results for Ivory Coast and Senegal showed less identity than the expected in same species for mtDNA CR sequences in GenBank (about 90%; e-value 1.33e-136), while Portugal and Tunisia showed the expected levels of sequence identity (about 99,7%; e-value 0). The analysis of mtDNA CR showed an extremely high differentiation comparing the southern individuals (Ivory Coast and Senegal) to the ones from the two northern locations (Portugal and Tunisia) ($\phi_{st} = 0.931$; $p = 0.000$), that is, 93% of the genetic variation in those individuals is due to differences among populations. This value, close to the maximum, indicates a lack of genetic flow between those populations and a degree of differentiation at species level. In accordance with these results, in each group of individuals (Northern vs. Southern) and for both nuclear markers we did detect specific SNPs (single nucleotide polymorphism) that differentiate each putative species. Furthermore, we found low genetic heterogeneity between Portugal and Tunisia individuals ($\phi_{st} = 0.007$; $p = 0.19$), as well low heterogeneity between Ivory Coast and Senegal locations ($\phi_{st} = 0.004$; $p = 0.162$), which, based on previous reports, are in agreement with the expected population differentiation for these species. In the case of *A. rochei*, the mtDNA CR BLAST analysis revealed the presence of 7 *Auxis thazard* individuals within *A. rochei* fishery in the Ceuta population (identity about 9%; e-value 6e-173). These individuals were also tested for the Tmo4c4 nuclear marker, their sequences were identical with previous annotated *A. thazard* Tmo4c4 sequences, further, the comparison of the region here analysed exhibit one specific SNP between both species. The analysis of the mtDNA CR for this species resulted in a lack of genetic heterogeneity among locations on the Iberian Peninsula - Portugal, Ceuta, Malaga, Cartagena, Valencia and Tarragona- ($\phi_{st} = 0,000$; $p = 0,472$), but significant differences between those locations and the two North-African locations, Algeria and Tunisia ($\phi_{st} = 0.034$; $p = 0.000$). In conclusion, the extremely high level of population structure within *E. alletteratus* that displays the mtDNA CR marker and the specific SNPs found for each group, sets the scenario in which no genetic flow occurs between those populations and thus, a possible event of speciation may or might have started to occur. Regarding *A. thazard*, this is the first evidence of this species within the Mediterranean waters but also, this warns us of a putative mixed fishery with *A. rochei*. Still, more research is needed in order to clarify why these individuals were caught in the Mediterranean Sea. If they are no fruit of a stochastic event but there is a displacement on this species distribution, will be necessary to upgrade the present management directives for this fishery

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Keywords: Fisheries research, speciation, population structure, conservation genetics, Scombridae, Genetic Markers

Are mussel attributes shaped by urbanisation?

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Urbanization and coastal development promote intense changes in marine ecosystems that jeopardize their ability to provide services, such as provisioning of food and other resources. The aim of this study was to explore the effects of urbanisation on different attributes of the mussel *Mytilus galloprovincialis* Lamarck, 119 in the Northern Portuguese coast: This species is harvested in natural systems as well as cultured on ropes suspended from rafts. Since mussel farming involves the capture of very large amounts of young wild mussels from rocky substrates to supply floating rafts, it is interesting to evaluate if urbanisation shapes the density, percentage of cover, depth of clumps, size-frequency distribution and condition index of *M. galloprovincialis*. To achieve this, a fully nested hierarchical sampling design was followed. Two conditions (urbanised and non-urbanised) were considered. At each condition, three different rocky shores were selected and two sites per shore were haphazardly chosen for the study of the different mussel attributes. Results pointed out that mussel density was significantly lower at urban shores. In terms of size, the abundance of mussels included in higher size classes was larger at urbanised than at non-urbanised shores. Moreover, percentage of cover, depth of clumps and condition index showed significant variability at different spatial scales.

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Keywords: urbanisation, *Mytilus galloprovincialis* L., Iberian Peninsula, Rocky shores, Hierarchical sampling design

A species distribution model for *Paracentrotus lividus*: predicted projections of habitat suitability

Ana Filipa Costa, Vania Freitas, Joana Campos and Francisco Arenas

INTRODUCTION The European purple sea urchin *Paracentrotus lividus* is the most common echinoid in Portugal (Gago et al. 2001), being present in the rocky intertidal and shallow subtidal habitats. It is a species with recognized importance both from an ecological and an economic point of view. In Portugal, for instance, the commercial exploitation of the European purple sea urchins, *P. lividus*, has increased more than 2000% since 2010 (INE 2011; INE 201). However, sea urchin's populations are exposed to several threats ranging from climate change (Miller et al. 201) to overfishing (Ouréns et al. 2015). Sea surface temperature has been increasing in the North Atlantic for decades (IPCC, 2014), which influences the metabolism and fitness of ectotherms as well as its distribution area (Shpigel et al. 2004; Byrne et al. 2009; Angilletta 2009; Schulte 2015). Understanding the impacts of future climate change on species distribution is key to help formulating conservation policies to reduce the economic and ecological risks of biodiversity loss. Species distribution models (SDMs) that characterize the species' niches based on environmental variables and species location data, are an important tool to predict species occurrence under particular climate change scenarios. In this study we used two algorithms (MaxEnt and Biomod2), as SDM tools, to assess the current and the future predicted habitat suitability of *P. lividus* in accordance with the worst climate change scenario, anticipating a reduction of its distribution area in the Atlantic coast. **MATERIAL AND METHODS** Species distribution model *Paracentrotus lividus* data occurrence was collected from existing online databases such as Ocean Biogeographic Information System (OBIS) and Global Biodiversity Information Facility (GBIF). Additional records were added from a literature review using the Web of Science. Duplicate records were eliminated using R code. A total of 266 georeferenced occurrence records were used in the SDM, for a given distribution area that considered European and North Africa shores. These coastal areas were masked using a bathymetric raster to include only a depth range from 0 to 200 meters (Bertocci et al. 2010). Data about environmental predictors were downloaded as raster layers from the online repository BIO-Oracle and then environmental predictors were selected in accordance with the potential influence on the distribution of *P. lividus*. Since correlation among environmental drivers is a potential problem in species distribution modelling (Elith et al. 2010), we only used predictors for which pairwise Pearson correlations between variables were less than 0.5: salinity, pH, maximum annual seawater temperature, minimum annual seawater temperature and seawater temperature range. The SDMs were construct-

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ed using two different algorithms. First, we used Maximum Entropy Modelling (MaxEnt software) (Phillips 2005) where the MaxEnt algorithm aims to maximize the entropy of the species probability distribution (Merow et al. 2013). This algorithm fits complex models as linear combinations of basic functions and we ran the models using the linear and quadratic features (Elith et al. 2011). Additionally, we built a generalized linear model (GLM) using the R package Biomod2, a regression-like method that relates presence records and pseudo absences with environmental layers (Guisan et al. 2017). The contribution of each predictor was examined using the permutation importance and percent contribution coefficients from MaxEnt software, as well as with the variable importance function of Biomod2. In the first case, the performance of the model was evaluated in accordance with the predicted area under the curve (AUC), where values higher than 0.5 indicated a good discrimination power (Phillips et al. 2006). Internal data-splitting validation was applied to confirm the variable importance of the final predictors in the training data (70% of presence = points) and the consistency of the above evaluation metric (AUC). MaxEnt was used to determine the habitat suitability index for all the study areas with the environmental conditions registered from 2002 to 2009, as well as to obtain future distribution projections by using rasters of forecasted physical conditions under the Representative Concentration Pathways (RCP) .5, the pathway with the highest greenhouse gas emissions (IPCC 2014). The layers extracted from Bio-Oracle contained the information from the UKMO-HadCM3 model, which represented the most severe among those provided by Bio-Oracle (Meehl et al. 2007). RESULTS Environmental predictors The species distribution models included the five initial predictors (i.e. salinity, pH, maximum annual seawater temperature, minimum annual seawater temperature and seawater temperature range). However, after the selection procedure, only three predictors were considered as most relevant in the modelling distribution of this species: salinity, seawater minimum temperature and seawater maximum temperature (Table 1). MaxEnt “functional responses” help to identify the thresholds of the species for each predictor (Figure 1). Thresholds were not clear in the functional responses to minimum and maximum temperatures. At minimum temperatures below 5°C, the presence of the species clearly reduces (Figure 1a) but no upper threshold was identified for maximum temperatures (Figure 1b). The functional response to salinity (Figure 1c) supports that the species is marine with a low probability of occurrence at salinities below 30 ppm. Habitat suitability The predictive accuracy of both models had a high evaluation score, with AUC = 0.97 for MaxEnt and AUC > 0.950 for Biomod2. The model prediction for the current distribution shows a species with affinities for fully marine warm waters with higher habitat suitability in the Mediterranean and southern shores of the Atlantic regions (Figure 2a). Future projections using the worst Representative Concentration Pathways (RCP) scenario (>900 CO2 ppm) suggest a general reduction of habitat suitability of the overall European population, except in the Black Sea. In Portugal the favourable index of 0.5 reduces to 0.2 by 2100 (Figure 2b). DISCUSSION Future of sea urchin population The response of a species to global warming depends on how different populations are affected by increasing temperature throughout the species’ geographic range (Gardiner et al 2010). Physiological thresholds and correlative functional responses from species distribution models performed quite accordingly in shaping the response of *Paracentrotus lividus* to temperature. Thus, our confidence in the performance of our modelling exercise is high. However, the species distribution model used did not take into account biological predictors such as food availability and/or biotic interactions, which can also compromise the existence/ absence of the species. Unfortunately, there were no available projections for algae distribution as well as other biological stressors. The projections from our model showed some worrying evidences of a decrease of the suitability of Portuguese coastal habitats for the sea urchin populations. In addition, contrary to what was expected based on temperature preferences, the species is not expected to move northwards, but to East instead, probably as a result of a combination of the other environmental factors considered in the model such as a predicted reduction of salinity in the North Atlantic due to the Arctic ice melting. Obviously, uncertainty of these models is high but the results are consistent with similar predictions for other coastal species (Martinez et al. 2015; Assis et al. 2016). We anticipate though a reduction on the area of habitat suitability for the species due to climate change. In particular for the Portuguese population, the reduced suitability might highly compromise the commercial exploitation which emphasises the need for a proper stock management, based on scientific monitoring, to assure a future sustainable harvesting of sea urchins.

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Keywords: Purple sea urchin, Climate Change, Species distribution model (SDMs), habitat suitability, temperature, Salinity

Effects of the parasitic pea crab *Afropinnotheres monodi* Manning, 1993, on commercial bivalve species of the Iberian Peninsula: results of the AFROBIV project.

Jose A. Cuesta, Marta Perez-Miguel, José E. García Raso, Jose I. Navas and Pilar Drake

Afropinnotheres monodi Manning, 1993 is one of the many African crustaceans that have been recorded on the coasts of the Iberian Peninsula. This crab belongs to the Pinnotheridae, a family that comprises 303 species of small marine brachyuran decapods. Pinnotherids live as symbionts in other invertebrates, very often in the paleal cavity of bivalve molluscs. The main objective of this project has been to determine the effect of *A. monodi* on bivalves of commercial interest in the Iberian Peninsula, especially mussels (*Mytilus galloprovincialis*) and cockles (*Cerastoderma* spp.), which are the two species presenting the highest degree of infestation by this crab. In this study the distribution of *A. monodi* has been researched in the Gulf of Cádiz, and beyond its known limits (the south of Portugal and Strait of Gibraltar) to monitor its process of geographic expansion. Relevant aspects of its biology, that affect its reproductive success and dispersive capacity, have been also studied experimentally in order to predict the potential evolution of this species in the Iberian Peninsula. The

current limits of the distribution of *A. monodi* have been determined by analysing samples from 69 localities between Gijón (Atlantic) and Sant Carles de la Ràpita (Mediterranean). Its current distribution in the Atlantic extends to the town of Cascais (Portugal), and in the Mediterranean, to the Caleta de Vélez (Spain). In parallel, the current distributions of three native pinnotherid species (*Nepinnotheres pinnotheres*, *Pinnotheres pectunculi*, and *Pinnotheres pisum*) on the coasts of the Iberian Peninsula have been updated. The information obtained has enabled confirmation of the distribution and hosts of several species, and the extension of their distribution of others. For each of the five European species of pinnotherids (including *A. monodi* and the recently-found new species *Pinnotheres bicristatus*), sequences of three genetic markers (16S, Cox1, and H3) have also been obtained, both to improve the accuracy in the identification of these crabs and to detect possible new species. To determine the optimal values and the limits within which *A. monodi* can live (respect to water temperature and salinity), experiments have been carried out in thermo-regulated culture chambers, at 5, 10, 15, 20, 25 and 30 °C, in both the larval (mobile) and adult (symbiont) phases. The complete development of larvae was successfully completed at 20 and 25 °C, but the highest rate of survival until the megalopa was observed at 25 °C. In a second experiment at 25 °C (optimal temperature), when larvae were cultured at salinities of 15, 25, 36 and 45, the maximum survival until reaching the megalopa phase occurred at a salinity of 25. In the case of adults, the results showed that all the demographic categories (males, hard females and soft females) can survive at the 6 temperatures tested except for the lowest temperature (5 °C). In addition, survival after 42 days of culture was always high (9.9% at 15 °C, 100% at 20 °C, .5% at 25 °C and 91.2% at 30 °C), except at 10 °C (survival of 69.4%). To estimate experimentally the effect of temperature on the fecundity of this species, ovigerous females were cultured at the 4 temperatures at which survival was high (> 50%). The effective fecundity decreases gradually as the temperature increases between 15 (95.94%) and 30 °C (3.23%). Finally, field samplings were carried out to estimate the degree of infestation and the condition index of *A. monodi* hosts. The bivalve target species, *M. galloprovincialis* and *Cerastoderma edule*, are of commercial interest for mussel and shellfish farming, and they have been sampled in three natural beds of the Gulf of Cádiz: Bay of Cádiz, and Carreras and Piedras river estuaries. The average prevalence of *A. monodi* infestation in cockles ranged from 4.0% in the Bay of Cádiz to 51.4% in the Carreras estuary; in the case of mussels, from 30.5% in the Bay of Cádiz to 45.4% in the Carreras estuary. In bivalves infested by *A. monodi*, we have estimated a condition index reduction of around 15% in the case of mussels, and of 3% in the case of cockles. In general, these studies demonstrate an energy disadvantage in infested mussels (with respect to non-infested mussels), suggesting that this infestation would cause negative commercial effects in the bivalve aquaculture. The habitat of the Andalusian populations of *A. monodi* coincides with those found at the Galician rias, where the most important mussel production in Europe is developed. In the case of a future infestation of this area, this parasitic crab could cause economic losses in the mussel raft cultures. For the moment, mussels infested by *A. monodi* have never been found in the Andalusian culture facilities. However, we found *A. monodi* in intertidal mussels less than 5 mm in length. Mussels of these sizes can be transported to be used as seeds in culture facilities; it could be a dangerous practice since infested mussel-seeds may contaminate previously unaffected sites. Currently, there is not enough evidence to consider this species as invasive; however, since it is not known how it has reached peninsular waters, it would be advisable to catalogue it as an allochthonous species. The thermal limits of survival of this African pinnotherid indicate its possible establishment in Galician rias, areas of special relevance for mussel farming. From this study, it has become clear that *A. monodi* is a parasitic species for the bivalves that infests, especially in the case of mussels, and that the species is currently in a period of expansion along the coasts of the Iberian Peninsula.

Keywords: pea crab, Mussel (*Mytilus galloprovincialis*), *Cerastoderma edule*, Aquaculture, Parasitism

Expansion history of the blue crab (*Callinectes sapidus* Rathbun, 196) in the Eastern Iberian Peninsula (Western Mediterranean Sea)

Carmen Barberá, Silvia Falco, Isabel Estesó, Andres Izquierdo, Jose M. Martín, Miguel Rodilla and Alfonso A. Ramos-Esplá

Abstract In the last five years (2014-2017), *C. sapidus* has expanded dramatically in the Iberian Levantine coast, from the Ebro Delta to the Mar Menor. The results of first occurrence in the different localities of Iberian Levantine coast and lagoons show two possible foci of expansion. This recent colonization could have happened by three different pathways: i) arrival of larvae by ballast waters of merchant vessels; ii) natural spread by current pathways; and/or iii) intentional introduction for commercial purposes. Key words: *Callinectes sapidus*, alien

species, vectors, E-Iberian Peninsula, Western Mediterranean Introducción The Atlantic blue crab *Callinectes sapidus* Rathbun, 196 (Brachyura: Portunidae) was recorded for the first time in the Iberian Mediterranean in 2004 (Giménez-Casaldueiro 2016, in the Mar Menor, Murcia). However, its impact on local fisheries started to be noteworthy in 2012 in the Delta del Ebro (Castejón & Guerao 2013). Later, its expansion has sped up in 2014 in the Valencia region (González-Wangüemert & Pujol, 2016; Izquierdo-Gómez & Izquierdo-Muñoz, 2016; Mancinelli et al, 2017) in the Albufera lagoon (Valencia, in October) and the mouth of Segura river (Alicante, in December); and the Balearic Islands in 2017 (García et al. 2017). Since the date of first occurrence the distribution of blue crab showed a fast expansion in the area of Valencia region, we discuss possible vectors of introduction. Material and methods The study area is the Valencia region, comprising the Gulf of Valencia (Castellón, and Valencia) and Alicante, with about 500 km of coast from Vinaroz as the northernmost harbour to Torreveja in the south. The information was obtained from sampling, interviews with professional fishermen, consultation of social networks and discussion forums for sports divers and fishermen. Moreover, the Centre for Marine Research (hereafter CIMAR) has a public database on exotic species from local knowledge in the area based in the collaboration between scientists and citizens. Results and discussion In the last five years (2014-2017), *C. sapidus* has expanded dramatically in the Iberian Levantine coast, from the Ebro Delta to the Mar Menor. The results of first occurrence in the different localities of Valencia region show two possible foci of expansion. One in the north, with a possible origin in the Delta of the Ebro and expanding quickly southwards, colonizing the coasts and coastal lagoons of Castellón and Valencia, during 2015. The second spread from the mouth of the Segura, but more slowly towards the north into the province of Alicante, reaching San Antonio Cape in 2017. In both cases, the progressive colonization from the two previous foci could be aided by the pattern of coastal surface currents of the Iberian Mediterranean (Fernandez et al. 2005). This recent colonization of the Iberian Levantine coast and lagoons could have happened by three different pathways: i) arrival of larvae by ballast waters of merchant vessels; ii) natural spread by current pathways; and/or iii) intentional introduction for commercial purposes. The most probable vector of the appearance in the Ebro Delta may be the transport of larvae in ballast water. There is a nearby cement factory and a cargo harbour where merchant ships arrive from America. The southward expansion (gulf of Valencia) from this point could have happened without external factors, simply following the coastal current. Arrival to the Segura River is not clear. Intentional introduction by man cannot be ruled out, as the Atlantic blue crab has high gastronomic interest and market value. Genetic studies of populations north and south of Cape San Antonio are necessary.

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Keywords: *Callinectes sapidus*, alien species, vectors, Iberian Peninsula, Western Mediterranean

First confirmed record of *Branchiommma luctuosum* (Annelida: Sabellidae) in marinas of southern Spain

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Introductions and spread of Non-Indigenous species (NIS) are one of the greatest threats to marine biodiversity around the world. One of the most investigated NIS is the fan worm *Branchiommma luctuosum* (Grube, 170). Its type locality is in the Red Sea and has been later reported as a NIS in the Mediterranean Sea for the first time by Bianchi (193). Since this finding, a dynamic non-stop invasion process has been recorded along the whole Mediterranean basin, from Turkish to Spanish coasts. In fact, *B. luctuosum* has been recently included in the list of the 100 worst invasive alien marine species in the Mediterranean Sea due to 1) its high densities in artificial and natural habitats, 2) ability to growth in nutrient-enriched water, 3) strategies of anti-predation, 4) ability to simultaneous hermaphrodite reproduction and 5) high capacity to outcompete other filter feeding organisms. However, in spite of its ability to successfully colonize new environments, its presence has not been confirmed yet on the European Atlantic coasts. The present study is within the framework of a project whose main objective is the characterization of the macrofaunal community associated with artificial structures in marinas. A total of marinas were sampled along the Bay of Cádiz between September and October 201. *B. luctuosum* was present in six of them being associated to the vertical surface of floating pontoons, very close to the water surface and usually in small aggregates. Some specimens have been detected associated to the fouling of ships hull; therefore, such finding involves the first evidence of its occurring in these biotopes. Anyway, all of them have been located on a few recreational ships with an obvious lack of maintenance. Thus, larval transport within water ballast generated by shipping traffic is probably the main introduction vector in the Atlantic Ocean coasts. To explore its invasion dynamics in these new colonized habitats, a monitoring program has been initiated aiming to 1) determining the presence and population densities of *B. luctuosum* both in marinas and natural habitats in the Atlantic coast of southern Spain and Portugal, 2) quantifying the biomass (wet weight) of *B. luctuosum* populations in comparison to other sessile species of the fouling on floating pontoons, and 3) describing the epibiont major taxa on tubes and aggregates of *B. luctuosum*. The present study represents the first documented record of *B. luctuosum* for the Atlantic marinas of the Iberian Peninsula. We also warn about its successful spread and establishment in these new environments along the east Atlantic Ocean. More studies are needed to understand the consequences of this invasion on the biodiversity of the Atlantic communities.

Keywords: *Branchiommma luctuosum*, Marine invasions, Marinas, Southern Spain, Bay of Cádiz, Fouling community, Polychaeta

Identification process of key elements for MPAs management. The case of the southeast of Iberian peninsula coast

Isabel Abel Abellan, Rosa M. Canales-Cáceres and Fancisca Giménez-Casalduero

The marine area located in the southeast of the Iberian Peninsula named Cabo Tiñoso y Escarpes de Mazarrón (CTEM), has a high diversity of species, habitats and communities. The studies carried out there point to an ecosystem of high ecological value and different protection sites converge there, like the Site of Community Importance (SCI) "Submerged Coastal Strip in the Region of Murcia" and Special Protection Area (SPA) "Marine Area of the Murcia Coastal Islets", as well as the SPA "Isla de Las Palomas", the Special Areas of Conservation (SAC) "Submerged valleys of the escarpment of Mazarrón", and the Tiñoso Cape Marine Reserve. This scenario of the confluence of protection figures is quite widespread in the rest of the Spanish coast. However, it is not an effective conservation guarantee of the environmental values protection. In fact, despite the numerous protection figures, the CTEM area presents a lack of knowledge on the values, processes and interrelations between the elements subject to protection and the socioeconomic activities that converge in it, which makes decision-making difficult and could generate two situations: I. An inadequate or absent planning and zoning justified by the information gap, which would lead to maintaining the fishing and other uses intensity in key areas with foreseeable consequences on habitats and species II. Oversized measures on fishing, which prevents a sustainable development of the activity and favors social conflicts To guarantee the effective management of the marine areas, it is necessary to consult all the environmental regulations that come together in the

area and that not always reply to the same objectives. Therefore, there is a previous step to the development of a management proposal that includes all the objectives established in the different protection figures. It is important to analyze, contrast, and standardize these management objectives defined in the regulations established in the study area and identify the main existing elements susceptible to preservation and conservation. In the case of the CTEM area, a systematic analysis of the regulatory framework has been carried out using an approach that goes from the global to the local, that is, from the international guidelines to the national legal system, allowing us to identify those key elements that converge in the area. (Fig. 1) As shown in Figure 1, in addition to the review of European policies, the most important directives at the Community level for the marine environment conservation have also been contrasted, such as the Habitats Directive, the Water Framework Directive and the Marine Strategy Framework Directive. Furthermore, it has been necessary for the Spanish legal framework to exhaustively select those regulation that provide for the species and habitats protection, such as the Spanish Constitution, Law 42/2007, of 13 December, Natural Heritage and Biodiversity; or Law 41/2010, of 29 December, on the Protection of the Marine Environment. This analysis has been carried out in parallel with the direct consultations that have been held with the national and local authorities on the management of the marine environment. The results obtained after the analysis show that nine different protection figures converge in the CTEM area. The analysis of the objective elements of these protection figures identifies as key elements: Four priority habitats, seven species of cetaceans, eight marine birds, one reptile and two other key elements identified as Maërl beds or sunfish. (Fig.1) The key elements identification of the CTEM study area from the analysis of policies and regulations can facilitate the management and planning of the marine protected area. The effort concentration on key elements simplifies management objectives, optimizes available resources, and facilitates the monitoring and evaluation of the effectiveness of the actions. In this way, hot spots for the conservation of species and habitats can be determined, for example, based on quantifiable and geo-referenced criteria. It is necessary to understand those objectives foreseen in the legal framework on the marine protected areas conservation to advance in the sustainable management of space. In order to help national and local authorities in the marine management, it is necessary to facilitate them some tools to achieve the conservation objectives, protection of biodiversity and sustainability of economic activities. The systematic analysis of management objectives on key elements can be used efficiently resulting in the improvement of the resources. It is expected from the stakeholders a better response to the limitations of marine protected area if decision-making is carried out through a merged managed plan.

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Keywords: Key element, planning, Marine Protected Area, regulatory and legal framework, Management

LIFE REMoPaF Project (Recovery of Endangered Mollusc *Patella ferruginea*)

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LIFE REMoPaF project, led by ACCIONA Ingeniería S.A (AI) as Coordinating Beneficiary and the consortium is also composed of University of Seville (US) and Port Authority of Melilla (APM), was chosen by the European Commission to be part of the LIFE15 program. The LIFE programme is the EU's funding instrument for the environment and climate action created in 1992 and is devoted exclusively to the conservation and protection of the environment. The project began in 2016 and will run until 2021 and it is located in Spain, specifically in the Autonomous City of Melilla and the Algeciras Bay (Strait of Gibraltar). The project is focused on the repopulation of the ribbed Mediterranean limpet (*Patella ferruginea*), a species in danger of extinction, through the recruitment of small juveniles at the Donor Area (Port of Melilla) where there is currently a large well-preserved population, and the subsequent translocation to the Receiving Area, in Algeciras Bay (La Linea harbour, APBA). Hence, the species can be introduced to an area characterized by relatively low density values, but with the

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potential to reach a population size that allows it to further development as a breeding population. The aims of this project are design and test new techniques to the conservation of marine mollusk *Patella ferruginea* through the development of a methodology that allows the settlement of the larvae and the metamorphosis in juveniles on mobile substrates. For its recruitment (natural capture of the species in its early stages) the design of small-sized Artificial Inert Mobile Substrates (AIMS) is proposed, designed using 3D technology and conventional methods that may facilitate its translocation from one area to another. These are shaped with a heterogeneity and structural complexity (surface roughness) similar to the rocks of the breakwater where the species naturally is settled. In addition, the adults specimens that established their home scars within the AIMS were also translocated. The project has been divided into two phases. The first phase has been focused as a pilot experience to correct and improve the designed methodology. During the second phase, a greater number of AIMS will be installed in the donor area in order to obtain the largest number of individuals of the species to be transferred. The first pilot transfer of AIMS with *Patella ferruginea* was carried out with a 100% of survival rate during the transfer (31 specimens distributed over 20 AIMS). Monitoring of the species has been weekly performed for the first months, and subsequently the monitoring was undertaken monthly. The survival rate was 1%, 77%, 75% and 6% at one, two, three and four months respectively. The survival rate has been higher for juveniles and recruits than for adult specimens (77% survival of recruits vs. 57% survival of adult specimens). Currently, the AIMS corresponding to the second phase have already been installed in the donor area and its transfer is scheduled to take place in October 2019.

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Keywords: *Patella ferruginea*, Life, limpet, translocation, Reinforcement

Monitoring Tropical Signals in the Tabarca Island MPA. Anthozoans as global warming indicators

Andres Izquierdo-Muñoz, Esther Rubio-Portillo and Alfonso A. Ramos-Esplá

Introduction: In the last decade marine heatwaves are becoming more frequent (Rivetti et al. 2014; Oliver et al. 201). Anthozoans (as corals and gorgonians) are very sensitive to warming events, since its tissues suffer from bleaching and even necrosis if the event is continued in time, so this species have proven to be good indicators (Verdura et al. 2019). Previous marine heatwaves showed high damage in gorgonians and corals populations along the Marine protected area of Tabarca Island (Rubio-Portillo et al 2015). Here, we study the shallow (-3 meters) and medium depth (-25) anthozoans populations after the summer months in 2017 and 201, together with seawater temperature tracking. **Material & Methods** We followed two different methodologies for gorgonian and coral sampling: - Hard corals: *Oculina patagonica* and *Cladocora caespitosa* colonies were monitored by free diving photography. - Gorgonians: Scuba diving censuses were used to sample gorgonian communities (*Eunicella* spp and *Leptogorgia sarmentosa* colonies). In hard corals, percentages of bleaching, necrosis and covering by other species (that show old necrotic parts) were calculated; in gorgonians the percentage of necrosis and covering were estimated. In situ temperature data were collected hourly at 5 and 25 m depth (from spring to early winter) by temperature data loggers (HOBO ProV2). Results Seawater temperature records showed no extreme events during 2017 nor 201 (as happened in 2015, for example). Maximum temperature at 5 m depth was reached in mid-August (2017, 27.9°C and 201 with 27.9°C). Seasonal thermocline at 25 meters depth has different trends from two years: in 2017 a temperature peak at mid-august was detected, and then it was reduced progressively; in 201 temperature stayed lower, and only after the second week of september the temperature raised to 27°C. Figure 1 Bleaching in shallow corals, measured as % of individuals affected and % of surface affected, was similar for the two species studied in both years. In 201, both species were more affected by bleaching than the previous year. Coral necrosis (tissue death) was strongly different between species and years. In 2017 *O. patagonica* showed medium affection of necrosis with low affected surface, and *C. caespitosa* had low affection of necrosis, but the colonies affected showed medium to high surface death. However, in 201 necrosis values were lower in both species. About covering by other species (usually macroalgae), results were dramatically different. *O. patagonica* had low affection in 2017 and medium in 201. *C. caespitosa* had high affection in 2017 and in 201 all colonies sampled showed some parts covered by macroalgae. In both years, *O. patagonica* showed more recovering capacity, having more fitness

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to survive after bleaching or necrosis events. This feature would be one of the reasons of the invasion success of this species in the MPA (Rubio-Portillo et al 2014). Other affected hard coral has been *Phyllangia mouchezii*, although the data has been insufficient to determine the degree of injury as a consequence of heat stress, due to the rarity of this species (Rubio-Portillo et al., 2016). Figure 2 In the case of medium depth gorgonian communities, no apparent necrosis was observed during fall sampling in 2017 and 201. Only few specimens had burned tips and covered parts (due to past years mortalities), but in general sea fans showed healthy status. In 201, there was a temperature abnormality in September, with the seasonal thermocline affecting the gorgonian community along this month and October (with temperatures near 27°C). We repeated the census in December, and at this time near the 20% of the gorgonians surface showed tissue damage.

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Keywords: Global Warming, Gorgonian communities, corals, Bioindicator, temperature

Rapid Assessment Survey of ascidians (Chordata: Tunicata) in marinas of SW Mediterranean

Julio Ubeda-Quesada, Bárbara Iváñez-Rugero, Elisa A. Martinez, Andrés Izquierdo-Muñoz and Alfonso A. Ramos-Esplá

Ports and marinas represent areas where alien species find adequate conditions to survive and resist and expanding at the local and regional level through shipping in the Mediterranean Sea (Ferrario et al. 2017; Ulman et al. 2017). In this regard, recreational boating is a major vector of the introduction of non-indigenous species (NIS) worldwide and ascidians are some of the most abundant introduced taxa (Lambert 2001). In order to know the ascidian community associated with marinas and its seasonal variation in the SW Mediterranean (Alicante, Spain), eight marinas have been selected and sampled using the RAS protocols (Pederson et al. 2003) in winter (March 201) and summer (August, 201), with a temperature range of 12.5 to 25.5°C, respectively. Fifteen ascidian species have been identified on the different marinas along 200 km of coastline, where they were sampled by scraping the surfaces of floating pontoons, and submerged structures, such as hanging ropes, buoys and defenses were also surveyed. The biofouling communities showed clear differences between winter and summer (serpulids and bryozoans dominance) that were reflected in the ascidiofauna, particularly in colonial species. Thus, *Clavelina lepadiformis*, *Botryllus schlosseri* and *Diplosoma listerianum* dominated in winter; while *Botrylloides* cf. *niger* and *Ecteinascidia turbinata* in summer. Regarding the solitary ones, only *Ciona intestinalis* showed clear seasonal differences (appearance in winter); *Microcosmus squamiger*, *Styela plicata*, *S. canopus* and *Ascidia aspersa* have been present all year. Between marinas, Guardamar (near the mouth of the Segura River) has shown great differences, with only one species (*C. lepadiformis*). Other interesting species, although less abundant, have been *Aplidium* aff. *densum* and *Polyandrocarpa* cf. *zorritensis*.

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Keywords: ascidians, alien, Biofouling, Marinas, SW Mediterranean

The thermal response of the brown shrimp *Crangon crangon* growth from the southern edge of geographic distribution

Ana Filipa Costa, Ingrid Peperstraete, Francisco Arenas and Joana Campos

INTRODUCTION The brown shrimp *Crangon crangon* is both ecologically and commercially important (Campos & van der Veer 200): it is highly abundant in European estuaries and represents over 31t landed in the North Sea fisheries (ICES 2016). The species wide geographic range extends from Morocco to the White Sea, and reflects its high capability of coping with a large range of environmental conditions, namely of temperature. The growth of the brown shrimp has been extensively studied both under natural (e.g. Amara & Paul 2003) and controlled conditions, in relation to several factors including food availability (Hufnagl & Temming 2011a), light intensity (Dalley 190), temperature (Campos et al. 2009a), and using modelling tools (Campos et al. 2009b; Hufnagl & Temming 2011b). Though the growth rate increases with temperature in an expected Gaussian curve, changes in moult time interval are known to be more consistent than the size increment ones in the species growth thermal response (Campos et al. 2009a). Despite the innumerable studies on the growth of the brown shrimp, yet, no information exists on the optimum temperature for growth, and on its sensitivity and tolerance range. Here we investigate the temperature dependence of the brown shrimp growth for a population near the southern edge of distribution and discuss results in respect to a global change scenario. **MATERIAL AND METHODS** The experiments were run indoors. Ten aquaria were prepared to independently test 10 temperature levels ranging from 5 to 27.5°C. Shrimps were collected at Minho estuary with a 1m beam trawl 10 days prior the experiment. After one week of acclimation at the prevailing temperature level, 27 shrimps were randomly chosen per level and measured (total length, TL, mm). Mean TL per aquarium did not differ (ANOVA: $F = 0.173$, $p = 0.99$) ranging 17-4mm, mean=26.96±4.00mm. During the trials, each shrimp was kept individually placed, separated from the others by perforated plastic cages within the aquarium. Water temperature, salinity (Table 1), light (12:12), food (mussel in excess), moult and death events were controlled daily. All trials took 61 days. In case of death, the shrimp was replaced by another one from the original pool. The survival was estimated as the proportion of live/dead shrimps over the total number of individuals at each temperature level, and used to estimate the lethal temperature for 50% of population (the temperature at which 50% mortality occurs, LT50). Whenever a shrimp moulted, its TL was measured the following day to determine the moult increment (MI) and the time elapsed was registered (intermoult period, IP). The growth rate per moult (GR) was calculated by $GR = MI/IP$ for each two consecutive moult events. A thermal performance curve (TPC) for GR was used to access the relationship between temperature and physiological performance. The GR data for the range of temperatures between 5 and 27.5 °C was fitted to a Gaussian function (Angilletta et al. 2006) which gave us the thermal optimum (the temperature at which performance is maximized) and the performance breadth – the range of temperatures over which physiological performance is at least 69% of the peak (Van der Veer et al. 2006). The effect of temperature on GR was evaluated by determining the activation energy following Brown et al. (2004) under the Metabolic Theory of Ecology (MTE). The relationship corresponds to a simple linear regression, with data represented through an Arrhenius plot with

$\ln(IM^{(-3/4)})$ against $1/kT$: $\ln(IM^{(-3/4)}) = -Ea/kT + \ln(i0)$ Where I is individual GR ($mm.day^{(-1)}$), M is the shrimp wet weight (g), T is the temperature (Kelvin) and k is the Boltzman constant. The Ea is the activation energy (eV) obtained as the (minus signed) slope coefficient of the linear regression, and $i0$ is a normalization constant independent of body size and temperature, which is given by the intercept coefficient. The range of selected temperatures corresponds to the near-exponential increasing phase of the biological rate as described by Sibly et al. (2012). The wet weight (WW) was determined with the equation: $Y = 0.019 WW^{(1/3)}$, $r^2 = 0.92$, $n = 5416$ obtained through linear regression with field data on individual TL and WW. Both lethal temperature for 50% of the population and the thermal performance curve for growth rate were performed with RStudio version 1.1.33. The relationship between survival and temperature was evaluated through a logistic regression model, using the glm (General Linear Model) function. The TPC were drawn through nls (Nonlinear Least Squares) function. **RESULTS** A total of 346 shrimps were used in the trials but only 73 gave results useful for the determination of the activation energy for growth. Within the chosen range of temperatures (5 to 20°C), moults that did not result in growth or for which the growth rate was negative were not considered. Additionally, at the two lower temperatures, moult events were insufficient to determine a growth rate, having only one record at 5 and 7.5°C. Survival Survival was higher at the lower temperature levels (Table 1). The logistic regression suggests a strong association between mortality and temperature, with p-value <0.001 for the two months experiments. The estimated LT50 occurred at 26.62°C (Figure 1). Growth The number of moult events, the IP and the MI varied significantly between temperature levels (ANOVA: $F=5.41$; $F=4.719$; $F=5.77$, $p<0.0001$, respectively). Up to four moult events were observed from 15°C and over, except at 22.5°C, while at the two lower temperature levels shrimps moulted only twice. The IP tended to decrease with increasing temperature, stabilizing from 17.5°C on. In contrast, the MI increased in the lower temperature levels up to aprox. 17.5°C, decreasing hereafter. Mean GR ranged from 0.05 $mm.day^{(-1)}$ at 5°C to 0.23 $mm.day^{(-1)}$ at 20°C (Table 1). The TPC for the brown shrimp GR estimated a thermal optimum of $1.4^\circ C \pm 0.^\circ C$ and suggested a species that can tolerate a large amount of temperatures changes, with a performance breadth ranging between 11.3 and 25.5°C, as depicted in Figure 2(A). Activation energy The activation energy obtained for brown shrimp GR within the selected temperature range was 0.51eV (p -value < 0.05) as shown in Figure 2(B). **DISCUSSION** The present study confirmed a general positive trend of the temperature impact on growth of the brown shrimp from Minho estuary, determined the optimum temperature for growth as near 19°C, and found a large temperature breadth. The positive trend means that the metabolic costs due to increasing temperature are smaller than the increase in energy ingestion and allocation into growth. Food is known to affect the growth of shrimps: living prey favour the size increase, while frozen food, like the one provided in this study, is less beneficial (Hufnagl & Temming 2011a). The initial nutritional condition of the animals was not evaluated. In the North Sea, about one third of the population is food limited but food limitation is not known in the Minho Estuary (Hufnagl et al. 2010). Yet, obtained growth rate values were within the range of previous studies suggesting healthy nutritional condition. The activation energy, which represents the sensitivity of a given process to changes in temperature, was within the expected range of 0.2-1.2 eV suggested by Gillooly et al. (2001) and close to the average referred by Brown et al (2004), as the typical activation energy observed for most biochemical metabolism reactions. Thus, 0.51 eV can be a sign of high temperature sensitivity and tolerance range confirming previous suggestions (Freitas et al. 2007), though impacts of temperature may have different trends. Countergradient growth compensation has been described in the brown shrimp, whereby northern populations from colder areas grow faster than southern populations (Campos et al. 2009a). Therefore, it is expected that in populations other than the one from Minho Estuary, the thermal optimum can differ, as well as the range of favouring temperatures. In the North Atlantic, sea surface temperature has been increasing (IPCC 2014), affecting the metabolism of ectotherms (Angilletta 2009; Schulte 2015; Van Donk & De Wilde 191), with consequences for several biological processes including growth. The population from Minho estuary is close to the southern edge of geographic distribution and hence expected to be close to the upper tolerance limit of temperature. Besides the temperature sensitivity, the growth trajectory in relation to temperature may condition future adaptation to water warming, as it also responds to the prevailing food conditions, which in turn also depend on temperature as well. Mismatch between growth season and periods of food abundance may constrain the population growth, and should be clarified in future assessments of climate change impacts on the species. List of table and figures captions Table 1. Experimental conditions of temperature (°C), salinity (ppm), total number of shrimps, maximum number of moults per shrimp, mean (\pm standard deviation, sd) growth rate ($mm.day^{(-1)}$) and number of deaths. Figure 1. LT50 curve for the *Crangon crangon* experiments along two months. Figure 2. *Crangon crangon* temperature dependence. (A) Thermal performance curve for growth rate ($mm.day^{(-1)}$) fitted to a Gaussian function, showing the thermal optimum and the performance breadth. (B) Arrhenius plot obtained for growth rate ($mm.day^{(-1)}$) along with the activation energy (Ea , Ev).

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Keywords: temperature, Climate Change, Growth, Moulting increment, Intermoult period

A new methodology for the study of biological invasions on coastal communities

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The compound effects of biological invasions and habitat destruction have long been considered major causes of biodiversity loss worldwide. Early detection monitoring is a critical component to mitigate non-indigenous species (NIS) threats. Although Portugal still lacks a national online database of NIS, a recent review reported a comparable number of NIS introductions to those reported in other European countries. However, these numbers are almost certainly underestimated. In order to obtain a better inventory of NIS, Madeira has implemented a monitoring program of marinas and harbours using PVC plates to assess the settlement and the diversity of fouling organisms. Nonetheless, these plates revealed not to be suitable for assessing diversity and structure of the whole hard bottom benthic community as they are not appropriate for macroalgae and mobile invertebrates. Moreover, despite the success of this program in the increased detection of NIS in known settlement areas (i.e. marinas and harbours), it is still unknown the potential expansion of NIS to the

surrounding coastal areas. In this context, we designed a pilot study conducted in Madeira Island focused on (i) developing a new and translatable settling methodology appropriate to be deployed under different environmental coastal contexts, including marinas/harbours, breakwaters, open coasts and estuaries/bays; and ii) evaluate and improve the capacity to collect samples that are more representative of the overall resident hard bottom benthic communities. We conducted an experimental test with three different methods (i.e. prototypes) constructed with a variety of settlement materials (e.g. PVC plates, collector mesh), to provide a larger availability of surfaces for colonisation and refuge habitat for a wider range of organisms. A total of 1 experimental units were deployed in June 2017 (3 replicates of each prototype per selected environment, namely marina vs open coast) and were collected in October 2017 (4 months). After the 4-month period, all units were transported back to the laboratory for careful inspection and catalogue of fauna and flora. Mobile organisms were preserved in ethanol 96% for posterior separation, quantification and identification. Settlement plates from each unit were photographed in vivo. Fouling organisms were identified and covering percentages of different species was estimated by point-count image analysis software. The deployment method showed a good performance for its use across a broad variety of the potential coastal environmental contexts, in terms of handling and durability. Results from the analysis of community composition and structure showed that one of the traps was more efficient as diversity collector, while a different prototype was more efficient for abundance estimates. Thus, a combination of features from two prototypes seemed to be more adequate to be used as collector of samples that better encompass the whole benthic community. Our findings suggest that comparative assessments of benthic biodiversity and/or mesocosm experimentation with marine communities will benefit from the use of this new standardised sampling method.

Keywords: Non indigenous species (NIS), Benthic ecology, Marine Biodiversity, coastal habitat, Environmental context

Creature from the Ria de Aveiro Lagoon. Did I just catch an alien species?

Francisco R. Maia

The present communication reports the occurrence of the alien soft-shell clam *Mya arenaria* (Linnaeus, 175) in the Ria de Aveiro lagoon (NW Portugal). A total of 47 living specimens were collected in spring 2013 (April) and winter 2019 (January) in an intertidal mudflat located at the upstream end of Mira channel (400 32' 23 N; 0 45' 56 W). In this area, the soft-shell clam was able to maintain a well-established population in close coexistence with another endobenthic bivalve species, the native clam *Scrobicularia plana*. The soft-shell clam *Mya arenaria* is native to the Atlantic coasts of North America and its probably the first marine invasive species recorded in European waters. Presumably, it was introduced in the North Sea by the Vikings in the 13th century (Peterson et al., 1992), from which it spread throughout its current distribution. At present, it is commonly found along the coasts of the North and the Baltic Seas, northern Adriatic Sea, the Black Sea, on all British and Irish coasts, France, Spain, Portugal and in the Mediterranean Sea. The ability to survive in a wide range of habitats; the capacity to tolerate a broad set of environmental conditions at all life stages; the high fecundity levels; and the long-lived planktonic larval stage, have been proposed to explain the long-distance dispersal of this species and its success as an invader. To our knowledge, there isn't any scientific evidence reporting negative effects of *Mya arenaria* on Ria de Aveiro ecosystems. Nevertheless, some authors refer that this species has the capacity to change the surrounding environment through its burrowing, feeding and excretory activities, and it is capable to outcompete native bivalve species for space and food, reducing diversity, distribution and abundance of those species. For this reason, more research is needed to better evaluate and fully understand the long-term impacts of *Mya arenaria* on the native bivalve assemblages of Ria de Aveiro lagoon.

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Keywords: *Mya arenaria*, soft-shell clam, alien species, Invasions, Ria de Aveiro lagoon, Portugal

High fusibility and chimera prevalence in a worldwide invasive colonial ascidian

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The formation of chimeras after colony fusion has been hypothesized to favour invasion success and resilience to multiple stressors in modular organisms. We studied prevalence of chimerism and performed fusion experiments in Mediterranean populations of the worldwide invasive colonial ascidian *Didemnum vexillum*. We analysed single zooids by whole genome amplification (WGA) and genotyping-by-sequencing (GBS) and analysed >2,000 loci. In the prevalence study, we analysed five fragments of each of nine colonies growing in oyster aquaculture facilities at the Fangar Bay (Ebro Delta, Spain). The percentage of shared genotypes (PSG) clearly identified samples with the same genotype ($96. \pm 0.14\%$ identical loci) and with different genotypes ($4.07 \pm 0.07\%$). We found 44% chimeras. The fusion experiment was carried out at the Venetian Lagoon (Italy). A total of 45 pairs (15 intra- and 30 intercolony pairs) were assayed but one or both fragments regressed and died in ~50% of the pairs. Among those that survived for the length of the experiment (30 d), 100% isogenic and 31% allogeneic pairs fused. Fusion was unlinked to global genetic relatedness. We did not detect any locus directly involved in allorecognition, however, the existence of an histocompatibility mechanism cannot be discarded. We conclude that chimerism occurs frequently in Mediterranean populations of *D. vexillum* and may be an important factor to enhance genetic diversity and promote its successful expansion.

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Keywords: invasive species, Chimera, Fusion experiment, Genomics, Whole genome amplification (WGA)

Historic marine traffic into an Atlantic island: temporal patterns evolution and Non-Indigenous Species (NIS) introductions

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Shipping in the past decades has increased globally, accounting for more than 0% of global trade by volume and more than 70% of its value being carried on board of cargo ships [1]. These introduction vectors involving ships and its movements (biofouling and ballast water) are often cited as high risk for the introduction of marine non-indigenous species (NIS) and this concurs with the finding that NIS hotspots are areas with a high volume of shipping traffic [2]. Previous work related to the study of maritime traffic as an introduction vector of NIS in Portugal are scarce [3,4]. In Madeira, until now, these studies were focused on recreational boating [5-7]. In this context, an historical compilation of maritime traffic data for the main harbor of Madeira island (Funchal) was carried out between 1935 and 2004 with data provided by Madeira's port authority. The most relevant records of the maritime routes with stops within Madeira were documented, regarding last port, ship type and size and duration of stay. Five shipping trends representing statistically different periods were observed, Period A: 1936-1939; Period B: 1940-1973; Period C: 1974-199; Period D: 1990-1996; Period E: 1997-2004. The most common sources of maritime traffic arriving in Funchal were Portugal (mainland) (NE Atlantic), Great-Britain (NE Atlantic) and Canary Islands (W Africa) that are in accordance with the frequency of the native distribution for the NIS listing for the region until 2004. NE Atlantic, W Africa and Mediterranean are the most frequent bioregions of the NIS established in Madeira, until 2004. Confirming shipping traffic as one likely vectors for the introduction of these species in Madeira. Although other vectors could account for some

NIS introductions (e.g. Aquaculture and floating marine litter).

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Keywords: Introduction vector, Madeira Island, biological invasions, Propagule pressure, Biofouling, ballast water

Non-native bivalve species from Ria de Aveiro lagoon, NW Portugal

Francisco R. Maia

This study reports the presence of three non-indigenous bivalve species in Ria de Aveiro lagoon. The manila clam *Ruditapes philippinarum* (Adams & Reeve, 150), the hard clam *Mercenaria mercenaria* (Linnaeus, 175), and the soft-shell clam *Mya arenaria* (Linnaeus, 175). Species occurrence data are the result of several field surveys conducted by the author in the region over the past 15 years. The manila clam *Ruditapes philippinarum*, native to the Pacific coast of Asia, is the most widespread alien invasive bivalve species in Ria de Aveiro lagoon. It was intentionally introduced by local professional fishermen (probably between 2005 and 2010) to replace the declining stocks of the native grooved carpet shell clam *Ruditapes decussatus*. Since then, it rapidly colonised the shallow brackish waters of the lagoon, revealing a great capacity to adapt to this new environment. The hard clam *Mercenaria mercenaria*, native to the eastern shores of North America, was first observed in Ria de Aveiro lagoon in 2012 (Maia, 2015). Two large living specimens were found in the middle section of Canal de Ovar, near Torreira village. Since then, no other specimen has been found which suggests that this species was unable to establish self-sustained populations in the region. The origin of these specimens is uncertain, probably they were introduced with oyster seed stocks imported for aquaculture purposes or released into the environment via untreated ballast water discharges. The soft-shell clam *Mya arenaria* is an alien species native to the North American east coast. It was first recorded in the north sector of Ria de Aveiro lagoon (Murtosa channel) in early 200. New findings of *M. arenaria* in an intertidal mudflat located at the upstream end of Mira channel extend the geographical distribution of the species nearly 25 km further south. In this new area, the soft-shell clam maintains a well-established population in close coexistence with the native clam *Scrobicularia plana*. The impact of these non-indigenous species on native fauna is not known locally. Nevertheless, only the manila clam displayed a clearly invasive behaviour and may thus pose a serious threat to local biodiversity, especially over autochthonous bivalve populations.

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de Aveiro lagoon, (NW Portugal). Portuguese Congress of Malacology, 1-2 May 2015, Portuguese Institute for Sea and Atmosphere (IPMA, IP).

Keywords: alien species, *Ruditapes philippinarum*, *Mercenaria mercenaria*, *Mya arenaria*, Ria de Aveiro lagoon, Portugal

Patterns of spatial distribution of *Callinectes sapidus* in invaded environments of the Valencian coast (Spain)

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Introduction *Callinectes sapidus* (Rathbun, 196) is a portunid crab native of estuarine systems in the Western Atlantic coast, capable of reaching sizes up to 20 cm in carapace width. This species typically lives segregated by size to avoid cannibalism and it has a complex life cycle in which males remain in estuarine systems and females move to saltier waters to spawn and hatch. Planktonic larvae live in open waters and return to estuaries to settle as juvenile crabs. It is a euryhaline predator with high diet plasticity, which gives its high invasive potential. Its presence in the Mediterranean has been documented since the 1950s, but in recent decades, the population started to expand rapidly. In the Mediterranean coast of Spain, the first crab was captured in 2012 in the Ebro's Delta. In the Albufera of Valencia, a coastal lagoon that is communicated with the sea by three channels equipped with floodgates, the first crab was captured in 2014, and since then catches have increased annually. This is of special concern because the Albufera of Valencia is a protected environment under Spanish regulation, and the presence of the blue crab might endanger the local fisher's livelihood, both by inflicting damage to the fishing gear and through competition with target species like *Anguilla anguilla*. There is another established blue crab population. In Gandia, a coastal locality 70 km south of the Albufera. Therefore, our aims were i) to determine the population structure and spatial segregation by sex in these two different areas and ii) to study if this species behaviour is different from the one it shows on its native distribution area. Methods In both locations, Albufera and Gandia, fishery independent (baited traps) and fishery dependent data (commercial catches) have been collected since 2017. Fishermen in the Albufera of Valencia use traps known as "mornell", whereas in Gandia blue crabs are caught in trammel nets located in the coastal area close to the harbour. Our baited traps were deployed in different locations inside the Albufera of Valencia and also in the channels that connect the lagoon to the sea. In Gandia, traps were deployed in the San Nicolás ravine that connects the inner part of the harbour with the sea. In both locations the salinity was recorded and used in the sampling design implemented in the Albufera of Valencia. Results Spatial segregation by sex and size were clear in both areas, with males showing a strong association to the lower salinity areas while females preferred saltier waters. In the lower salinity areas, between 0‰ and 90% of the catch were males, whereas in the higher salinity areas up to 90% of the catches were females. However, salinity ranges were very different between locations, varying from 0‰ to 2‰ inside the Albufera lagoon and up to 24‰ in the channels that connect the lagoon to the sea whereas in Gandia, salinity ranged from 20‰ - 30‰ in the part of the port connecting to the ravine to 37.5‰ in the coastal area. Spatial segregation by sex happened both at salinities between 0‰ and 24‰ in the Albufera, and between 20‰ and 37.5‰ in Gandia. Discussion In its native distribution area *C. sapidus* lives in big estuaries with gradual salinity changes, but in the Mediterranean coast there are only strongly stratified estuaries, areas with sudden salinity changes (floodgates) or small salinity variations due to the contribution of small rivers, ravines or groundwaters. In the estuaries of the Atlantic coast of America, females do not move away from the mouth of estuaries and return in these after the reproductive events. However, off Gandia, females remain in the marine environment after the reproductive period and spend a period of inactivity buried in shallow sandy sediments. Salt waters are essential to ensure egg viability and larval survival, but it is not necessary to have large estuaries to complete the life cycle of the species. The segregation by size is a strategy to avoid cannibalism and besides the need of females for saltier waters, these factors could explain the apparent change in the life cycle of the species. It would be very interesting to deepen the knowledge of the migration patterns and the spatial segregations linked to the need for habitat use (underwater vegetation in juveniles, etc.) to complete the life cycle in an invaded environment which is so different from the original environment.

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Keywords: blue crab, Invasive specie, Reproductive phenology, spatial segregation, structure population

Tunic microbiome of a global ascidian invader

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The tunic of the ascidians shows a high diversity of prokaryote symbionts, which may play an important role in the species' biology, adaptation and survival. The global invader *Didemnum vexillum* provides a unique opportunity to assess the patterns of variability of ascidian microbiomes. In particular, do they remain stable as the host species colonises new habitats or do they shift according to local environment? We have sequenced 16S amplicons of the microbiome of 60 *D. vexillum* colonies from 2 native (Japan) and 10 introduced populations spanning the known distribution of the species. We found 2,94 zero-radius OTUs (ZOTUs) belonging to 36 bacterial and 3 archaeal phyla. The microbiome had a markedly different composition from surrounding seawater. Only ZOTUs (0.27%) were present in all colonies and 47 (1.5%) were present in all localities. However, these ZOTUs represented the 71.31 and 9.60%, respectively, of the total number of reads obtained. Thus, the species showed a high-abundance but low-diversity core microbiome. The variable component is quantitatively much less important but comprises a highly diverse assemblage. This component differs within localities (0.59 average Bray-Curtis distance), but even more between localities (0.70) and between described genetic groups of the ascidian (0.74). The average of exclusive ZOTUs was 11.95% in each colony and 22.05% in each locality. The combination of a quantitatively dominant core component and a highly diverse variable fraction in the microbiome of *D. vexillum* can contribute to the success of this global invader in their expansion to different environments.

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Keywords: invasive species, microbiome, Symbiosis, adaptation, *Didemnum vexillum*

Updating the list of recent non-indigenous ascidians (Chordata: Tunicata) and its spreading in the Mediterranean Sea. Ten years later (2009-2019)

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Introduction: A decade ago, a revision of the recent non-indigenous ascidians in the Mediterranean Sea was made (Izquierdo-Muñoz et al 2009). The study showed ten species considered as new aliens to the Mediterranean sea, since the revision of Pérès (195); we consider the Pérès study as temporal mark (after 1950's) to consider one species as recent invader or not. Ten years later, a deeper revision of the literature shows a total of 20 recent NIS ascidians. In 2009 we consider 14 species (Izquierdo et al. 2009) belonging to 7 families. Ten years later, 6 new NIS have been added to the list (*Aplidium accarense*, *Didemnum vexillum*, *Polychinum constellatum*, *Ecteinascidia thurstoni*, *Botrylloides anceps*, *Micocosmus anchylodeirus*) and related to 9 families. The species are classified into the categories proposed by Zenetos et al (2005), and discuss status, vectors, and habitats. Results and discussion We have considered recent NIS ascidians, those recorded later to the 1950 (Pérès, 195) and whose date of first record is known. In this regard, we can establish the following categories (Zenetos et al. 2005): established, casual and questionable. a) Casual: Some species were cited once in

the Mediterranean Sea, in a specific habitat: *Ecteinascidia styeloides*, *E. thurstoni*, *Perophora multiclathrata*, *Botrylloides anceps*, *Botrylloides violaceus* and *Microcosmus anchylodeirus*. b) Established: Those species that have been located in 2 or more sites and spread across the Mediterranean Sea. We can consider two categories: i) located in anthropic and natural environments; and ii) located in anthropized environments (ports, marinas, aquaculture zones). In the naturalized ones we find: *Distaplia bermudensis*, *Herdmania momus*, *Microcosmus exasperatus*, *M. squamiger*, *Phallus nigra*, *Rhodosoma turcicum*, and *Symplegma brakenhielmi*. In those located in anthropic environments: *Aplidium accarense*, *Ascidia cannelata*, *Didemnum vexillum*, *Polyandrocarpa zorritensis*, *Polyclinum constellatum* and *Styela clava*. Ghobashy & Abdel Messeih (1991) cited *Aplidium accarense* and *Styela clava* in Alexandria and Port Said, respectively, a further verification of these records would be necessary. Figure 1: Location of non-indigenous ascidians in the Mediterranean Sea. Families are indicated by the following symbols: Δ Polyclinidae Didemnidae Polycitoridae \circ Corellidae Perophoridae Ascidiidae \times Styelidae Pyuridae c) Questionable: Species were cited once but its description or the lack of it, require further verification and possibly not found in Mediterranean waters. In this category we include: *Eusynstyela hartmeyeri* cited by Harant (1939) in Port Said; *Ascidia corelloides* by Pérès (1959) in the Balearic Islands; and *Aplidium retiforme*, *Didemnum moseleyi*, *Eudistoma angolanum* and *Polyclinum saturnium* by Halim and Abdel Messeik (2016) in Egyptian Mediterranean waters. These last authors also find boreal species (*Ascidia obliqua*, *Cnemidocarpa mollis*, *Leptoclinides faeroensis*), never mentioned in the Mediterranean. *Polysyncraton amethysteum* has recorded in Balearic Islands by Pérès (1957), and cited in Egypt by Halim & Abdel Messeik (2016), however, we also consider this species as questionable, since they have not been observed elsewhere in the Mediterranean. d) Cryptogenic: We have considered cryptogenic species those ones in which the first record in the Mediterranean is not clear. Some of them were introduced centuries ago and are already naturalized; or by genetic studies represent different populations that have subsequently been NIS, coming from other regions: i) Tropical Atlantic and NW Temperate, coming in the hulls of ships from America (*Botrylloides niger*, *Clavelina oblonga*, *Ecteinascidia turbinata*, *Perophora viridis*, *Molgula manhattensis*, *M. occidentalis*) ii) cosmopolitan distribution (*Ciona robusta*, *Styela canopus*, *S. plicata*); iii) Indo-Pacific distribution (*Cystodytes philippinensis*, *Trididemnum cf. savignii*); and iii) originally native to the Atlantic NE (*Ascidia aspersa*, *Botrylloides leachii*, *Botryllus schlosseri*, *Clavelina lepadiformis*, *Ciona intestinalis*). We exclude them from this revision by failing to comply with the temporal criterion discussed at the introduction. The main vector of transport of these organisms is maritime traffic, attached to the ships as hull fouling. These NIS ascidians occur in harbours and there is some dispersion mainly by colonization of degraded and/or artificial habitats. A few species are associated to aquaculture, being the most likely introduction vector adhered to the shells of cultivated bivalves. This would be the case of *Styela clava* and perhaps *Botrylloides violaceus*, *Didemnum vexillum*, and *Polyandrocarpa zorritensis*. In these cases, its habitat is currently restricted to coastal lagoons or deltas, where there are marine farms. The range of distribution of some species considered here would be greater than described. This could be because of the difficult of proper identification of the species of the group; and there are very large areas (as the African coasts of the Mediterranean) that are under-sampled.

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Keywords: ascidians, invasive species, exotic species, Mediterranean sea, Harbours

Poster session 2

The structural role of canopy-forming macroalgae in modeling macrobenthic communities

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In marine environments, macroalgae acts as a biological structural substrate, creating a particular set of conditions for macrobenthic colonization. By creating a tridimensional space, they are able to modify their surroundings and therefore influence the community's structure [1-3]. Considering that complex and productive habitats lead to higher abundance and diversity of organisms, we can ask ourselves what processes drive the community assembly? What kind of dynamics arise during these processes? Are those based only on resources availability? Does the complex habitat structure benefits certain specific traits? This is the point at which the characterization of the macrofaunal communities it's crucial, in order to evaluate the marine biodiversity and to understand the ecosystem functioning and state. As to better understand the dynamics of community assembly, four patches of artificial algae were deployed in a natural intertidal habitat for a 6-month experiment. Here, we examine the relative importance of a complex habitat structure for the development of macrofaunal communities through a species-trait approach.

After evaluating the temporal patterns of the substrate colonization, a consistent increase of the abundance and richness until the 3 months mark was observed, with the macroinvertebrates assemblages reaching a considerable level of stability (specific richness, diversity and equitability) up from that time. This translated into a gradual increase of the functional diversity and the distribution of the functional space, representing a continuous improvement of the macroinvertebrates community structure. It was also noticeable the dominance of key functional groups like tube builders, borrowers-borrowers-dwellers, suspension-deposit feeders and epiphytic-epibenthic organisms, revealing their bond to the habitat and their capability to influence ecosystem processes. The use of a different set of metrics related to a species-trait approach and a new sampling methodology, allowed to obtain a higher level of information, making it possible to understand the origin of certain relationships of several groups of organisms with the structure of the habitat. Ultimately, understanding the relationship between species and functional diversity is fundamental in ecology in order to obtain key information about the stability, resistance and resilience of the ecosystems and their processes [4][5].

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Keywords: Macrobenthic communities, Functional diversity (FD), Species-Trait approach, Macrobenthic colonization, Artificial substrates

Ascidians (Chordata: Tunicata) from circalittoral and upper-bathyal soft bottoms of the Iberian Mediterranean. Bottom trawling impact

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The ascidiofauna of the MEDITS 2010 and 2017 cruises in the Iberian Mediterranean (from the Alboran Sea to NE-Iberian), has been studied, showing a high homogeneity with certain particularities according to the

areas and fishing pressure. Murcia and Catalonia harbor a relatively high abundance and diversity, both in solitary and colonial species. While in Alboran and the Gulf of Valencia, diversity and abundance have been lower; as well as the colonial strategy. With regard to the bathymetric distribution, a progressive decrease in abundance and diversity is observed with the increase in depth, giving the highest values between 40 and 100m. Based on the results, the ascidians, with their strategies (solitary, colonial), can be good indicators of the state of the Mediterranean soft circalittoral bottoms impacted by trawling. Introduction The soft circalittoral and upper-bathyal bottoms (between 35 and 00 m depth) of the Mediterranean Sea are subject to heavy fishing pressure due to bottom trawling. Among its effects, the destruction of the habitat complexity and homogenization of the sediment with an increase in the fine fraction (Jones, 1992, Thursh et al., 1995, Gray et al., 2006). The sessile and filter-feeding epifauna (sponges, anthozoans, bryozoans, ascidians ...) is particularly vulnerable to this impact, representing an excellent indicator (de Juan et al., 2007). In this regard, the ascidians form aggregations in soft circalittoral bottoms known as "ascidian bottoms" (Costa, 1960, Pérès & Picard, 1964, Monniot, 1965, 196, Pérès, 195), creating a complex habitat and increasing biological diversity with new substrate (epi and endobionts), food and shelter. In addition, they present two types of organization (colonial and solitary) with a different response to sedimentation (Millar, 1971; Ramos & Ros, 1990). In the present work, ascidiofauna is studied from the MEDITS 2010 and 2017 cruises (International Bottom Trawl Survey in the Mediterranean) in the Iberian Mediterranean, from Alborán to Catalonia and between 3 and 693m depths. The results show homogeneity of the ascidiofauna according to previous studies (Cornet & Ramos, 190; Ramos, 19; Turón, 19), with certain biogeographical particularities. Also, the impact of trawling on the structure of taxocenosis is demonstrated, particularly in those areas with a wide continental shelf (in the case of the Gulf of Valencia). Material and methods The study area has been the Iberian Mediterranean, from the Alborán Sea to Catalonia, during the MEDITS 2010 and 2017 cruises aboard the B / O 'Miguel Oliver', at depths between 3 and 693m (Fig. 1). The epibenthic megafauna has been sampled using an experimental fishing trawl (GOC-73: 16-1m width, 2-3m height, 20mm cod end mesh). The presence of ascidians in the catch has been about 32% of the trawl samples. Two types of analysis have been carried out (PRIMER 5 program): i) structure of the ascidian community (specific richness, abundance, Shannon-Viewer diversity and Pielou equitability); and ii) multivariate analysis (Cluster, MDS), with species whose abundance has been > 3 (0.1% of the total abundance). Figure 1. Studied zones with the number of samples (lances). Image from Google Earth Results and discussion Taxonomic study: A total of 310 ascidians belonging to 39 spp. and 10 families have been determined (Table 1). The Styelidae family stand out (56.7% of the total abundance) with *Polycarpa mamillaris*, *P. pomaria* and *P. gracilis*; following by *Pyuridae* (14.4%) with *Microcosmus vulgaris* and *Pyura microcosmus*, *Asciidiidae* (9%) with *Ascidia mentula*, *Molgulidae* (.7%) with *Molgula appendiculata*, and *Didemnidae* (6%) with *Didemnum maculosum*. Regarding the strategies, the solitary over the colonial dominates with 7.6%. Table 1 Structure of the ascidiofauna. SE and NE-Iberian zones have presented the highest species richness (26 spp.), an average abundance per sample (1-3 ej.), and a diversity > 2.0 (Table 2). On the contrary, Alboran and Gulf of Valencia have presented 12 and 16 spp., an average abundance between 11 and 33 ind./sample, and a diversity <1.. Regarding the range of depths, a progressive decrease of the species richness and the relative average abundance with the increase of the depth is observed. However, diversity has been slightly higher in the 100-199m range. Table 2 Multivariate analysis: The MDS species/zone analysis discriminates 4 groups (Fig. 2). Group A encompasses the species that normally appear in soft circalittoral bottoms. In the remaining groups (B-D) appear the species that have only appeared in some of the sampled areas, or have been very abundant: (B) SE-Iberian; (C) NE-Iberian; (D) Gulf of Valencia and NE-Iberian. Figure 2. Aggregation of ascidians/zone (MDS): (Aa) *Asciidiella aspersa*; (As) *A. scabra*; (Am) *Ascidia mentula*; (Ac) *Aplidium conicum*; (Ae) *A. areolatum*; (Br) *Botryllus renieri*; (Bs) *B. schlosseri*; (Cd) *Cystodytes dellechiaiei*; (Dc) *Didemnum coriaceum*; (Dm) *D. maculosum*; (Dva) *Distomus variolosus*; (Dvi) *Diazona violacea*; (Hp) *Halocynthia papillosa*; (Ma) *Molgula appendiculata*; (Mp) *Microcosmus polymorphus*; (Ms) *M. savignyi*; (Msb) *M. sabatieri*; (Mv) *M. vulgaris*; (Pb) *Polysyncraton bilobatum*; (Pma) *Phallusia mamillata* (Pg) *Polycarpa gracilis*; (Pm) *P. mamillaris*; (Pp) *P. pomaria*; (Pmi) *Pyura microcosmus*; (Ps) *P. squamulosa*; (Rn) *Rhopalaea neapolitana*; (Sc) *Styela canopus*. The observed ascidiofauna corroborates homogeneity in the soft circalittoral bottoms of the Iberian Mediterranean (Cornet & Ramos, 190, Ramos, 19, Turón, 19, Díaz-Valdés, 2009). Large solitary ascidians have dominated (Costa, 1960, Monniot, 1965); while colonial species have been frequent in sheltered or protected areas (Alboran, Murcia, Catalonia), which are not accessible to trawling. On the contrary, in the Gulf of Valencia (Valencia, Castellón) they have been very rare. The ascidiofauna and its strategies (solitary and colonial) are good indicators of the state of the soft bottoms (Ramos & Ros, 1990), particularly the impact of trawling.

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Keywords: ascidians, circalittoral, upper-bathyal, Soft bottoms, Mediterranean Sea, Iberian Peninsula, Bottom trawling impact

Associated fauna of Sponge grounds in the Cantabrian Sea. SponGES Project. Crustacea Decapoda. Expansion of distribution and new records of unusual species.

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Introduction Sponge grounds in the North Atlantic are the object of study by the Sponges project in which an international consortium of scientific institutions participates, which deal with the multidisciplinary study of these ecosystems of great importance. Of the seven study areas of this project, two are located on the Iberian continental margin, Le Danois Bank and the Aviles Canyons System, in the Cantabrian Sea (Southern of Biscay Bay), Le Danois Bank is a submarine mountain that rises from the abyssal plain of the Bay of Biscay from 5000 to 450 meters at its summit. It has been well characterized, as an area of great ecological importance and Marine Protected Area since 200. The Aviles Canyon System consists of three main canyons with different morpho-structural characteristics; Aviles, El Corbirio and La Gavierra and their valleys. This system has been designated since 2014 as Site of Community Importance (SCI) as defined in the EC Habitat directive. Different multispecific sponges grounds have been observed in this area and their taxonomic characterization is being studied. Aggregations dominated by the hexactinellid *Pheronema carpenteri* (Thomson, 169) as well as large specimens of *Asconema setubalense* Kent 170, *Phakellia* spp., *Geodia* spp. and *Lithistids* sponges. Also in these areas, important reef structures have been observed, dominated by the scleractinians *Desmophyllum pertusum* (Linnaeus, 175) (formerly *Lophelia pertusa*) and *Madrepora oculata* Linnaeus, 175, among others with the hexactinellids sponges *Aphrocallistes beatrix* Gray, 15 and *Regradella phoenix* Schmidt, 10 growing on. Dense aggregations of megafauna, such as the sponge fields, constitute biogenic habitats that increase the three-dimensional heterogeneity of the seabed, forming microhabitats that harbour a great biodiversity. (Klitgaard, 1995, Bo et al., 2012, Beazley et al., 2013). The biodiversity associated with these sponge grounds is one of the objectives of the SponGES Project. Results This communication presents the results corresponding to the taxonomic study of the decapod crustaceans, obtained in the SponGES 0617 cruise in the Aviles Canyon System and Le Danois Bank and the study of samples collected in different previous surveys carried out in the same areas developed by the Life+ INDEMARES project. Highlighting among the samples belonging to these surveys, the presence of the following species: *Uroptychus cartesi* Baba & Macpherson, 2012 only known to date at Galicia Bank, this record represents an expansion of its distribution and first record in Biscay Bay. *Munidopsis vaillanti* (Milne-Edwards, 11), with known distribution in the Bay of Biscay but with scarce records (Milne-Edwards & Bouvier, 194; 1900 and Lagardère, 1973) as *Munidopsis media* Milne-Edwards, 14, invalid name. *Munidopsis acutispina* Benedict, 1902 species of wide distribution in the NE Atlantic; western Sahara and Azores Islands, (Milne-Edwards & Bouvier 194, 199, 1900), Mid Atlantic Ridge, (Macpherson and Segonzac, 2005), Galicia Bank (Cartes et al., 2013) and Mediterranean Sea (Frogliata et al., 2002 ; Olu-Le Roy et

al., 2004) *Parapagurus nudus* (Milne-Edwards, 1911), species of wide distribution Western Atlantic, off United States, Bahamas, Gulf of Mexico, Caribbean Sea, and eastern Atlantic, Azores Islands, Cape Verde Islands, Canary Islands, Gulf of Guinea (Lemaitre, 196; 199) and off Mauritania (Matos-Pita & Ramil, 2015). This new record represents a northern extension of its known distribution in the Eastern Atlantic and first record for the Bay of Biscay. *Paralomis microps* Filhol, 14, to date only known by the type specimen obtained in the Bay of Biscay at 45°59'N / 06°29'W depth of 140m. A complete redescription of the type and summary of the taxonomic history of this species was made by Macpherson, 19. The specimen obtained this study it is the second record since its description.

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Keywords: associated, Fauna, Crustacea, Decapoda, sponge, Ground, Cantabrian, sea, new, records

Cannibalism behavior in the brown shrimp *Crangon crangon*

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INTRODUCTION The brown shrimp *Crangon crangon* is a common decapod crustacean in European estuaries

and a valuable fisheries resource in the North Sea. Due to its high abundance, the species is an important component of the trophic web, both as prey and predator. Recently, a list of up to 291 prey species were identified through DNA metabarcoding of the species' stomach contents, confirming its highly opportunistic feeding behavior (Siegenthaler et al. 201). Yet, this list does not report cannibalism (i.e. the process of killing and consuming either all or part of a conspecific individual) which can account for up to 20% of the species' diet in wild populations (Pihl & Rosenberg 194, Marchand 191, Evans 193, Del Norte-Campos & Temming 1994, Feller 2006). Like in other crustaceans (Abdussamad & Thampy 1994, Fernandez 1999, Marshall et al 2005), both predation and cannibalism pressure are expected to vary with size and density of predator and prey, and also with food availability and temperature. Cannibalism may then contribute to control or regulate the natural recruitment (Campos & Van der Veer 200). Additionally, cannibalism is one of the major difficulties which compromise the rearing of *C. crangon* (Delbare et al. 2014). In the Dutch Wadden Sea, recent settlers of about 6mm suffer high cannibalism pressure from adults larger than 30mm (Del Norte-Campos & Temming 1994), suggesting size-selectivity. Here, we investigate the size-related susceptibility to cannibalism of *C. crangon* juveniles and adults. The relationship between the sizes of the cannibal-shrimp and its prey-shrimp is studied using not only live prey but also by offering a dead conspecific, in order to determine the opportunistic character of cannibalism. MATERIAL AND METHODS The experiments were run indoors at CIIMAR facilities. Two observational aquaria were prepared for the trials. Each aquarium was divided into 32 individual compartments (7x9cm) by a perforated acrylic structure. Water temperature (13-15°C, mean = 14.3±1.4°C) and salinity (26-30, mean = 29.0±2.0) were independently controlled. Photoperiod was kept at 12:12h and the food provided consisted of mussel meat. Live juvenile and adult shrimps were collected in the Minho estuary (northern Portugal), with a 1m beam trawl, 10 days prior the start of the experiment. All animals were kept in maintenance aquaria in the same conditions and fed ad libitum. Trials were designed to favor cannibalism. Shrimps of known size (total length, TL, mm) were placed individually (in each of the 32 compartments) to act as 'cannibal-shrimp' (CS) and kept under starvation for 4h before the observational trials. 'Prey-shrimps' (PS) were kept in the maintenance aquaria up to the observational trials and fed ad libitum. After the starvation period, 5 PS (all with the same TL but smaller than the CS) were transferred into the respective CS compartment along with an extra dead shrimp of the same size as the live PS. After 24h, the animals were separated, measured and the sex of CS was identified. The number of remaining PS was registered. When ecdysis occurred during observations, these were excluded from data treatment. Shrimps used in the trials were not re-observed. Results were tested to determine size-selectivity and to quantify the cannibal-prey length ratio, as well as possible differences between predator sex. All tests were run in R version 3.5.3 (R Core Team 2019). RESULTS A total of 366 observations were made, using 27 female (TL: 21 to 51mm, mean = 33.4±6.0mm) and male (TL: 19 to 49mm, mean = 32.±7.2mm) shrimps as potential cannibals. As expected, nearly all CS showed cannibalistic behavior: 97.5% of the females and 95.5% of the males (no sex differences were found, p>0.05). Cannibalism was more common upon the dead PS (observed in 94.2% and 93.2% of the females and males, respectively; no sex differences, p>0.05) than upon live PS (observed in 61.5% and 55.7% of the females and males, respectively; no sex differences, p>0.05), while about 3% of the CS (all females) ate all the PS provided, both dead and live (Fig. 1). Only 6% of the shrimps that showed cannibalism did not eat the dead PS. Cannibal behavior was found in all size classes, starting right at 19mm TL. Size ratio varied from 0.31 to 0.99, i.e. up to similar sizes between CS and PS (Fig. 2). DISCUSSION In the present work we confirmed an extremely intensive cannibalism behavior in juvenile and adult *Crangon crangon*. However, no size selectivity was found as feeding on conspecifics was observed in potential cannibal-shrimps over the entire size range (19-51mm), upon prey-shrimp of up to the same size of the cannibal. Cannibalism is common in a wide range of animals, including Crustacea (Abdussamad & Thampy 1994, Fernandez 1999, Marshall et al. 2005), and has been documented both in field studies and in laboratory experiments. The evolution of cannibalism most likely resulted from a 'tradeoff' between its benefits (nutrition in case of food shortage, elimination of competitors) and its costs (risk of injury, acquisition of parasites and diseases, and potential detriment of a cannibal's inclusive fitness). Cannibalistic populations may persist when food is severely scarce, whereas a non-cannibalistic but otherwise identical population would go extinct. In crustaceans, cannibalism is often a response to a stressor such as limited food availability, high population densities (Abdussamad & Thampy 1994), limited refuge accessibility for vulnerable prey (Luppi et al. 2001) and low habitat complexity (Marshall et al. 2005). Prey vulnerability to predation is also relevant and is influenced by prey size (Fernandez 1999, Marshall et al. 2005), developmental stage and moult stage (Marshall et al. 2005). Favorable conditions to feed on conspecifics in this study included prior starvation, the lack of alternative prey, the relatively high densities and the absence of refuges for the prey-shrimp, including bottom sand. As a form of density-dependent mortality, cannibalism functions as a self-regulating mechanism in many populations under natural settings, though the role of cannibalism in the recruitment control or regulation of *C. crangon*'s natural populations is not entirely clear (Campos & Van der Veer 200). The stocking densities used in this study (11.1 ind m⁻²) were much lower than the highest reported for juvenile shrimps in their natural areas (about 0 ind m⁻²) (Bodekke et al. 196). Under controlled settings, even higher densities of up to 52 ind m⁻² have been used, and the resulting increased mortality (up to 66%) was attributed to cannibalism (Regnault 1976). Feeding on conspecifics is associated with size heterogeneity between the cannibal

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and its prey (Marshall et al. 2005). The handling time of prey is expected to decrease with increasing size differences between cannibal and prey, resulting in higher profitability. Surprisingly, size disparity (ratio between the total length of cannibal and prey-shrimps) did not influence the results, as cannibalism was found in all size classes and upon prey-shrimps of up to similar size of the cannibal-shrimp. In other animals, cannibalism occurs between different life stages (and hence differing sizes): adults cannibalize their own eggs, larvae, juveniles, or other adults, and juveniles consuming eggs or other juveniles. Already during larval stages, food unpalatability or insufficient quantity can condition the brown shrimp's cannibalistic behavior. During settlement, extreme cannibalism of *C. crangon* juveniles causes very high mortalities (Dalley 190). The starving condition must then be a strong trigger for cannibalism in *C. crangon*, even at relatively low densities. Cannibalism in crustaceans is often considered an opportunistic behavior associated with moulting (Marshall et al. 2005, Romano & Zeng 2017). During ecdysis, crustaceans are particularly vulnerable to predation and hence to cannibalism, because they must remain immobile and soft up to the end of the hardening process of the new carapace. It is still unknown which factors trigger the cannibalistic behavior, i.e. the release of signal products during molting or simply the presence of newly molted individuals (soft carapace). In the present work, the effect of molt stage was not investigated. Yet, the higher impact of cannibalistic behavior upon the dead prey-shrimp suggests that it is an opportunistic behavior. Nevertheless, active cannibalism upon the live prey-shrimp, which required agonist encounters, occurred in more than half of the observations. Besides density-dependent, cannibalism is always a direct cause of mortality. Therefore, cannibalism by brown shrimp should be carefully considered when rearing brown shrimp under controlled conditions. In the rearing of other crustaceans, cannibalism poses similar problems. In *Penaeus monodon*, the cannibalism rate increases with stocking densities and decreases with increasing feeding frequency (Abdussamad & Thampy 1994), while in *Litopenaeus vannamei* higher mortalities are associated with cannibalism upon molting shrimps (Romano & Zeng 2017). Future research should compare cannibalism for different population densities. Multiple-choice studies with alternative prey can clarify the preference for other prey. The impact of other factors requiring further investigation include: the shrimp molt cycle, the nutritional state, and the availability of refuges (sand, vegetation, shell, crevices).

Acknowledgements

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Keywords: Cannibalism, size-selectivity, Opportunistic feeding, Trophic relationship, predator/prey

Cephalopod component of Snares Penguin (*Eudyptes robustus*) at Snares Islands, New Zealand in the 190's: relationships with local fisheries and implications on the penguin's conservation

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In the Southern Ocean, cephalopods play an important role in food webs where penguins are known to feed considerably on them. The Snares penguin, *Eudyptes robustus*, an endemic penguin species from Snares Islands, New Zealand (4°1'32"S, 166°36'54"E), was used as a biological sampler to evaluate the cephalopod component of the diet of penguins around the Snares Islands during the 190's and to assess the habitat and trophic level of cephalopods (through stable isotope analyses of beak material). Snares penguin is classified as "vulnerable" according to the IUCN due to its restricted habitat and it is susceptible to anthropogenic activities. As historical diet data is rare for this species, we carried out detailed analyses of the diet of Snares penguins in the breeding season 196-197 (Nov.-Feb.). The results obtained show that they feed on three cephalopod species (*Nototodarus sloanii*, *Onykia ingens*, and *Octopus campbelli*) (99, 1221 and 294 beaks, respectively, from 36 samples). All specimens were juveniles, but with *N. sloanii* (mean mantle length (ML) = 12.9 mm; mean mass (M) = 63.7 g) individuals being larger than *O. ingens* (mean ML = 73.9 mm; mean M = 23.6 g) and *O. campbelli* (mean ML = 0.4 mm; mean M = 0.6 g). By frequency of occurrence and mass, *N. sloanii* was the most important species (F% = 97.2; M% = 72.5) and *O. ingens* was the most important species by number (N% = 4.6). Mattern et al. (2009) found a higher diversity of cephalopods in the diet of Snares penguins in samples collected in Oct.-Nov. 2002 (n = 11 taxa versus 3 species in our study). The most important cephalopod species were the same in both studies (*Nototodarus sloanii* and *Onykia ingens*). Also, Mattern et al. (2009) found *Histioteuthis atlantica*, which is a squid associated with warm waters, and the pelagic octopod *Ocythoe tuberculata*, both still numerically relevant. These results suggest that Snares penguins may forage more in pelagic waters in the 2000's than in 190's. According to stable isotope analyses, our study showed that all three species, *N. sloanii*, *O. ingens*, and *O. campbelli* occupied similar habitats (mean $\delta^{13}C \pm SD$; $-17. \pm 0.2$, -17.4 ± 0.5 , -1.5 ± 0.3 , respectively) on the continental shelf of Snares Islands. Moreover, *O. campbelli* prey on significantly higher trophic level prey (mean $\delta^{15}N \pm SD$; 6.7 ± 0.5) when compared to *N. sloanii* and *O. ingens* ($4. \pm 0.9$, 4.3 ± 0.9 , respectively). *O. campbelli* is a benthic octopod species and in a benthic system recycling of nutrients is typically enhanced which usually translates into elevated $\delta^{15}N$ values at the base of the trophic chain, but more studies on its diet are required to understand this. In relation to local cephalopod fisheries, *N. sloanii* was already targeted commercially, in the 190's at the Snares Shelf, catching bigger size squids (ML: 150-36mm) (Uozumi, 199) than those found in the diet of Snares penguins, despite both overlapping in their fishing/foraging areas. In the 190's and at these days, local fisheries occur during Snares penguins breeding season, but as they breed only at Snares Islands, 200 km south of the mainland, the risks of these species to interact with fisheries is lower when compared to other species, but can't be overlooked (Crawford et al. 2017). Climate change, which may affect the future availability of their prey, including cephalopods, might be a real threat in the near future (Trathan et al. 2014).

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Keywords: Snares Penguin, Diet, *Nototodarus sloanii*, *Onychia ingens*, *Octopus campbelli*

Co-engineering in mussel beds. First results on north of Portugal

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When spatial distribution of species overlaps, different kinds of interaction between organisms are possible (e.g. predation, competition). Sometimes, different species live on a very close relationship even as epibionts. This is the case of some macroalgal species which live as epiphytes above mussel beds. Specifically, in the intertidal zone of the north of Portugal, red algae of the genus *Caulacanthus* occasionally live above mussel beds of *Mytilus galloprovincialis* Lamarck, 1819.

Both algae and mussels are recognized as ecosystem engineers, since these organisms, due to their morphology, activity and/or their life-style, provide habitats for many species. Although the role of mussels and algae as ecosystem engineers has often been analysed separately, few studies have still explored the effect of co-engineering due to these two groups of organisms together.

The objective of our study is to explore how the presence of *Caulacanthus* spp. as co-engineer on *M. galloprovincialis* beds can affect their associated macrofauna. For this, two sites were selected from a rocky shore in the north of Portugal and at each site, five quadrat (10x10cm) replicates of mussels with and without alga were collected. Univariate analyses were done to explore differences between the abundance and diversity of the fauna, as well as the most abundant taxa. Moreover, a multivariate analysis considering the whole structure of assemblage was done.

Results showed that the presence of *Caulacanthus*, as co-engineer on mussel beds, did not affect the total abundance and diversity of the associated fauna with *M. galloprovincialis*. However, the abundance of crustaceans and molluscs increased when the alga was present. Within crustaceans, amphipods were the taxa, which most likely benefited from the presence of the alga, since their abundance doubled when the algae is present. Finally, considering the entire assemblage structure, the multivariate analysis showed marginal significant differences between macrofauna on mussels with and without alga.

Overall, we can conclude that *Caulacanthus* spp. as co-engineer of *M. galloprovincialis*, did not showed an important effect on mussels' associated fauna, although molluscs and crustaceans seemed to be positively affected by the presence of this alga.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Keywords: Co-engineer, *Caulacanthus* spp. , *mytilus galloprovincialis*, Associated macrofauna, North of Portugal

Contribution to the differentiation of Iberian Hermit Crabs species (Crustacea: Anomura) of the genus *Anapagurus* Henderson, 16

Bruno Almón, Jacinto Pérez, Eva García-Isarch, Enrique García-Raso and Jose A. Cuesta

Hermit crabs are among the more familiar organisms in shallow-water and intertidal areas worldwide. But within this group, there are plenty of forms, habitat preferences and habits. Taking into account that the taxonomy of the group encompasses several difficulties derived from the inherent variability of the species, the characteristic bodily asymmetry and, in many cases, the reduced size of the specimens, there are some genera in which these difficulties are even more challenging. The genus *Anapagurus* Henderson, 16 is composed by 19 species worldwide (García-Gómez, 1994), 11 of which have been recorded in the Iberian Peninsula (although the single Iberian record of *A. smythi* Ingle, 1993, still has not been confirmed). All are small species, with a homogeneous general morphology and sometimes very subtle differences between species, which makes ticklish their correct differentiation. Similarly, live color patterns are considered characteristic of each species in hermit crabs, but nevertheless is an aspect of the taxonomy that, in most of the cases, has been neglected (see Zariquiey, 1949, 1954 for some exceptions). Within a wider project oriented to the complete revision of the Iberian hermit crabs, both from the morphological and molecular perspective, the revision of the genus *Anapagurus* arise as one of the main challenges. Given that more than 50% of the existing species of the genus *Anapagurus* are present in Iberian waters, there is an unsurpassable opportunity to study them and try to clarify several conflicts regarding their systematics, which has being problematic for a long time. Specimens studied have been collected by a variety of methods and come from different sources, including Biological Reference Collections (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 collection from Prof. J. Enrique García-Raso- Málaga University), from scuba diving, and from scientific cruises devoted to the evaluation of fisheries stocks (ARSA 2017-201 and MEDITS 2017-201). Samples were studied under the stereomicroscope and then photographed and sketched to define forms and relative proportions, based on similar cephalothorax length in order to make the comparison reliable. Drawings were then digitalized in Inkscape, with the aid of a digital tablet. Color patterns have been drawn in Photoshop. Molecular samples were then taken from at least a pair of individuals of each of the species, one male and one female. Sequences of two mitochondrial genes (16S and COI) were obtained and others from GenBank were also included in the comparative. A complete Maximum Likelihood phylogenetic tree was obtained for the genus *Anapagurus* along with other species of the family Paguridae in order to examine the place occupied by this genus in the family and corroborate morphological identification. During the morphological revision of the specimens, a series of features have revealed as especially useful for the accurate differentiation of the species. Given the fact that other characters must always be also checked when dealing with these species, a certain level of clarification in relation with some cephalothoracic parts could lead to assign greater importance those characters and help to improve the differentiation process within the group. For this work, ocular peduncles shape and size have been studied in relation with ophthalmic scales shape and size for all Iberian *Anapagurus* species, revealing itself as a great tool that allows improving the accuracy in the differentiation of this complex species when comparing similar cephalothoracic lengths. From the samples studied we can highlight that, although the shape of the scales is not always easily observable, their size is generally noticeable. The proportion between scales and peduncles are for some species definitory, as is the case of *A. longispina* A. Milne-Edwards & Bouvier, 1900, where the scale is unusually large, reaching the half-length of the ocular peduncle, which is relatively short and wide, somewhat narrowed in the middle. In other cases, the proportions are not so evident, but preliminary results show that even in those cases, there are traits that can help in the differentiation. The validity of the species has been also tested by means of the combination of molecular and morphological information. Although this is still a work in progress, the main species within this genus appear to be valid so far, with 7 species already confirm and 4 more (including *A. smythi*) waiting for the final sequences to be obtained. Live color patterns have been also studied and described for most of the Iberian species, describing the unique traits and patterns that can be of major utility especially when dealing with images or field sampling works, where we have no access to microscopy facilities. These preliminary results are oriented to facilitate both field and laboratory taxonomic work, improving the differentiation

accuracy when dealing with *Anapagurus* species, which should lead to a better understanding of the natural populations, their numbers, and conservation status.

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Keywords: Hermit crabs, Taxonomy, Phylogeny, DNA, *Anapagurus*, Paguridae

Effects of harvesting on annelid assemblages associated with mussel beds

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The mussel, *Mytilus galloprovincialis* Lamarck, 119 is an ecologically and economically relevant species on European coasts. It is very abundant in the Atlantic intertidal fringe of the Iberian Peninsula where is harvested in natural systems and/or cultured intensively on rafts. Aquaculture production is dependent on their abundance in natural environments because juveniles are collected in rocky shores and then attached to ropes on rafts. Moreover, it provides habitat for many species, enhancing local biodiversity. The aim of this study was to explore effects of harvesting on the annelid assemblage associated with mussels. To achieve this, two rocky shores, where mussels are intensively harvested, were selected in Galicia (NW Iberian Peninsula). At each shore, two different sites were considered; at each site, 4 replicates (10x10cm) of harvested mussel clumps and 4 replicates of non-harvested clumps (control) were collected. Size and number of mussels at each replicate were compared between treatments (harvested-control). Moreover, annelids at each replicate were identified to species level. A total of 1351 individuals and 27 species were found. Abundance, taxon richness, Margalef index and assemblage composition were compared between treatments by means of univariate and multivariate techniques. Results pointed out that harvested clumps showed a lower and more homogenous mussel size than control clumps, as expected. Regarding annelids, the interaction between treatment and site was significant for the multivariate assemblage structure and for the total abundance. Therefore, the effect of harvesting is dependent on the spatial scale considered.

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Keywords: *Mytilus galloprovincialis* L., Harvesting, Annelids, Iberian Peninsula, Rocky shores

Energetic cost derived of short-term starvation on marine crustacean *Palaemon elegans*

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Starvation has a major impact on physiological processes in marine plankton and these processes have a major impact on the energy transfer through marine ecosystems. However, quantifying and understanding how starvation changes different aspects of the energy transfer has been retarded because planktologists have largely relied on biomass fractionation to shed light on it. Here, we experiment with the cellular energy allocation approach (CEA index), the respiratory electron transport system activity (ETS), and organic analysis of lipids (Lip), carbohydrates (Carb) and proteins (Prot) to help further our understanding of the energy transfer in the caridean shrimp, *Palaemon elegans*. We analyzed the effect of 72 hours of starvation using the CEA index that is calculated as the ratio between energy available to energy consumption. It allows us to evaluate the net energy budget (Verslycke and Janssen, 2002). The energy available (Ea) was determined from Lip, Carb, and Prot content, where $Ea = Lip + Carb + Prot$. Energy consumption (Ec) was calculated from the activity of ETS. All the parameters were transformed into energy equivalents using their respective energy of combustion (Gnaiger, 1993). Proteins were the main energy source ($1450. \pm 355.7 \text{ mJ} \cdot \text{mgWW}^{-1}$), followed by lipids ($727.2 \pm 124.4 \text{ mJ} \cdot \text{mgWW}^{-1}$) and carbohydrates ($2.1 \pm 17.9 \text{ mJ} \cdot \text{mgWW}^{-1}$). After 24 hours of starvation, the carbohydrates dropped by 3.2%, a significant decrease. After 4 hours, the proteins had decreased by 36.0%. The lipids were the last to decrease. After 72 hours they had dropped by 42.3%. Adding these organic constituents to calculate the Ea revealed a significant decreasing trend of 34.42% at 72 hours (from 2260.1 ± 45.5 to $142.3 \pm 120.9 \text{ mJ} \cdot \text{mgWW}^{-1}$). In contrast, the Ec, as ETS activity, showed a significant increase at 24 hours (from 5.2 ± 16.6 to $15.0 \pm 40.4 \text{ mJ} \cdot \text{h}^{-1} \cdot \text{mgWW}^{-1}$) that stabilized after 72 hours. Overall, the energy balance (Ea/Ec) showed that the energy available was higher than the energy consumption during the experiment. Ea/Ec ratio decreased for the first 24 hours, then remained stable for the next 4 hours. This portrait of energy regulation shows a rapid shift mechanism of *P. elegans* under sudden adverse conditions.

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Keywords: CEA index, Energy available, energy consumption, *Palaemon elegans*, Starvation

Factors driving waterfowl feeding preferences and foraging impacts on submerged aquatic vegetation ecosystems

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In aquatic ecosystems such as coastal lagoons, lakes and deltas submerged aquatic vegetation (SAV) often plays a vital role, influencing nutrient dynamics and water chemistry, modulating the structure and dynamics of food webs, and increasing habitat diversity. Several studies have reported long-term changes in SAV coinciding with changes in the size of waterfowl populations. To investigate the generality of this relationship, we present comparative results from studies in The Mediterranean (Ebro delta lagoon, Spain) and in the Gulf of Mexico (Mobile Bay, Alabama, US). Waterfowl feeding preferences and consumption of different SAV species were evaluated by tethering experiments and exclusion cages. In the Mediterranean, exclusion-cage experiments showed intense summer herbivory by coots (*Fulica atra*) that affected the biomass, canopy height and flowering of *R. cirrhosa* (flowers abundance was times higher inside exclusion cages; $1015.7 \pm 269. \text{ flw m}^{-2}$).

In GOM, coots (*Fulica americana*) were significantly more abundant over Eurasian watermilfoil than native wild celery and experiments showed greater grazing effects and consumption rates in milfoil. Overall, our results suggest that waterfowl effects on submerged macrophytes in ecosystems can be significant and are strongly influenced by seasonal changes in the availability of food resources. In addition, waterfowl do not always feed on their preferred or most nutritional food resources presumably because other factors, such as proximity of refuges from predators or competition for food resources, are of overriding importance.

Keywords: Waterfowl abundance, Herbivory, Cages experiments, Submerged macrophytes ecosystems, food choice, feeding behaviour

Feeding behaviour of the clown crab *Platypodiella picta* upon two species of the genus *Palythoa*

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Platypodiella (Decapoda, Xanthidae) is a genus of relative small crabs, called clown crabs, mainly known for living in close association with Zoantharia (Hexacorallia, Cnidaria), especially with those belonging to the genus *Palythoa* (Den Hartog and Holthuis 194). Different kinds of associations are frequently found among marine organisms, such as mutualism, symbiosis or commensalism, including species that become poisonous only after feeding from their prey (Cheney et al. 2016). For example, it has been found that specimens of *Platypodiella spectabilis* exhibited huge concentrations of palytoxin in their body tissues, an extraordinary toxic compound of zoantharian, mainly of the genus *Palythoa* (Gleibs et al. 1995). Direct observations of *Platypodiella* crabs feeding on zoantharians, which could help elucidate whether specimens are able to acquire these palytoxins by feeding on *Palythoa*, have not been up to date reported. *Palythoa* spp. are organisms very common in tropical and subtropical benthic ecosystems, where some species are currently expanding their populations and even replacing altered hard-corals ecosystems (Cruz et al. 2016). Given the inherent features of zoantharians, such as their prolific mat-forming growth and asexual reproduction, these population outbreaks can alter the structure of whole ecosystems, so there is a need to improve our understanding of any ecological forces that can directly or indirectly control populations. The increased dominance of *Palythoa* spp. in the habitat may also lead to an increase in associated *Platypodiella* spp. populations. In this sense, the aim of this study was to provide new information about the nature of this particular relationship between clown crabs and Zoantharia, in order to establish if there is any predatory control of colonies by the crab and a potential to buffer such zoantharian populations. In order to do so, colonies of the two most common *Palythoa* spp. found in the Canary Islands, *P. caribaeorum* and *P. aff. clavata* (López et al. 2019), were collected and immediately transferred to the laboratory in humid and dark conditions. Fragments of 6–cm² of *P. caribaeorum* and 7 to 10 polyps of *P. aff. clavata* were glued in artificial substrates and placed in tanks with running filtered sea water and 12 h light / 12 h dark cycle under LED lighting. Four colonies of *P. caribaeorum* and four of *P. aff. clavata* in a total of four tanks were separately exposed to one individual of *Platypodiella picta*. We included crabs of different gender and sizes to avoid any possible bias related to sexual dimorphism and/or ontogenetic changes. To standardize feeding status between individuals, crabs were not fed during two days previous to experiment commencement (Scheibling and Robinson 200). Respective controls without predatory crabs were established using another set of four colonies per species and tank. Susceptibility of zoantharians to the predatory crab *P. picta* was assessed by means of calculating predation rates upon experimental specimens as the difference in the weight of colonies between the commencement and the end of the experiment (5 days). A distance-based permutational analysis of variance (ANOVA) (Anderson 2001) was used to evaluate differences in predation rates and feeding behaviour among crabs due to their intrinsic characteristics of gender and size. Additionally, a total of 60 *P. picta* were collected for morphometric measurements of the carapace width and claw lengths to determinate if there are in fact any differences between males and females. Results of this study showed that *Platypodiella picta* is an active predator of *Palythoa* species (Fig. 1). Experimental colonies showed a significant decrease in weight in the presence of *P. picta* ($F= 15.4$, $p= 0.001$ and $F= 15.04$, $p= 0.001$ for *P. aff. clavata* and *P. caribaeorum*, respectively), being more noticeable in the case of *P. caribaeorum* (-1.079 ± 0.75 g) (Fig. 1). We found a positive linear relationship between carapace width and claw length in both sexes of *P. picta*, without differences between males and females ($13,70 \pm 2,16$ and $13,91 \pm 2,51$ in average, respectively, Fig. 2a). In fact, no significant effect of either crab sex or size were detected in analyses and several bites were observed in the experimental colonies, as well as crabs directly eating pieces of both *Palythoa* spp. *Platypodiella* specimens have been observed associated to zoantharians in several sites around the Canary Islands and, although they usually remain cryptic in their habitats, high abundances of *P. picta* are easily observed in

large colonies of *P. caribaeorum*. This fact may be related the greater predation activity of the crab observed upon this species in the present study. Results suggest that crabs may not only find a source of food in these kinds of habitats but also shelter from predators, due to the intrinsic characteristics of this species with polyps immersed in a well-developed coenenchyma (Pax 1910). Additionally, preferences of *P. picta* could be related to concentrations of palytoxins, which is specie-specific, being *P. aff. clavata* 90% more toxic than *P. caribaeorum* (Sawelew et al. 201). Nevertheless, feeding on the high biodiversity of mesoinvertebrates that also live in association with zoantharians (González-Delgado et al. 201) cannot be excluded and further multiple choice experiments should be considered. *P. caribaeorum* has high environmental tolerance and northward expansion of this species is expected to occur under future mild climate scenarios of change (Durante et al. 201). Given the nature of the close relationship reported here, changes in population structure and distribution of *P. caribaeorum* could be also joined to changes in *Platypodiella* spp. abundances. Fig. 1. Results of predation experiments with *P. aff. clavata* and *P. caribaeorum*, showing mean differences in colony weight (\pm SD) after 5 days of exposure to the crab *Platypodiella picta* (E: experimental colonies) and respective control conditions without crab (C: control colonies). Specimen of *P. picta* with a polyp of *P. aff. clavata* in its claw is also shown. Fig. 2: Relationship between carapace width and claw length of the clown crab *Platypodiella picta* (a).

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Keywords: predation, Zoantharia, commensalism, *P. aff. clavata*, *P. caribaeorum*

First data about marine tardigrades from Andalusia (South Spain) and first record of the genus *Archechiniscus* Schultz, 1953 in the Iberian Peninsula

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Marine tardigrade fauna of Andalusia remains unexplored despite the high diversity and the high number of endemic species of other taxa found in this area. A first exploratory study to fill this gap is presented here. Tardigrades were collected from sand samples collected from the intertidal at Punta Umbria beach, Huelva (Atlantic coast) and from samples of *Mytilus galloprovincialis* Lamark 119 and *Perforatus perforatus* (Bruguère, 179) scraped from rocky natural and manmade substrates at Algeciras harbour (Mediterranean coast). All the tardigrades were mounted in microslides using glycerine and sealed with nail varnish. Animals were measured and photomicrographed under x100 oil immersion, using a Zeiss Differential Interference Contrast Microscope (DIC) equipped with digital camera and Zen Imaging Software. Two species of tardigrades were found in sand samples *Batillipes pennaki* Marcus, 1946 and *Batillipes phreaticus* Renaud-Debyser, 1959. Probably these two species of *Batillipes* are the commonest at intertidal sandy beaches of the Iberian Peninsula. Recently, the cosmopolitan nature of *B. pennaki* was refuted and it is now considered a species complex, with specimens recorded from the Atlantic coast of the Iberian Peninsula showing important morphological differences from Atlantic American and Mediterranean individuals. The specimens recorded from Punta Umbria fits well with individuals from the Atlantic coast of the Iberian Peninsula in all traits, namely in the morphology of primary clavae and leg IV sensory organs. In the rocky substrates, we found two species undescribed species of the genus *Styraconyx* Thulin, 1942 and one individual of the genus *Archechiniscus* Schultz, 1953. This is the first record of the genus *Archechiniscus* for the Iberian Peninsula. Results suggest that marine tardigrade fauna for Andalusia can be diverse and may harbour many undescribed species.

Keywords: Tardigrada, intertidal, soft bottom, hard bottom, Andalusia (Southern Spain)

First record of the epibiont barnacle *Platylepas hexastylus* (Crustacea: Cirripedia: Platylepadidae) from Galician coast

Bruno Almón, Patricia R. Ariza and Jacinto P. Dieste

The presence of organisms growing over any kind of surfaces is common, especially in marine environments, where the larvae are transported by the currents long distances searching for suitable conditions to settle. These communities, known generally as fouling, are frequently composed by a complex assemblage of species living together. A special type of fouling communities are those associated with other living organisms, which adds to the equation the variable of displacement and migration between areas that are sometimes very distant. Sea turtles provide a suitable substrate for the settlement of many organisms, especially on the hard parts of the body, providing numerous advantages in relation to other types of inanimate surfaces, and being in occasions considered as "floating ecosystems". As pelagic organisms travelling through long distances, Sea turtles could hence function as a vector for transfer and introduction of foreign species in new habitats. On May 1, 2017, a male of loggerhead sea turtle, *Caretta caretta*, was found trapped in a gill net near the locality of Aguiño in the Ría de Arousa estuary, Galicia, Spain. The specimen was recovered alive and transferred to the facilities of the Grupo de Estudio do Medio Mariño (GEMM) to check his health condition and posterior release in a safe zone. On examination of the specimen, tens of specimens of the barnacle *Platylepas hexastylus* were found attached to the turtle. The barnacle genus *Platylepas* is represented by eight living species worldwide. These all have a large distribution in world's ocean and have often been found as epibionts on marine turtles, manatees and sea snakes. In the former case, its ability to attach to any part on the turtle has been reported, with a frequency of occurrence ranging from 10 to 70 percent. *Platylepas hexastylus* is a cosmopolitan species, and has been reported in Japan, Australia, Netherlands, Aegean sea, Senegal, Malta, Algeria to Balearic Islands, USA, Brazil, Levantine Basin, South China Sea, Mexico, Uruguay, Hawaii, Mediterranean sea and Gambia. Here-

in, we report the occurrence of *Platylepas hexastylus* for the first time in Galician coasts as epibiont of *Caretta caretta*. During the examination of the turtle, a sample of the barnacle species was taken in order to identify them in the laboratory. The barnacles were removed from different parts of the turtle using a scalpel blade, focusing on the adherence zone of the epibiont, enabling it to be detached in its entirety without suffering morphological damage. Once collected, all the specimens were identified and preserved by immersion in alcohol 70°. All collected specimens were studied under a stereomicroscope, then photographed and measured with digital caliber. The turtle was also measured (standard carapace length and width 55.4 cm x 52.1 cm) before being immediately released. Barnacles were found attached mainly in the plastron, but also on the carapace, flippers, head, neck and cloacal region of the specimen of *Caretta caretta* (Fig 1). Their size ranged from 5 to 20 mm in diameter in the longest side and agreed with the description of *P. hexastylus*, being this species the only barnacle species present (Fig 2). This species present the capability of producing serrated striations that allows the barnacle to penetrate on the skin and hard parts tissue, burrowing the surface. Many of the specimens where in this case deeply incrustated in the tissue of the turtle. Moreover, other taxa were also identified in the samples as part of the fouling community associated with the barnacles, being the most manifest those belonging to the Algae, Hydrozoa and Caprellida. The algae and hydrozoa were too damage for identification, but over the seaweed carpet, several specimens of the caprellid *Caprella andreae* Mayer, 190, were found. *C. andreae* is also a well-known epibiont of *Caretta caretta* and has been recorded in different areas attached to a variety of floating objects on high sea. Turtles represent a refuge for the epibiont fauna against predation; it could be the reason for the many associations between epibionts and their host. This kind of association allows the study of the ecological and evolutionary factors involved, thus obtaining more information about the structure of these communities. Hence, the occurrence of *P. hexastylus* is reported in Galicia for the first time associated with Loggerhead turtle, *Caretta caretta*.

Keywords: epibiont, *Platylepas hexastylus*, *Caretta caretta*, *Caprella andreae*, Barnacle

Description of fouling population emerging after the episode of eutrophic crisis in the Mar Menor coastal lagoon

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The Mar Menor is a hypersaline coastal lagoon, located in the southeast of the Iberian Peninsula, is one of the largest coastal lagoons in the Mediterranean basin. Over the years, Mar Menor has suffered widespread environmental degradation due to multiple factors causes of Anthropogenic Origin. The surroundings of the lagoon have suffered in the last decades, a strong drive towards the conversion of the crop water regime. The transformation from rain-fed agriculture to intensively-irrigated agriculture has resulted in increased flows of water and nutrients from the basin, which has led to eutrophication processes. From the second half of 2015 the waters of the Mar Menor have undergone a drastic change in quality due to the massive proliferation of phytoplankton, since then this situation has been maintained which has led to a considerable reduction in light at the bottom of the lagoon, which has prevented photosynthesis of the phanerogamous meadows. In 2016, the ecological status of the Mar Menor went through an unprecedented anthropogenic crisis that developed in record time. The increase in nutrients caused changes in the structure and function of the lagoon ecosystems and the epibenthic and filtering organisms benefited from this imbalance - growing rapidly and reaching a high level of population density. In this study, described the filtering community found in the collectors maintained for a period of more than two months located in two locations in the Mar Menor: El Barón (N 37° 41'45.9 "O 0° 45 '9.4") and Encañizata (N 37°46'9.69 "O 0° 45'7.79") during the years 2017 and 201. The presence of 33 species belonging to 27 genus and phylum has been observed. The phylum Annelida is the best represented species with 12 identified species, the relative abundance in 2017 was 1.2%, while in 201 it rose to 43.6%. Closely following, is the phylum Arthropod, with 6 species identified, had a relative abundance of 79.2% in 2017 and dropped to 45.1% in 201, Phylum Mollusca, with 6 species identified, had a 0% presence in 2017, but in 201 it rose to 2.1%, Phylum Cnidaria, with 3 species identified, had a relative abundance of 2.5% in 2017, while in 201 it rose to 4.%, Phylum Porifera, with an identified species (*Sycon raphanus* Schmidt, 162), which was also 0% in 2017, increased to 3.6%, Phylum Echinodermata, with one species identified (*Ophiura ophiura* Linnaeus, 175), had also increased in density from 0% in 2017 to 0.01% in 201, For phylum Chordata, an identified species of ascidia sp, also surfaced in 201 with a 0.046% relative abundance, and a species of the genus *Nemertea* sp, turned up with a 0.01% relative density in the same year. (Figure 1,2). Within Annelida family, the genus with the most significant wealth of species is the *Hydroides* sp. with three species: *Hydroides norvegica*

(Gunnerus, 176), *Hydroides dianthus* (Verrill, 173), *Hydroides elegans* (Haswell, 13). Followed by the genus *Serpula* sp. with two species: *Serpula vermicularis* (Linnaeus, 1767) and *Serpula concharum* (Langerhans, 10). In the present work, some specimens detected were considered as bi-operculate, that is, with the presence of two operculi. Among the polychaetes found in 2017 the relative abundance of bi-operculate was 1.4%, while in 201 it was 1.01%. In some of the bi-operculate specimens found, the operculum presented different morphological characteristics and matched the characters of different species, while in other organism they presented operculums with similar morphological characteristics (Figure 3A, B). In addition, two specimens with three operculums have been found (Figure 4). Bi-operculated and tri-operculated organisms are associated with an advanced ontogenetic stage of polychaetes. However, in no case is it considered a different taxon. The study of Mar Menor has allowed to understand how the emerging communities are structured in the lagoon after the eutrophic crisis and how they respond to the different anthropogenic stresses. Mar Menor is characterized by extreme values of temperature and salinity and the species that inhabit them must adapt to fluctuations. The process of eutrophication causes a high availability of nutrients, a drop in pH and as a consequence an increase in dissolved calcium carbonate. This situation favors the growth and excessive development of the filtering community and especially those with calcareous tube.

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Keywords: Ecology, Lagoons, Eutrophication, polychaetes, Bi-operculate

Free living marine nematodes from A Cabana cove in Ría de Ferrol, Galicia (NW Iberian Peninsula)

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Ría de Ferrol is one of the Galician Rías Altas, in the province of A Coruña and is located at the mouth of the Xuvia river. It is considered as a good place for the marine wildlife due to its coverage and hydrodynamic conditions, that produces plenty of different habitats. A Cabana cove was formed by a granite tidal dike of 51 m length and 15 m width, concealing the area from the strong north winds and increasing the marine richness. The study area is located in the inner third part of the Ría de Ferrol, on its northern margin. This area is mainly formed by sandy mud sediments. There is a mechanical alteration of the sediment in this area due to shell fishing, as well as a split of domestic waste water in the cove. This study was conducted to determine the diversity of free living marine nematodes in A Cabana cove. The studied material consists of one sample with 100 cc. sediment that was taken in A Cabana cove (43°29'12"N; 0°15'02"W) using a PVC core. The meiofauna present in the sample was treated with MgCl₂ before extraction. After that treatment, it was washed with filtered tap water through a sieve of 50 µm. The nematodes present were picked up from the rest of the sediment and preserved in flasks using ethanol 70%. Nematodes were mounted on microscope slides to their study. An initial taxonomic list of nematodes was constructed based on the presence of species. In A Cabana cove, a total of 1612 specimens were found, belonging to 11 different families and 13 different genera. The order Desmodorida was best represented with 32 specimens from 2 genus followed by the order Araeolaimida with 256 specimens from 3 genus. Species belonging to the orders Chromadorida, Monhysterida and Enoplida were also recorded. The dominance of genera *Spirinia* was remarkable, with 2 specimens present in the sample. Genera such as *Pareurystomina*, *Metoncholaimus*, *Thalassironus*, *Odontophora*, *Paracomesoma*, *Sabatieria*, *Chromadora*, *Chromadorella*, *Chromadorita*, *Monoposthia*, *Siphonolaimus*, and *Linhomoeus* were also present. Moreover, there were 135 specimens that could not be identified and 206 were deteriorated and not identifiable. From the reviewed literature, 2 new species of free living marine nematodes have been reported for the first time in Galician waters: *Thalassironus britannicus* (Fig.1) and *Paracomesoma dubium* (Fig.2). The high number of nematodes that are deposit-feeders either selective and non-selective, representing the 6.5% of the sample, were related to the influence of the split of domestic waste water in the zone. Due to this split, the diversity of nematodes is also low in comparison to areas with similar conditions.

Keywords: Taxonomy, Free living marine nematodes, Nematoda, Galicia, A Cabana

Home selection and regular welcome (and not so much) guests in Iberian Hermit Crabs

Bruno Almón, Jacinto Pérez, Eva García-Isarch, Enrique García-Raso and Jose A. Cuesta

Hermit crabs, known as paguroids, are relatively common along the shores of the Iberian Peninsula. They are easily recognizable due to their peculiar characters, as the soft twisted abdomen, usually protected inside a gastropod shell, although some species can use also sponges, bivalves, scaphopoda shells and even polychaeta tubes, among other objects. In other cases, they can also establish symbiotic relationships with a variety of Anthozoa species who serve as a refuge for them. By using of a wide range of objects and organisms to protect their body, hermit crabs create an optimal moving substrate for the settlement of other organisms, both on the outside of the shells and inside them. In recent years, some interesting research about this matter has been released, increasing to a large extent, the knowledge about this interesting biological feature on hermit crabs, describing up to 550 symbiotic species of marine invertebrates permanently or temporarily associated with paguroids (Williams and McDermott, 2004, McDermott, 2010). However, there is very little information about these relations within the Iberian Peninsula framework. As part of an ongoing taxonomic revision of the hermit crabs of the Iberian Peninsula, information on the associations they establish with other organisms is being collected. Samples included in this study, come from direct sampling by scuba diving mainly along the coasts of Galicia and Andalucía. Fourteen species belonging to 6 different Phyla have been identified so far as frequent associates of at least 9 hermit crab species within the Iberian shores. The purpose of this work is to review the available information on the habitual tenants of the Iberian species of hermit crabs, adding new data obtained during this preliminary work, serving as a start point, baseline, for future revisions. Two species of sponges are commonly found in Iberian waters associated with at least three different species of hermit crabs. *Suberites domuncula* (Olivi, 1792), is frequently found covering the shells occupied by *Pagurus cuanensis* and *P. bernhardus*, while *Suberites pagurorum* Solé-Cava & Thorpe, 196, has been found associated with a wide range of species in other areas, although just two species have been registered as hosts in Iberian waters so far, *P. cuanensis* and *Anapagurus hyndmanni*. Both sponges are first records for Galicia. The hydrozoan *Hydractinia echinata* (Fleming, 12), build colonies over shells of hermit crabs on regular bases; although it can be found also on rocks, this behavior is by far less frequent. Polyps are disposed covering all the shell but tend to be placed in higher densities near the aperture of the shell. It doesn't seem to show a great specificity regarding the species of hermit crab, although it can be found frequently associated with *P. bernhardus*. Due to the presence of the hydrozoa on the shell, the sea slug *Cuthona nana* has also been recorded feeding on them, temporarily associated with the community supported by the hermit crab presence. *Podocoryna carnea* M. Sars, 146 and *Podocoryna exigua* (Haeckel, 10) are small hydrozoans living in colonies connected by a basal stolon. These species have preference for *Nassarius* shells, which can be occupied by a hermit crab. This relationship can be characterized therefore as "casual", rather than specific symbiosis. However, colony structure changes depending on the substrate, with defensive polyps only present when the colony is placed on shells occupied by a hermit crab. *Adamsia palliata* (O.F. Müller, 1776) has a predilection for gastropod shells of the genus *Gibbula*, and maintains a commensal relationship with hermit crabs, especially with *Pagurus prideaux*. It is exceptional to find this anemone on a shell inhabited by another species of hermit crab; the anemone feeds on the hermit's leftovers, while the anemone offers protection with its cnidocytes. This anemone secretes a chitinous substance at its base that allows it to grow at the same rate as the hermit crab. It presents an ectoparasite, the amphipod *Tryphosella sarsi*. *Calliactis parasitica* (Couch, 142) is not an obligate symbiont and can be found on rocks or old empty shells. However, in most of the cases, they appear associated with shells occupied by hermit crabs, often *P. bernhardus*, since the crab is able to facilitate the transfer of the anemones from the old shell to the new one using their claws. The anemones feed on zooplankton, hermit's leftovers and organic matter. *Hippoporida lusitania* Taylor & Cook, 191 is an encrusting colonial bryozoan that can be found covering gastropod shells occupied by several species of hermits. So far, two Iberian species have been found associated with these colonies, *Anapagurus curvidactylus* and *Cestopagurus timidus*. *Neanthes fucata* (Savigny, 122), a polychaete worm, invades the hermit's shell (usually those of *P. bernhardus*) and remains inside poking up occasionally to remove the food from the crab claws. Its presence, although it does not cause great damage, doesn't seem to bring any known benefits either. *Peltogaster paguri* Rathke, 142, *Peltogasterella sulcata* (Lilljeborg, 159) and *Clistosaccus paguri* Lilljeborg, 160 are parasitic cirripeds infecting each of them several species of hermit crabs, being the first found in *P. bernhardus*, *P. cuanensis* and *A. chiroacanthus*, the second in *A. chiroacanthus*, *A. hyndmanni*, *A. laevis*, *P. cuanensis* and *P. prideaux* and the last only in *Diogenes pugilator* so far. The ectoparasite *Athelges paguri* (Rathke, 143) can be found fixed to the abdomen of the hermit crabs. It has been recorded so far on *P. bernhardus*, *P. cuanensis* and *A. laevis*. *Pebrilla paguri* Giard, 1 is a suspension-feeding ciliate originally described on *P. bernhardus*, although it has been located on other species ever since. These preliminary results, here summarized, are a starting point heading to a more complete

description of this type of interactions in the near future, improving the knowledge about these unusual communities. At this moment, *P. bernhardus* holds the most diverse community of associates, but it may be due to the differences in sampling effort since *P. bernhardus* is one of the most common species in the sampled area.

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Keywords: Hermit crabs, Symbiosis, Associates, Iberian Peninsula, Taxo nomy

Molluscs of the Gazul mud volcano (Gulf of Cádiz)

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The Gulf of Cádiz represents an area with an important seepage activity with ca. 70 mud volcanoes (MV). MVs of the Spanish margin were included in the Natura 2000 network and declared as "Site of Community Importance" (SCI) in 2014 (Volcanes de fango del golfo de Cádiz, ESZZ12002). Among these MVs, Gazul MV stands out for the presence of vulnerable deep-sea habitats (e.g. cold-water coral banks, sponge aggregations) and a potential high biodiversity when compared to other bathyal areas of the Gulf of Cádiz. Molluscs are one of the most diverse faunistic groups in marine environments and represent a good indicator group for the evaluation of benthic biodiversity in a particular area. Because of this, molluscs collected during the INDEMARES/CHICA 0610, 0412 and ATLAS/MEDWAVES 0916 oceanographic expeditions on Gazul MV were studied through the analysis of samples collected in different areas (summit, flanks, seafloor depression, adjacent bottoms) of Gazul MV, using different methods such as box-corer, Shipek grab, benthic dredge and beam-trawl (Figure 1). Previous studies of the malacofauna of the Gulf of Cádiz have focused on infralittoral and circalittoral habitats, or mainly addressed taxonomical and ecological aspects of the deep-water species, including the chemosymbiotic bivalves of MVs. The present work focuses on bathyal vulnerable and non-vulnerable habitats of Gazul MV, analyzing their molluscan taxocoenoses and thanatocoenoses and exploring the relationships between the detected molluscan assemblages and some environmental and anthropogenic features of the area. A total of 266 mollusc species were identified, of which 47 are new records for the Spanish margin of the Gulf of Cádiz (six of them are new records for Spanish waters altogether). This reflects the uniqueness and the richness biodiversity of Gazul. The high species richness observed could be mainly related to the combination of different sampling methods, taking into account the thanatocoenoses, to the high habitat heterogeneity and to the geographical location of Gazul MV. The best represented species were the bivalves *Bathyarca philippiana* and *Asperarca nodulosa*, in both the taxocoenoses and thanatocoenoses, followed by the polyplacophoran *Leptochiton* sp. as well as the bivalves *Astarte sulcata* and *Limopsis angusta* (Figure 2). These thanatocoenoses also harbors less frequent species that are either typical of northern latitudes, indicators of past fluid expulsion, transported from the shelf, or very restrictive regarding their host. The taxocoenoses from different areas of Gazul were somewhat similar, with significant differences observed in some cases between those from the MV, where the highest evenness and diversity values were observed, and from the depression in relation to those from adjacent areas. On the other hand, those environmental variables displaying a higher correlation with the distribution of molluscs were generally indicators of environmental complexity (e.g. proportion of gravels, presence of methane-derived authigenic carbonates) and food availability (e.g. proportion of organic matter) for a large number of the collected species.

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Keywords: Mollusca, species richness, mud volcano, Gulf of Cádiz, Bathyal

New advances in the study of the biodiversity of the SCI "Volcanes de fango del golfo de Cádiz" (south-western Spanish Margin)

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The Gulf of Cádiz represents an important seepage area with ca. 70 mud volcanoes (MV), of which one third are located in European waters (Spain and Portugal). Previous projects and expeditions resulted on a large amount of information on different aspects of MV of the Moroccan margin, which seem to have a higher seepage activity than those of the Iberian margin. Those studies mainly focused on their geological characteristics, whereas others offered novel information on their associated biota, especially the endosymbiont-bearing invertebrates as well as non-previously described species (Vanreusel et al., 2009). In 2014, the MV of the Spanish margin of the Gulf of Cádiz were included in the Natura 2000 network (Site of Community Importance - SCI "Volcanes de fango del golfo de Cádiz" - ESZZ12002) under the framework of the Life + project INDEMARES. Nowadays, the LIFE IP PAF INDEMARES project represents an opportunity for improving the knowledge on the biodiversity of this SCI. Samples and underwater images were obtained in MV and their adjacent bottoms from 300 to 1100 m water depth in the SCI, during the INDEMARES/CHICA and ISUNEPCA expeditions (Figure 1). Sampling was carried out using box-corer/Shipek, benthic dredge, beam-trawl and otter-trawl. Samples were sieved (10, 5, 1 and 0.5 mm) and individuals separated from the sediment, preserved in 70% Ethanol-4% Formaldehyde and identified. Transects with the ROV LIROPUS 2000 and photogrammetric sleds (APHIA 2012, HORUS) were done in different areas and completed the faunistic list with other species. More than 1000 species have been listed so far in the SCI, with molluscs displaying the highest number of species, followed by fishes, annelids, decapod crustaceans, cnidarians, sponges, echinoderms and bryozoans in decreasing order. Other groups are less represented such as brachiopods, sipunculids, nemerteans and ascidians or could not be studied yet (e.g. peracarid crustaceans). Recent revisions of some groups, mainly poriferans, bryozoans and molluscs, may result in the description of more than 10 new species to science such as the sponge *Myrmekeiderma indemaresi* (dedicated to the INDEMARES project) (Sitjà et al., 2011) or the bryozoan *Reteporella victori* (dedicated to the mud volcano specialist Victor Díaz del Río, Ramalho et al., 2011) (Fig. 2). Other species are new records for the area (the echinoderm *Hacelia superba*, the decapod crustacean *Calliax lobata* (García Raso et al., 2011), the molluscs *Pseudosimnia flava*, *Neopycnodonte zibrowii*, *Solemya elarraichensis*, *Lucinoma asapheus*, the fish *Zenion hololepis*, *Opisthoproctus grimaldii*). Around 20 species are included in local, national and international lists of threatened or vulnerable species, such as the cnidarians (*Madrepora oculata*, *Dendrophyllia cornigera*, *Lophelia pertusa*), echinoderms (*Centrostephanus longispinus*), molluscs (*Charonia lampas*) and fishes (*Centrophorus granulosus*). Also, an interesting 'ascidian garden' with the species *Polycarpa mamillaris* has been observed on carbonate structures, between 229 and 459 depth. Gazul, Pipoca and Chica displayed the highest species richness values, but Anastasya displayed low ones due to its extremophile sedimentary conditions with chemosynthetic-based communities and also the high trawling activity. The role of cold seeps and the formation of methane-derived authigenic carbonates for increasing biodiversity when compared to adjacent soft bottoms was noticeable in Gazul, Anastasya and Chica. A high number of species have been recorded in the SCI, as a result of the geological, geochemical, oceanographic and habitat heterogeneity of this area. The faunistic list of this area has increased considerably in 10 years from 30 spp. (Díaz-del-Río et al., 2009) to more than 1000 spp. (this study) due to the high sampling effort using different techniques targeting the infaunal, epifaunal and demersal species, new multidisciplinary sampling expeditions and the contribution of experienced taxonomists on different phyla, among other factors.

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Keywords: Biodiversity -, Gulf of Cádiz (10.5 - 5.5 W / 33.5 - 37.5 N), mud volcanoes, *Myrmekioderma indemaresi*, *Reteporella victori*

Regional and interannual recruitment variation of the stalked barnacle *Pollicipes pollicipes* in SW Europe by using a new artificial substratum

David Mateus, David Jacinto, Joana Fernandes, Alba Aguión, Gonzalo Macho, Katja Geiger, Caroline Broudin, Eric Thiébaud and Teresa Cruz,

The stalked barnacle *Pollicipes pollicipes* (percebe) is a sessile marine invertebrate that inhabits extremely exposed intertidal and shallow subtidal rocky shores along the Eastern Atlantic coast from Brittany to Senegal. This species is a very important shellfish resource and intensively harvested (mainly in the Iberian Peninsula) by recreational and professional fishers. Larval settlement and recruitment of *P. pollicipes* is very intense on conspecifics and has been mostly studied in the past by counting recruits attached to adult conspecifics collected in the field. This method for measuring recruitment of this species involves scrapping clumps of barnacles attached to the rocky substrate which impact intertidal habitats and natural populations. But this method has some subjectivity on it because it is very difficult to standardize the analyzed surface since individuals vary in length and width. The use of artificial substrata with a standardized area is an alternative to destructive sampling and allows to measure recruitment to a maximal age of juveniles (maximum time of deployment of substrata in the field). Previous studies have described null/low values of recruitment of this species onto artificial substrata. Recently, a new artificial substratum (hereafter named as barticle) was developed in Portugal and has proved to be efficient for larval attachment, survival, and growth of juveniles of *P. pollicipes* in the field. The aim of this study was to compare for the first time recruitment of *P. pollicipes* within its entire European distribution range (Brittany-France, Asturias-Spain, Galicia-Spain, and SW of Portugal) using barticles, along two recruitment seasons (2017 and 201). In this study, recruitment of *P. pollicipes* was measured on two years (summer of 2017 and of 201) in three (in 2017) and four (201) regions of SW Europe (150 to 1500 km apart). In each region, three shores were chosen (50 to 100 km apart) and three plots of twelve barticles were randomly deployed (5 to 50 m apart) in each shore, in the middle of intertidal distribution of *P. pollicipes* and close to natural clumps of this species. In each year, barticles were collected after approximately 3 months in the field during the peak of the recruitment season, and the cyprids and juveniles of *P. pollicipes* per barticle were counted. Regional and interannual variability of recruitment of *P. pollicipes* was detected and patterns will be shown and discussed.

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Keywords: Intertidal rocky shores, Cirripedia, Post-settlement processes, Artificial substratum, Atlantic Arc

Show us your beaks and we tell you what you eat: Different ecology in sympatric Antarctic benthic octopods under a climate change context

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Sympatry can lead to higher interspecific competition under climate change and other environmental pressures, and this may be particularly relevant in polar regions. This study focused in the ecology of two sympatric benthic Antarctic octopod species, *Adelieledone polymorpha* and *Pareledone turqueti*, highly abundant around South Georgia region located on the northern limit of the Southern Ocean. With a novel approach combining carbon (d13C) and nitrogen (d15N) isotope signatures, total mercury (T-Hg) analysis and biomaterials engineering techniques (Scanning Electron Microscopy, X-Ray Diffraction, Micro Computerized Tomography and Nanoindentation Test) applied to their beaks, we aimed to assess interspecific competition by evidencing the different beak adaptations that both species possess and estimate their niche overlap. An isotopic niche overlap of 95.6% was recorded during the juvenile stage (represented by upper beak's rostrum) of both octopod species, dropping to 19.2% during the adult stages (represented by the upper beak's crest). Our results also show that both species inhabit benthic ecosystems around South Georgia throughout their lifecycle (d13C: $-1.5 \pm 0.50\text{‰}$), whilst *A. polymorpha* and *P. turqueti* explore trophic niches partially different during adult life stages (d15N: $7.01 \pm 0.40\text{‰}$ and $7.4 \pm 0.65\text{‰}$, respectively). Besides differing greatly in morphology, *A. polymorpha* beaks' microstructure is less dense and significantly less stiff than *P. turqueti*, confirming our expectations of an adaptation to a more specific trophic niche. The T-Hg concentrations in flesh were lower in *A. polymorpha* ($0.322 \pm 0.0\mu\text{g g}^{-1}$) than *P. turqueti* ($0.434 \pm 0.12\mu\text{g g}^{-1}$). Beaks showed lower mercury uptake rates relative to flesh (*A. polymorpha*: $0.052 \pm 0.009\mu\text{g g}^{-1}$; *P. turqueti*: $0.03 \pm 0.009\mu\text{g g}^{-1}$). Overall, both species, although exhibiting similar habitats, have different trophic niches related to differences in morphology and function of beaks, and their digestive apparatus. In addition, due to mercury bioaccumulation throughout trophic levels, the high T-Hg concentrations in both octopod species might have negative consequences on Antarctic top predators that feed considerably on these organisms, an effect which will likely increase under the present climate change context.

Keywords: Sympatry, cephalopods, South Georgia, Stable isotopes, Mercury, Biomaterials

Spatial and seasonal dynamics of zooplankton assemblages in the Douro Estuary

Inês Duarte, Cláudia Mendes, Sérgio C. Costa-Dias and Adriano Bordalo

Introduction Zooplankton plays an extremely important role in aquatic food webs, representing a link from primary producers to higher trophic levels. Therefore, it is crucial to understand this compartment in estuaries in order to feed conservation and management strategies. The present study aims the understanding of spatial and temporal dynamics of zooplankton assemblages in the Douro Estuary. Location and methods The Douro Estuary is the end-member of the largest Iberian watershed. The freshwater flow is controlled by a network of 50+ large dams, the last one located just 21.5 km from the river mouth. For this study, a seasonal (spring-summer-fall-winter) sampling program was implemented in three locations corresponding to the lower ($41.14631^{\circ}\text{N}/66677^{\circ}\text{W}$), middle ($41.13990^{\circ}\text{N}/6149^{\circ}\text{W}$), and upper ($41.06945^{\circ}\text{N}/50057^{\circ}\text{W}$) estuary. At each sampling site, vertical tows were performed with a $150\mu\text{m}$ mesh net for zooplankton collection. Key environmental descriptors (salinity, temperature, dissolved oxygen, pH, turbidity) were measured with an YSI 6000 series multiprobe CTD. Zooplankton individuals were sorted into major taxonomic groups, namely Copepoda, Cladocera, Hydromedusae, Oykopleura, Polychaeta larvae, and fish eggs and larvae. Abundances were determined (individuals.m⁻³) for each taxonomic group. The Shannon-Wiener index H' was determined as a measure of diversity of the zooplankton community in the Douro Estuary. Main results The zooplankton community of the Douro Estuary presented low diversity, according to the Shannon diversity index ($H'=0.56\pm 0.15$) across all sampling locations and seasons. Furthermore, there was no consistent spatial trend of zooplankton diversity throughout the year. The diversity varied seasonally, reaching a minimum in spring ($H'=0.47\pm 0.11$), when no clear salinity gradient from the lower (0.1) to the upper (0.10) estuary could be observed. The highest

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diversity occurred during the summer ($H' = 0.67 \pm 0.17$), with a peak in the lower estuary ($H' = 0.6$), with salinity in the polyhaline range (> 1). The abundances of zooplankton also varied seasonally, reaching a peak in summer. Copepoda was the most abundant taxonomic group, representing over 60% of the zooplankton identified, followed by Cladocera (2%). The nauplius phases of Copepoda and Cirripeda represented an important component of the zooplankton community, especially during summer. Moreover, the structure of the zooplankton community followed spatial and temporal patterns. The Copepoda were the most abundant group from summer to winter, while Cladocera (54%) were more abundant than Copepoda (43%) in the spring. The Copepoda were more abundant in the lower and middle stretches, than in upstream areas. Conversely, Cladocera abundances were higher at the upstream areas. Conclusions This study contributed to the understanding of the zooplankton assemblages of the Douro Estuary. In summary, few taxa dominated the zooplankton community, namely Copepoda and Cladocera, whose distribution was determined by the salinity gradient. In the context of climate change, increasing drought due to low precipitation and exacerbated by the large number of dams in the Douro River, may disrupt the present seasonal and spatial salinity patterns. Therefore, the productivity and functional roles played by the different components of the estuarine food web, including zooplankton, may be compromised.

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Keywords: Plankton, Copepoda, Cladocera, diversity, estuarine

Spatial distribution of *Donax trunculus* (Linnaeus, 175) populations along the intertidal area of the Gulf of Cádiz (SW, Spain)

Sara Román, Raimundo Blanco. Pérez, Luis Silva, Ana Rodriguez-Rua, Miguel Cojan, Esperanza García and Marina Delgado

The wedge clam (*Donax trunculus* Linnaeus, 175) is widely distributed along Atlantic-Mediterranean exposed beaches at depths ranging from 0 to 6 meters. This species inhabits of the superficial fine sand biocenosis, with beach characteristics such as beach profile, wave energy, tidal range and grain size being important drivers controlling the distribution of the populations (Bayed and Guillou, 195). This species has high commercial importance on the southwest coast of Spain, and *D. trunculus* is harvested by licensed fishermen using hand-dredges in shallow waters (IDAPES, 2016). However, it is common to observe unauthorized fishermen hand-dredging and tourists hand-gathering this valuable fishing resource from beaches, making official statistics unreliable and making difficult stock assessment and resource management. Thus, knowledge of resource situation through estimation of abundance by direct methods is essential to improve suitable management measures, and for understanding the ecology of the bivalve beds. In this context, in the framework of the research project VENUS, the main objective of this study was providing a preliminary estimation of spatial distribution for *D. trunculus* in the intertidal areas along the littoral of Huelva to adopt optimal resource exploitation strategies compatible with its sustainability. The study area comprised the intertidal zone of the Huelva province coast. This area was divided into six sections of sandy beaches from Doñana to Ayamonte (i.e. between the mouths of the Guadalquivir and Guadiana rivers): Doñana, Mazagón, Huelva, Punta Umbría-La Bota, Isla Cristina-El Rompido, and Ayamonte. These beaches included different levels of anthropogenic pressure (fishing, tourism and pollution), fishing reserves and the protected area of Doñana National Park where fishing activity is under control. A total of 5 sampling sites, separated by 2 km, were sampled along the coast during low tide in June 201. Samples were collected from the lower part of the upper sublittoral area of the beaches. Sampling was performed using a hand-dredge similar to those used by local bivalve harvesters. The hand-dredge has an iron structure with a 44.5 cm wide opening that digs deeply into the sediment (upper 15 cm), using an experimental mesh size (3 x 3 mm) to allow for the collection of the smallest individual and juveniles. At each sampling site, hand-dredges were towed diagonally to the shoreline for 5 min, each transect being geo-referenced by means of a GPS. The number of retained individuals was recorded to estimate pop-

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ulation density (ind. m⁻²) and biomass (g m⁻²), and shell length (SL) with a digital vernier caliper to produce length-frequency distributions. Environmental variables such as sediment grain size and T^a were analyzed. A total of 1619 individuals were collected and analyzed from the beaches. In general terms, highest values (density and biomass) were reported on Doñana section (National Park), where fishing activity is controlled, particularly in Site (53.6 ind. m⁻² and 7.3 g m⁻²). On the contrary, low values corresponded to beaches with higher touristic and illegal fishing pressures which are clearly affected the wedge clam populations. These values were observed on Mazagón beach (touristic zone together with river mouths of the rivers Tinto and Odiel), in Site 24 (0.04 ind. m⁻² and 0.1 g m⁻²). Additionally, low values were also found on Ayamonte beaches where there is fishing zone (legal and illegal) and touristic pressure. Sedimentary classification ranging from coarse to fine sand. These maximum values reported from *D. trunculus* were related with fine sand and well sorted fine sediments being the minimum ones associated with medium sand and poorly sorted sediments. Regarding length-frequency distributions, protected areas together low fishing activity zones showed more complete population structure compared to other areas. These results highlighted the important role of protection measures on the population conservation management. In order to preserve this important resource monitoring and new management measure should take into account the different types of pressure: authorized and unauthorized fishermen hand-dredging and tourists hand-gathering.

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Keywords: *Donax trunculus*, sandy beaches, spatial distribution, Bivalves, hand-dredge fishery

Spatial partitioning of Zoantharian species (Suborder Brachycnemina) at intertidal rocky platforms of the Canary Islands

Sonia Fernández Martín, Cataixa López Batista and Sabrina Clemente Martín

Zoantharians (Cnidaria: Anthozoa) are benthic cnidarians commonly found in marine habitats, from intertidal rocky platforms to the deep sea and in tropical and subtropical marine regions worldwide. Members of the genera *Palythoa* and *Zoanthus* (Brachycnemina) are particularly ubiquitous in rocky coastal zones, where some species are able to cover extensive areas. Recent studies have demonstrated that zoantharians are more resistant than hard corals to habitat degradation, pollution, oceanic acidification and the ongoing ocean warming. In fact, some species, such as *Palythoa grandiflora*, have been able to rapidly increase their populations causing phase-shifts in degraded coral reef ecosystems (Cruz et al., 2016). Their fast growth rates due to the lack of carbonate in their body walls, combined with an effective asexual reproduction and the production of powerful palytoxins, are probably involved in such colonization success. However, the effects of the actual increase in zoantharians populations at subtropical and temperate zones, where main structural elements of ecosystems are macroalgae, are still unknown. Despite zoantharians are common sessile organisms, few

studies have paid attention to this group in the subtropical region of the Canary Islands. Some of them have focused only in their toxins or taxonomy (Fraga et al., 2017; López et al., 2019) and ecological studies are still scarce. Understanding the causes of marine communities' dynamics has gained the attention of many researchers interested in making species range predictions and their consequences for community structure. Given that zoantharians populations are predicted to increase northward in a climate change scenario (Durante et al., 2011), the aim of this study was to evaluate spatial partitioning among intertidal zoantharians populations, mainly considering their role in determining levels of macroalgae coverage which are main benthic ecosystem engineers in the Canary Islands. Surveys were conducted in 30 intertidal rocky platforms around the Canary Islands (Spain). At each location three belt transects (20 x 4 m) were set parallel to the coastline, in order to count the number of colonies of the different zoantharian species found. Moreover, four quadrats (50 x 50 cm) randomly placed at each transect were photographed to in depth examine relationships between percentage of coverage of zoantharians, macroalgae and sessile invertebrates, as well any relation with free space (sandy or rocky substrate) by means of correlations analyses. Also, the number of tidepools was evaluated as potential habitats available for zoantharian species. Finally, substrate rugosity and inclination were evaluated by means of testing their effect over zoantharian populations by means of a distance-based permutational ANOVA. Four Zoantharian Brachycnemina species were recorded along the transects throughout the Canary Islands: *Palythoa* aff. *clavata*, *P. caribaeorum*, *Zoanthus pulchellus* and *Isaurus tuberculatus*. Zoantharian assemblages studied in terms of species composition and their number of colonies, were not influenced by the inclination of the rocky platform ($F= 0.49$, $p= 0.654$), and not significant relationship was detected between the number of colonies recorded and the number of the tidepools available ($F= 2.52$, $p= 0.116$). These results are probably due to the fact that *P. aff. clavata* was the dominant species throughout the Archipelago and it seems to tolerate desiccation better than the other species, with several colonies found outside tidepools. Species-specific analyses showed a significant effect of substrate rugosity over abundances of *P. aff. clavata* and *Z. pulchellus* ($F= 4.54$, $p= 0.019$; $F= 6.99$, $p= 0.001$, respectively), with increased numbers of colonies among the higher levels of rugosity. Previous studies have found a positive correlation between substrate complexity and the colonization of corals, that mainly favour larval settlement and development, which may explain the greater abundance of zoantharians colonies (Carleton & Sammarco, 197). However, other factors such as shelter availability to cope with hydrodynamics or nutrient availability must be considered and may not be excluded as factors determining populations substrate partitioning. The abundance of zoantharians was not related to the coverage of other sessile invertebrates ($F= 1.41$; $p= 0.251$), probably because of the small sizes of the species found. Most of the organisms recorded, such as *Anemonia sulcata* and *Chthamalus stellatus*, are not large enough to show a negative effect by the settlement of zoantharians species in the substrate, since colonies seem to leave enough free substrate for their growth. In fact, the density of zoantharians was positive correlated with the percentage of substrate available (Figure 1), with higher number of colonies found in places with greater free-space. However, when other sessile organisms with the ability to cover extensive substrate areas occur, such as macroalgae, spatial competition seems to greatly increase. Large coverage of erect macroalgae may also hinder small zoantharian colonies from growing due to a shading effect on these zooxanthellate species. In fact, the coverage of zoantharians showed a negative correlation with macroalgae cover, showing lower densities when algae dominated the substrate (Figure 2). Among the most common algae sharing the substrate with zoantharians we found *Ellisolandia elongata*, *Jania rubens*, *Dictyota* spp., *Padina pavonica* and *Cystoseira* spp., as well as undetermined turf algae. Spatial partitioning and processes of competition for space between macroalgae and hard corals have been extensively studied (Cruz et al., 2016), but studies that involve zoantharians species are still scarce. Our results indicate that zoantharian species may not take advantages of all substrate free-space and are greatly limited by intertidal macroalgae communities. However, altered ecosystems, either by the effect of climate change that may favour zoantharian growth or locations with high abundances of grazers that may leave wide unvegetated extensions available for zoantharians, may favour zoantharian populations extensions in the Canary Islands.

Acknowledgements

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cf. *variabilis* on coral reefs. *Marine Environmental Research*, 115:2-35 Durante, L.M.; Cruz, I. & Lofuto, T. 201 The effect of climate change on the distribution of a tropical zoanthid (*Palythoa caribaeorum*) and its ecological implications. *Journal of the Marine Biological Association of the UK*, 95(2):301-309 Fraga, M.; Vilariño, N.; Louzано, M.C.; Molina, L.; López, Y.; Poli, M. & Botana, L.M. 2017 First Identification of Palythoxin like molecules in the Atlantic Coral Species *Palythoa canariensis*. *Analytical Chemistry*, 9(14):743-7446 López, C.; Reimer, J.D.; Brito, A.; Simón, D.; Clemente, S. & Hernández, M. 2019 Diversity of zoantharian species and their symbionts from the Macaronesian and Cape Verde ecoregions demonstrates their widespread distribution in the Atlantic Ocean. *Coral Reefs*, 3(2):269-23

Keywords: Climate Change, intertidal, Rocky platforms, Spatial partitioning, Zoantharians, Canary Islands

The effects of temperature on embryonic and larval development of the stone crab *Xantho poressa* (Decapoda, Brachyura)

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Temperature is known to largely influence survival and the total duration of larval development in many brachyuran species (Anger, 2001). Animals in the intertidal zone are subject to environmental challenges posed by both aquatic and aerial climatic regimes, and thus intertidal crabs are model species for the effect of climate change (Helmuth et al., 2006). Previous investigations on a number of crab species highlight that temperature has an effect on the survival and development time of larvae (Marco-Herrero et al., 2016). However, the information on the influence of temperature on survival, growth and variability of the number of larval instars in *Brachyura* is limited. The aim of this research was to analyse the effects of temperature, on the duration of embryonic development, and survival and duration of larval development of *X. poressa*.

Tests were done through laboratory experiments under controlled conditions of salinity (35), photoperiod (12:12), and food (freshly mixed of *Brachionus plicatilis* and hatched *Artemia nauplii*), at three temperature treatments (17, 21 and 25°C). Six ovigerous females were collected in the intertidal rocky shore from Torregorda beach (Cadiz, Spain) and maintained at ambient temperature and light-dark regime until hatching. From each female, 24 active swimming larvae were reared in individual vials where water and food were changed every day, and moults and dead larvae were recorded daily. For brood development experiments, six females per temperature treatment (18 females total) were maintained under the same salinity and photoperiod conditions. Each crab was kept in an individual tank with oxygen and monitored every two days.

Larval survival rates was higher at 25°C than at 17°C and 21°C, and varied from 47,92 ± 27,98% at 17°C, 52,08 ± 36,49% at 21°C and 63,19 ± 35,59% at 25°C. At 17 °C, none of the megalopae moulted to the first juvenile crab. Meanwhile, at 21°C and 25°C, the average megalopal survival rates were 4,86 ± 8,09% and 32,64 ± 23,19%, respectively. Most of the larval mortality occurred during the moulting process to the second zoea and megalopa stages (the two bottlenecks in larval developments) and varied between larvae hatched from different females (see standard deviations in figures). Larval development was completed until zoea IV in the three temperatures and an additional stage (Zoea V) was observed at the lowest temperature treatment 17°C from some females. From this extra stage, some larvae moulted to the megalopa stage although they did not survive to the first juvenile crab. The total duration of larval development was longer at 21°C and decreased at 25°C and varied (in days) from 19,67 ± 2,08 (21°C) and 15,02 ± 0,68 (25°C). At the lowest temperature treatment (17°C), zoeal development time was 36,62 ± 1,43 days (from zoea I to zoea IV) and 42,16 ± 1,18 days including zoea V (Figure 1). The duration of the intermolt periods at 25°C was similar to the days reported for Rodríguez and Martín (1997), although these authors reared larvae at 26°C. Mean brood incubation period was longer at 17°C than at 21°C and 25°C and varied from 23,4 ± 1,92 days at 17°C, 12 days at 21°C and 8,66 ± 1,15 days at 25°C (Figure 2). At the three temperatures, some females lost their broods before the embryos were fully developed.

The data indicate that temperature affects brood incubation, larval development time and survival in *X. poressa*. Our results point to important maternal influences on survival and development. The additional zoea stage V is probably related to the interactive effects of unfavourable culture temperature conditions and maternal influences through quantity or quality yolk.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Keywords: Xantho poressa, temperature, larval survival rate, larval development time, Brood development time

Tolerance to salinity changes in four species of Veneridae Bivalvia, a laboratory assay

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Estuaries and coastal areas close to river mouths and fluvial discharges are strongly influenced by flooding, temperature and salinity. Climate changes may induce alterations in these factors (salinity reduction, ocean surface temperature increases and sea level rises) and consequently may induce alterations on benthic communities that inhabit these ecosystems. In the last decades, the Iberian Peninsula climate has undergone significant changes translated in the occurrence of extreme weather events, altering precipitation patterns and seasonal temperature. Because of this, intertidal faunal species, as bivalves, can be affected by changes in salinity. The main goal of this study is to evaluate the response of four clams submitted to different salinities. Specifically, *Ruditapes decussatus* (Linnaeus, 175), *Ruditapes philippinarum* (Adams and Reeve, 150), *Chamelea gallina* (Linnaeus, 175) and *Venerupis aurea* (Gmelin, 1790). *R. decussatus* is a burrowing bivalve largely distributed on the Atlantic coast, from England to Mauritania and is found in the Mediterranean Sea. It is cultivated in the coastal lagoons near the Gulf of Lyon and in the Venice Lagoon. Natural populations of *R. decussatus* can be found in sandy and muddy-sand sediments in bays, estuaries coastal lagoons and other sheltered environments, living on tidal flats and below tidal marks. *R. philippinarum* is native to the Indian-Pacific region, but due to its high growth rates and its ability to tolerate a wide range of environmental conditions, it was introduced into European Atlantic and Mediterranean coastal waters for commercial cultivation, occupying a habitat that overlaps that of the native species *R. decussatus*. The striped venus *C. gallina* occurs in shallow waters from intertidal areas to 20 m depth, preferentially with well sorted fine sandy bottoms. *C. gallina* distributes on the eastern Atlantic coast, across Norway and the British Isles, Iberian Peninsula, Morocco, Madeira, and the Canary Islands. It is also found in the Mediterranean, Black Sea and Adriatic seas. *V. aurea* occurs on the eastern Atlantic coast, from Norway to Morocco and in the Mediterranean and Black seas. It lives in a variety of sediment types from gravel to muddy bottom and it can be found from intertidal area to 36 m depth. This study aims to evaluate the response of these bivalve species submitted to different salinities. Six assays of 21 days for each species were performed using tanks with 15 L of filtered seawater and diluted to salinities 0, 1.9; 5.9, 10.9, 15.9, 20.9, 25.9, 30.9 and 35.9 g/L. The water was continuously aerated and maintained at a constant temperature (20.3 °C) in a germination chamber. For each tank, six specimens of two species were placed. Clams were fed every 2 days using a mixture of algae *Isochrysis galbana* Parke, 1949 and *Chaetoceros gracilis* Schütt, 195, diluted in water with the same salinity than the treatments. Mortality was registered every 12h. For data analysis, median lethal salinity to 21 days (LC50) and median lethal time for each salinity (LT50) were determined from Probit analysis. The results showed that mortality in *R. decussatus*, *R. philippinarum* and *C. gallina* was higher than 90% in the salinities among 0-10.9 g/L. Mortality in *V. aurea* was lower than 90%, only in the salinity trial of 35.9 g/L. It seems to be much more sensitive to salinity changes or to remain in laboratory conditions. Regarding the survival shown by the species, *R. decussatus* has a greater resistance capacity, in fact mortality values were lower than 10% in all the assays with salinities higher than 15.9 g/L, while *R. philippinarum* and *C. gallina* need to be in salinities higher than 20.9 g/L and 30.9 g/L, respectively, to reach this survival values. Significant differences in mortality mean values among species in each salinity were observed. However, these differences did not occur throughout all the experimental period, but they appeared at varying intervals according to salinity. With low salinities (0-15.9 g/L), significant differences were observed from 36 hours after the start of the experiment to 240-34 hours. Since then, the distribution of mortality remained similar among species. In salinities above 20.9 g/L, differences were observed from 60-4 hours from the beginning until the end of the assays. The values of LT50 for each salinity were always higher in *R. decussatus*, mainly in salinities lower than 10.9 g/L. *V. aurea* reached the highest values of LT50 for all salinity assays. LC50 values for each time also showed differences among the species. E.g. 10 days after the

start of the assays LC50 for *R. decussatus* was 2.5, while it was 16.5 and 10.5 for *R. philippinarum* and *C. gallina*, respectively. At the end of the experiments (21 days), LC50 values were 16.7, 1. and 24.1 for *R. decussatus*, *R. philippinarum* and *C. gallina*, respectively.

Keywords: Salinity effect, Bivalves, intertidal, Mortality, Lethal concentration (LC50), Lethal time (LT) 50

Trophic plasticity in *P. lividus*: herbivory, detritivory and omnivory as a function of resource availability

Judith Camps, Javier Romero and Patricia Prado

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 and foraging experiments, stomach contents and stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) were conducted in the Alfacs Bay (Ebro Delta). Our results show that sea urchins strongly prefer *A. verticillata* over *C. nodosa* and *C. prolifera* (Fig. 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 (Fig. 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.

Keywords: *Amathia verticillata*, Seagrass beds, *Pinna nobilis*, *Paracentrotus lividus*, Pseudo-indigenous species

Updated checklist of the marine gelatinous macrofauna from the Azores

Bruno I. Magalhães, João M. Gonçalves and Carlos J. Moura

The marine macroplankton and micronekton fauna of the Azores, which includes gelatinous creatures, have been little investigated, despite their recognized importance in trophic interactions, both as preys or predators. Probably, such lack of knowledge reflects the difficulty of sampling these fragile animals, associated with a lack of taxonomic expertise. The Azorean jellies may thus be referred as “forgotten fauna”. Jellies are characterized by the gelatinous transparent body with high water levels and low carbon content. These oceanic drifters, ranging from small to large-sized organisms or colonies, comprehend several taxonomic groups of invertebrates: cnidarians (hydromedusae, scyphomedusae and siphonophores), ctenophores and pelagic tunicates (appendicularians, doliolids, pyrosomes and salps). Some jellies are voracious predators of zooplankton (competing for food with fish) and ichthyoplankton (eggs, larval and juvenile stages of fish), affecting recruitment of commercial important species. Therefore, they may severely impact fisheries. On the other hand, many jellyfish species may represent an important component of the diet of several species with commercial interest or sensitive conservation status, such as the loggerhead sea-turtle *Caretta caretta* and the sunfish *Mola mola*, which are specialized predators of these organisms. Additionally, for example salps are efficient filter-feeders, which may consume most of primary production in short time periods, responding quickly to microalgae blooms. Some gelatinous species with stinging capability may severely affect tourism activities. We therefore intended to update the checklist of the marine gelatinous macrofauna occurring within the Azores

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EEZ (exclusive economic zone). We reviewed and analyzed the occurrence of gelatinous macrofauna biodiversity in the Azores Sea, through available records in scientific literature, taxonomic collections, databases of photographs and video footages, and online digital platforms. Our updated list of jellies occurring in the Azores comprehends around 0 species (cnidarians, ctenophores and chordates), ranging from very common species as the mauve stinger (*Pelagia noctiluca*) and the Portuguese man-o-war (*Physalia physalis*), to more unusual species such as pyrosomes (*Pyromoma* sp.) and deep-sea medusae (*Deepstaria enigmatica*). To the best of our knowledge, the first report of jellies for the Azores was given by Maas (1904), who curiously registered three scyphozoan species (*Atolla wyvillei*, *Periphylla periphylla* and *Aurelia aurita*) not that common, presently, in the archipelago. Some important contributions to our knowledge on Azorean jellies were provided in the last half of the last century, highlighting works of Kramp (1959), Baker (1967) and Soest (1973). The most recent species checklists including pelagic jellies in the Azores reported solely a total of twenty two species (Morton et al. 199, Costa 2010, ImagDOP 2013, SRMCT 2014). Although, our own investigations of total reports/sights indicate: 41 species of Hydrozoa (nine Anthoathecata, 10 Leptothecata, nine Narcomedusae, 13 Trachymedusae), seven Scyphozoa (four Coronatae and three Semaestomeae), 21 Siphonophora (16 Calycophorae, one Cystonectae, four Physonectae), four Ctenophora (one Nuda and three Tentaculata), two Thaliacea (one Pyrosomatida and one Salpida). While we expect much more biodiversity to be found in the archipelago, the hydrozoans and siphonophores thus seem the most speciose groups of jellies in Azorean waters. This study has relevant impact in fisheries and tourism, representing an important leap forward towards the comprehension of the gelatinous faunistic diversity and associated dynamics in Azorean waters.

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Keywords: jellyfish, Macroplankton, cnidarians, Ctenophores, pelagic tunicates

Effects of hydrodynamic conditions and feeding period on size, growth and cirral behavior of the stalked-barnacle *Pollicipes pollicipes*

Maria Inês Seabra, Maria Santos, David Jacinto, David Mateus, Joana N. Fernandes, Ana Pombo, Teresa Silva, João J. Castro and Teresa Cruz

The stalked barnacle *Pollicipes pollicipes* is abundant in extremely wave-exposed rocky intertidal habitats along the Northeast Atlantic coast, being rare in sheltered conditions. Environmental hydrodynamics might

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shape barnacle assemblages by affecting individual filter-feeding behaviour and growth. A recent study performed under laboratory conditions indicated that hydrodynamic conditions did not affect survival, but had consistent effects on size and growth of *P. pollicipes*, which grew faster at high turbulence. In that previous study, barnacles had been fed daily with *Artemia nauplii* during 2 hours (feeding period) and hydrodynamic conditions (turbulence) had been manipulated through the use of a flow pump according to three treatments: low" - no pump; "feed" - pump turned on only during feeding period; and "high" - pump permanently turned on. Results from the described experiment could be explained by the enhancement of respiratory efficiency due to cirral extension under high turbulence, and consequently by a higher allocation of energy to growth. The aim of the present study was to test if cirral behaviour, size and growth are affected by hydrodynamic conditions and by the length of the feeding period. In October 2011, wild juvenile barnacles, that were fixed on artificial substrata (barticles) deployed in July 2017 on the intertidal zone of a very exposed rocky shore (Cape of Sines, Portugal), were collected, counted, marked with calcein and randomly allocated to twelve well-aerated seawater aquaria. Hydrodynamic conditions were set-up by using a flow pump to increase water turbulence within each aquarium. The following experimental orthogonal design was considered: turbulence (fixed factor, 4 levels: "off" - pump permanently turned off; "feed off" - pump turned off only during feeding periods; "feed on" - pump turned on only during feeding periods; and "on" - pump permanently turned on); feeding period (fixed factor, 3 levels: 2h feeding period followed by 2h immersed period; 2h feeding period followed by 2h emerged period; 4h feeding period). *Artemia nauplii* at a concentration of 2 nauplii per ml was supplied daily to all treatments. One aquarium was randomly allocated to each of the twelve conditions. Each aquarium contained 6 barticles with an average of 12 barnacles per barticle. On December 2011, all barticles were removed for analyses of barnacle size (maximum rostrum-carina distance, RC) and growth. Individual growth was measured as the monthly increment of RC relative to the calcein mark. Additionally, on 6 dates during the experimental period, behaviour of the cirri was investigated by filming barnacles attached to a barticle during periods of 2 minutes before the feeding period and 2 minutes during the feeding period. Results will be analysed and discussed.

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Keywords: Marine aquaculture, Optimization of growth, Stalked-barnacles, Cirripedia, water flow

Four-spot megrim (*Lepidorhombus boscii*) in Atlantic waters: growth parameters and age corroboration in Porcupine Bank

Carmen Hernández and Jorge Landa

The four-spot megrim (*Lepidorhombus boscii*), a flatfish distributed in European waters, has relevant commercial importance in the bottom fisheries of Spain and Portugal. Although the biological aspects and state of its Iberian Atlantic population are annually studied, information from northernmost waters is lacking. In recent years, probably influenced by climatic variations, the size of these northernmost populations of *L. boscii* (northern Bay of Biscay, Celtic Sea, west of Ireland) has increased, with annual catches of about 600 t (~ 6 M €) of which Spain captures around 70%. ICES (International Council of the Exploration of the Sea) requires information of the biological aspects of these populations, mainly growth, to be able to properly assess its stock state, allowing an adequate management of this resource. The present work studies the age and growth of *L. boscii* in one of the main fishing areas for the Spanish fleet and relevant in the European demersal fisheries, the Porcupine Bank (west of Ireland). The growth parameters obtained are here compared with those from other areas, mainly Bay of Biscay and Portuguese waters, offering a broader knowledge of the global growth of the species. These parameters obtained are available for the upcoming analytical stock assessment. The age and growth of *L. boscii* is here estimated based on the interpretation of the translucent bands (annuli) of the sagittal otoliths, following internationally standardized protocols. Otoliths were obtained from specimens caught in five annual bottom trawl research surveys (200-2012) carried out by the Instituto Español de Oceanografía (IEO). The age interpretation consistency is here evidenced by analyzing the regularity of the distances of the annuli to the otolith primordium and by the back-calculation of the annuli. The growth patterns ob-

tained from direct otolith age estimation and from back-calculation are also compared. The tracking of the strength of the year-classes in the survey abundance matrix at age and year of *L. boscii* allows corroborating the age estimation and showing the preponderance of the abundant 200 year-class.

Keywords: Age Estimation, Growth, *Lepidorhombus boscii*, Northeast Atlantic, Porcupine Bank, otolith

Growth performance and feed intake of the juvenile sea urchin *Paracentrotus lividus* fed on three diets

Silvia de la Uz, Jacobo López¹, Natalia Pérez¹ and Carmen Rodríguez¹

The progressive depletion of sea urchin populations, due to overfishing, habitat destruction and natural diseases, reinforces the need for effective aquaculture practices. This interest in sea urchin aquaculture increased in the last decades and produced an important number of studies aimed to improve the rearing conditions. However, most research attention was focused on the development of effective feeds for the grow out of adults promoting and enhancing the gonadal production. The feasibility of producing adult sea urchins is still hindered by the availability of excellent feeds, able to guarantee the fast growth of juveniles. Therefore, there is a great need for improving diets in order to accelerate somatic growth and reduce rearing time to market size. Traditionally, the aquaculture of sea urchins have depended exclusively on seaweeds found locally. The macroalgae species are not permanently available throughout the year and their chemical composition change according to the season. Alternatively, formulated feed provide constant availability and nutritional composition. Feed formulations are essential to aquaculture but proteins from animal sources are relatively expensive and not sustainable. For these reasons, modern aquaculture is turning to alternative products, in particular land vegetable products, which are constantly available and more sustainable. Spinach has emerged as one of the best options for alternative ingredients. Its use as source of antioxidant has already been proved in gonadal growth, but its effectiveness has not been proved in somatic growth. The aim of the present study was to evaluate the effect of three diets on feeding and growth rates of juveniles of the purple sea urchin *Paracentrotus lividus*. We tested three different diets: the first composed of the brown algae *Saccorhiza polyschides*, kelp fronds were collected on the beach and stored frozen until they were used; a second diet based on pellet normally used in aquaculture for abalone, and finally, a land vegetable diet based on spinach. Hatchery produced *P. lividus* were reared at the Centro de Experimentación Pesquera in Asturias, Spain. In the study were used 360 six-month-old juveniles (14.9±0.9 mm; 1.7±0.3 g) from the same cohort. Sea urchins were starved for 3 weeks to ensure acclimatization and uniform nutritional status. Three diets were tested: a macroalgal diet (M) composed of frozen kelp fronds, a formulated diet (F) based on dry pellet, and a vegetable diet (V) based on fresh spinach leaves. Each one of the three diets was randomly assigned to triplicate batches and each batch was fed twice a week. The feed ration was adjusted depending on the absence or presence of non-ingested feed. The experiment was conducted for 1 weeks and biometric measurements of test diameter and body weight were taken for each sea urchin every 6 weeks. The food intake of the three diets was calculated once in each sampling period. Uneaten food was removed and dried in oven at 105°C until a constant dry weight. Mean consumption per sea urchin per day (mg) and mean daily consumption as percentage of body weight (%) was calculated for the number of surviving sea urchins per treatment. The mean water temperature gradually increased from 15.2±1.2°C in the first period to 16.5±1.1°C in the last one. All diets were well accepted by sea urchins and no significant difference was found in the survival between the different treatments. There was 100% survival in the three diets during the 1 weeks-experimental period. There were not significant differences in either diameter or weight of the sea urchins between treatments at the beginning of the experiment. But there were significant differences in both test diameter and wet weight in all subsequent sampling dates. The mean final test diameter was 20.±1.5 mm (M), 21.1±1.4 mm (F) and 25.2±2.1 mm (V), with a significantly greater test diameter exhibited by sea urchins of V diet compared with M and F groups. No significant difference in the final test diameter was recorded between the sea urchins fed on M and F diets, but the sea urchins fed on formulated feed had a significantly higher final weight (4.4±0. g) than sea urchins fed on macroalgae (3.9±0.7 g). The highest final weight was obtained, once again, by sea urchins fed on spinach (6.3±1.4 g). Feed intake, expressed in dry weight, varied substantially depending of the food provided. Overall, ingestion rates were greater with the artificial diet (F) than for the other two natural diets (M and V). The maximum rate of feeding was reached by F diet in the first sampling period, decreasing in subsequent periods. By contrast, daily consumption of M and V diets increased progressively with the size of the sea urchins throughout the experimental period. In the present study, the results demonstrate that the diet based on land vegetable was the most successful diet in promoting the growth of sea urchins. The good

performance of the juveniles fed on spinach, regarding test diameter (1.75 µm day⁻¹) and total wet weight (36.5 mg day⁻¹), suggest that spinach is a very promising ingredient for sea urchin feed in order to maximize the somatic growth of *P. lividus* juveniles.

Acknowledgements

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Keywords: *Paracentrotus lividus*, sea urchin, Juveniles, feeding, Growth

Morphological and quality variation of the stalked barnacle *Pollicipes pollicipes* (Gmelin, 1790)

Alina Sousa, David Jacinto, Nélia Penteado, Diana Pereira, Teresa Silva, João J. Castro and Teresa Cruz

The stalked barnacle *Pollicipes pollicipes* can be considered the most important ecological resource on rocky shores of North Spain and Continental Portugal [1], [2], and has a high commercial value [3]. *P. pollicipes* inhabits very exposed rocky shores from Brittany (France) to Dakar (Senegal)[4]. There is an overall perception, namely by the fishers, that there is variability of the quality of *P. pollicipes*, namely by the existence of thinner and longer barnacles that have a lower quality. Quality variability is important as low quality barnacles have a lower commercial value. The main objective of the present study was to address the complex issue of the existence of different morphologies/qualities of *P. pollicipes* by evaluating the perception of the problem by the harvesters, and by analysing the morphology of the barnacles and its biochemical composition in three Portuguese regions ("Reserva Natural das Berlengas" (RNB), the coastal area between Cape Carvoeiro and Cape Raso (CENTER) and the "Parque Natural do Sudoeste Alentejano e Costa Vicentina" (PNSACV)). Finally, a manipulative experiment was performed to test the effect of putative factors causing this morphological/quality variation. The harvester perception was evaluated by performing individual interviews to professional barnacle fishers from the three regions CENTER(n=26), RNB (n=32) and PNSACV (n=49) in 2013. Most of the harvesters of the three regions considered that there are barnacles with different qualities and have defined barnacles with a lower quality the ones that were "thin", "long", with "high water content" and with "few muscle". A collection of 10 stalked barnacles with different morphologies/qualities was shown to the fishers and it was asked that they classify the barnacles according to their quality. All the barnacles from the collection had a same maximum distance between the rostrum and the carina plate (RC), but the ratio between the maximum distance between the base of subcarina and the base of the subrostrum (CB) and their maximum length (TL) was different and was used to describe the barnacle quality (lower values of CB/TL were indicative of a lower quality). More than 70% of harvesters of the three regions correctly identified the three good quality barnacles (CB/TL~ 0,43) and the three lower quality barnacles (CB/TL ~ 0,17). In order to analyse the morphometry (CB/TL) and biochemical profile (e.g. water content, fat content, total protein) of low and good quality barnacles, *P. pollicipes* of both qualities were collected by members of the research team and by professional harvesters in two sites of each region (RNB, CENTER and PNSACV). Analysis of variance to CB/TL revealed no significant effect of type of collector and of site, but a significant effect (P<0.05) of quality. Good quality barnacles presented an average value of 0.35 in the CB/TL ratio, while the low-quality ones had an average value of 0.23. Regarding the biochemical analysis, the results showed that the low quality barnacles had a lower fat and total protein content. The manipulative experiment was performed at PNSACV, where "low quality barnacles" were transplanted to "good quality barnacles" habitat and vice versa, as well, as the density of barnacles (a putative factor to affect morphology) was artificially reduced in areas with "low quality barnacles". One year after the set-up of the experiment, the most relevant result detected was that "low quality barnacles" that were transplanted to "good quality barnacles" habitat became larger and shorter than control ones.

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Keywords: Stalked barnacle, quality, Morphology, Portugal, *Pollicipes pollicipes*

Reproductive and population aspects of *Macra stultorum* (Linnaeus, 175) in the littoral of Huelva (SW Spain)

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The surf clam *Macra stultorum* (Linnaeus, 175) is widely distributed in sandy bottoms at depths ranging between 1 and 30 m along the Black Sea, Mediterranean Sea and Atlantic Ocean, from France to Senegal. This species is part of the accompanying fauna of commercial species such as *Chamelea gallina* and *Donax trunculus*. *M. stultorum* is presented with two different and well-defined morphotypes, easily distinguishable by the colour of the shell: the white variant and the brownish radiating bands variant (Guarniero et al., 2010). Despite its abundance and commercial importance in many countries, no studies regarding its reproductive cycle have been carried out to date in the Atlantic coasts. The aim of this study is to provide information on aspects of the gametogenic cycle and the population structure of *M. stultorum* in its two morphological variants by means of histological analysis, physiological indices and length-frequency distribution. To achieve this objective, populations of *M. stultorum* in its two variants were monitored during one year. Samples were collected monthly from the lower part of the intertidal area of Doñana beach (36°42'06"N/6°23'29"W) using two hand-dredges, one with an experimental mesh size (3 x 3 mm) to allow the collection of the smallest individuals and juveniles and the other with a commercial mesh size (7x7 mm). Shell length (antero-posterior axis) of retained individuals was recorded using a digital vernier caliper to the nearest 0.01 mm to produce length-frequency distributions. Additionally, twenty five individuals for each morphotype were sampled monthly for histological analysis. A piece of visceral mass was taken for each individual and fixed in buffered formaldehyde (4%), dehydrated through ethanol series and subsequently embedded in hydroxyethylmethacrylate (Technovit 7100). Sections (5 µm thick) were cut and stained with Harris Hematoxylin and eosin (H-E). Histological preparations were observed under a light microscope (Nikon Eclipse 90i) and the stages of gonad development were scored according to the scale proposed by Delgado et al. (2013) for *C. gallina*. To determine the condition index twenty five individuals for each morphotype were sampled per month. Body soft parts and valves were separated and dried at 100° C during 24 h (until constant weight). Soft tissues and valves were then weighted with a precision balance to the nearest 0.0001 g. The index used was: CI = Soft tissue dry weight/Valves dry weight Data on surface sea water temperature was collected in situ and samples of water were taken for chlorophyll a determination. The reproductive cycle of marine bivalves is influenced by a number of environmental factors including temperature and food availability. Water temperature showed high fluctuations along the study year, with maximum values in August (26°C) and minimum in December (10°C). The histological results suggest that spawning occurs during spring and summer when sea water temperature was higher. The condition index showed a similar pattern, with a high peak in spring and a decrease during the summer that can be related to a spawning event. The sexes in *M. stultorum* were clearly separate and sexual dimorphism was not observed. No significant differences between both morphotypes were found. These results are in concordance with a previous study carried on *M. stultorum* from Tunisia (Chetoui et al., 201). Several cohorts were identified in the monthly length-frequency distributions throughout the study period.

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Keywords: *Macra stultorum*, length-frequency distribution, Gametogenic cycle, Huelva (Spain), condition index

Reproductive characteristics of European anchovy, *Engraulis encrasicolus* (L.), off Western Iberia

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European anchovy, *Engraulis encrasicolus* (L.), has exponentially increased its abundance off the Western Iberia in recent years to maximum historical levels. The stock component was assessed by ICES for the first time during 201, but the biological data of the species in this area is very scarce due to the low abundance in the past. The correct maturity stage is crucial to an accurate calculation of maturity ogives (and therefore of the SSB). Misclassification of immature and/or resting (mature) individuals will lead to a sub- or overestimation of the proportion of these maturity stages and consequently the spawning stock biomass. In this work, samples of European anchovy were monthly collected in the western Iberia during 201 and 2019. Histological sections of male and female gonads were analysed in order to i) describe the seasonal reproductive activity of the species and ii) microscopically identify immature from resting stages. Macroscopic and microscopic maturity ogives are also presented and its implication in estimating the spawning biomass is evaluated.

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Keywords: European anchovy (*Engraulis encrasicolus*), Maturity stages, Histology, Maturity ogive, Reproductive season, Western Iberia

Spatiotemporal distribution and growth pattern of *Venus nux* (Gmelin, 1791) in the Gulf of Cádiz (Southern Spain)

Marina Delgado, Ana Rodríguez-Rua, Luis Silva, Esperanza Garcia, Miguel Cojan, Sara Roman and Raimundo Blanco

The venerid clam *Venus nux* (Gmelin, 1791) is a species distributed along the Atlantic from southern Portugal to Senegal, and is also present in the Mediterranean (Poppe & Goto 1993). Dense populations seem to be restricted to the Alboran Sea and the Ibero-Moroccan Gulf (southern Spain), usually inhabiting muddy sand bottoms from 30 to 350m in depth (Salas 1996). The decline of catches of commercial bivalves in southern Spain has pointed out an overexploitation of these resources. For this reason, a new research line of shellfish resources have been opened in the last years among them this venerid *V. nux* (Tirado et al., 2011). In this sense, Tirado et al. (2011) have studied the reproductive cycle of Atlantic and Mediterranean populations of *V. nux* from southern Spain. The main objectives of the present work are intended to complete this previous study, an particularly were (i) to analyse the spatiotemporal distribution and (ii) to provide an updated age-length key for *V. nux* from the Gulf of Cádiz (Atlantic waters). First the spatiotemporal distribution of this species was investigated using time series data from trawl surveys conducted in the Gulf of Cádiz. The geographic and bathymetric variability in the population distribution was analyzed. Secondly, an in-depth analysis of the shell and updated estimation of growth parameters of the *V. nux* population were performed using thin-layer cross section methodology. Data on species abundance are derived from 1993-2015 monitoring bottom-trawl surveys (ARSA surveys) conducted to evaluate demersal habitats of the continental shelf and slope of the Gulf of Cádiz. We calculated spatial indices of location in longitude, latitude and depth (i.e. center of gravity, CG) to explore and analyze trends in the 21 yr time series of scientific surveys for each year as described by Woillez et al. (2009) and using the RGeostat package in R software (Renard et al., 2014; R Core Team 2015). CG is the mean location of a population and also the mean location of an individual clam taken at random in the field (each data location was weighted by clam density) The inertia is the variance of the location of individuals in the population, that is, the mean square distance between an individual clam and the centre of gravity of the population (Bez, 1997). Additionally, a total of 40 *V. nux* individuals (size range: 20-43 mm) were collected from 2017. Shell length, height and width from each individual were measured to nearest 0.1 mm with a digital vernier caliper to produce length frequency distributions and to estimate relative growth. To determine the species microgrowth pattern by means of the thin-layer technique, a modified protocol of Schenk et al. (194) for calcified tissues was applied. The left valve of each clam was embedded in poly methyl methacrylate, and thin sections from the umbo to the ventral edge of 100 µm thickness were obtained using low-speed diamond (ISOMET 4000) and grinding and polishing with sequential grinders (sandpaper from 120 to 1 µm) (Delgado et al., 2017). After the identification of annual growth bands, the distance between the umbo and the annual ring was measured using the image analysis software NIS-Elements AR 3.2. Because these measurements are relative to shell height, they were transformed into shell length using the morphometric relationship. Figure 1 shows the spatial distribution of *V. nux* densities as well as the center of gravity (CG) and inertia (latitude and longitude) in 2015. Geographical range distribution varied between 36°53'40" and 36°25'31" in CG latitude, and -6°35'3" and -7°5'43" in CG longitude. Figure 2 displays the time series of changes in the center of gravity in depth. CG depth varied between 66 and 103 m and a trend towards deeper waters can be observed. The morphometric relationships between shell height (SH), shell width (SW) and shell length (SL) were: SH = 1.096 SL 0.936 (r²: 0.94) and SW=0.37 SL0.937 (r²:0.5). The embedding in poly methyl methacrylate and the subsequent procedures of sectioning, grinding and polishing until obtain thin sections allowed for highly detailed direct observations and analysis of the inner structure of valves. *V. nux* shell growth pattern is printed in the external prismatic layer. Growth bands were clearly visible in sections of analyzed shells (Fig. 3). Wide translucent zones alternate with opaques zones, probably showing the alternation of fast and slow growth periods (Fig. 3). Translucent growth lines were also present either on the umbo or close to the edge.

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Keywords: *Venus nux*, spatiotemporal distribution, Gulf of Cádiz, Growth pattern, geostatistics

The PERCEBES project: science for the spatial management of the stalked barnacle fishery in the Atlantic Arc

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In the Atlantic Arc, the stalked barnacle fishery is managed using a variety of spatial tools ranging from open access, through Territorial User Rights for Fishers (TURFs) to Marine Reserves (MR). In addition, co-management in some places allows the emergence of new management practices, which makes this fishery an interesting example to investigate the implications of distinct spatial management options on the biology and ecology of exploited coastal species. It also provides a learning platform to guide marine spatial planning of Small Scale Fisheries in other contexts in the EU. The PERCEBES project is a Portuguese, French and Spanish initiative co-funded by the ERA-Net BiodivERsA intended as a scientific and practical demonstration of the effects of Stalked Barnacle (SB) harvesting on the biodiversity, productivity and connectivity of SB populations/stocks? both at local and landscape level. This is done through two approaches: 1-A continental-scale, Human Exclusion Experiment (HEE). By using steel cages that simulate the effects of 1 and 2 year bans and open plots as controls where harvest continues unimpeded. The HEE tests the effect of those treatments on the biodiversity, productivity and economic value of SB stands and on their potential to produce larvae. 2-Construction of regional, spatially explicit Bioeconomic Models (BM). We are building biophysical larval dispersal models to visualize the seeding effects of fallow or protected areas on other regions and the patterns of connectivity among managed or co-managed spatial units. These models are being validated by direct measurement of recruitment distributed in time and space. Additionally, the biophysical models will be coupled with landings and sales data to assess the sustainability and cost effectiveness of distinct spatial management options. These two objectives are carried out at four experimental sites: coast of Alentejo (Portugal), NW coast of Galicia (Spain), Western Asturias coast (Spain) and South Brittany (France), covering the latitudinal range where the barnacles are exploited in the EU. PERCEBES benefits from/has the collaboration of stakeholders, fishers, administration and NGOs for site selection, experiment surveillance and sample collection. The project has established a consortium with the fishers to secure a large number of recruitment observations distributed in

time and space. PERCEBES combines results from experiments, hydrodynamic models and landings data into spatially explicit bioeconomic models which will allow generation of “policyscapes” to optimize the conservation/exploitation tradeoff. This is of interest for Administrations seeking to manage the SB fishery. At a final collaborative workshop, fishers, scientists, administrators and ONGs will discuss the potential for crossbreeding of good management practices across the Atlantic Arc. PERCEBES is also producing a video documentary focused on the effects of harvesting on biodiversity patterns, intended for a general audience, and focused on the science of the interaction humans-ecosystem.

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Keywords: Marine reserve (MR), Territorial User Rights Fisheries (TURFs), Co-management, biophysical model, Bioeconomic model, human exclusion experiment

Assessing the role of benthic filter feeders in the food web of the Gulf of Gdansk (Sothern Baltic) – an Ecopath with Ecosim exercise

Aleksander Drgas and Joanna Całkiewicz

Benthic filter feeders are an important link in energy and matter flow between pelagic and benthic part of the ecosystem. Their role is particularly important in highly eutrophicated water bodies, where they may control phytoplankton biomass. On the other hand, benthic organisms are important part of benthivorous fish diet and may affect the availability of food for zooplankton. Possible changes in the biomass of benthic filter feeders due to changes in environmental conditions, changes in the structure of the food web or the appearance of alien species may cause effects on different trophic levels of the ecosystem. The analysis of biomass changes of individual food web elements in the Gulf of Gdansk (Southern Baltic) in response to various scenarios of filter feeders biomass was carried out using the dynamic Ecosim model. This model is based on the Ecopath trophic model constructed for the biomass values of individual ecosystem components observed in 2004-2006. The model is composed of 17 groups, including detritus. The pelagic part consists of fish (cod, flounder, herring, sprat, other predatory fish, other non-predatory fish, and round goby), zoo- and phytoplankton. Benthos is represented by four groups (deposit feeders, predators, filter feeders and meiofauna). The model was also completed by piscivorous and benthivorous birds. Subsequent runs of the Ecosim model were carried out for different biomass values of benthic filter feeders, starting with a drastic decrease through intermediate states to a fourfold increase in biomass in relation to the real values observed in 2004-2006 period. In addition, model runs were carried out at a set of vulnerability values to simulate different control mechanisms (top-down vs bottom-up). The simulations carried out for the period 2017-2050 showed a significant effect of benthic filter feeders biomass change on the other elements of the food web. It concerned not only those groups of organisms directly affected by filtrators (phytoplankton) and those for whom this group of organisms is food (benthivorous fish) but also elements more distant trophic levels such as herring or sprat.

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Keywords: Benthic filter feeder, Gulf of Gdansk, food web, modelling, Ecopath with Ecosim (EwE)

Bivalve contamination with high paralytic shellfish poisoning toxin's levels derived from *Gymnodinium catenatum* ingestion: understanding its decadal-like periodicity

Paulo J. Vale

In Portugal, bivalves can accumulate paralytic seafood poisoning (PSP) toxins after feeding upon the toxic dinoflagellate *Gymnodinium catenatum*. Despite bivalve harvest bans, a few rare reports of this neurological syndrome in humans have taken place along the years. Accumulation of these high toxin levels, capable of affecting seriously humans, has occurred spaced by several years and less often in consecutive years. These PSP contamination events in bivalves of the Atlantic Iberian coast have followed a decadal-like periodicity since the 1970s. Years of very high contamination have coincided with years in which solar activity (derived from the 11-year sunspot cycle) was at its minimum (Vale, 2013). Retrospective data analysis between 1997-2017, showed monthly PSP maxima exceeding half the current regulatory limit (400 µg STXequiv./Kg) at Ria de Aveiro's mussels was associated with solar radio flux, often below 0 F10.7 units (a proxy for solar activity derived from sunspots). It was also associated with low geomagnetic activity (terrestrial effects derived from solar wind) (Fig. 1). During in vitro experiments, *G. catenatum* survival to hypo-osmotic shock or extracellular hydrogen peroxide administration was reduced when solar or geomagnetic activity increased. In short duration experiments, survival followed a hormetic dose-response curve (Vale 2017, 201). Cultures of *G. catenatum* were also exposed to an extremely low-frequency (0.5 Hertz) and weak (central amplitude 7 µT) magnetic field. After 3-4 days, its population was only 91% from control. In a comparative exposure with a related dinoflagellate, the PSP producer *Alexandrium pacificum*, its population was 100% from control (Fig. 2). This marked sensitivity to solar activity, and its subsequent geomagnetic unrest, might result from *G. catenatum* being a recent migrant to the Atlantic Iberian coast, as it does not appear in the recent cyst record. Migrants might be less adapted to novel geomagnetic environments than indigenous organisms. At Mar del Plata (Argentina), where *G. catenatum* was first described, the Main Field Total Intensity is currently 23 µT, whereas at the Atlantic Iberian coast it is 42-46 µT.

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Keywords: *Gymnodinium catenatum*, Paralytic shellfish poisoning (PSP) toxins, Space weather, Geomagnetic activity, Weak magnetic fields

Use of individual early life traits in larval dispersal models: a multispecies approach

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Dispersal is a key process shaping species population structure. In demersal marine species, which usually have sedentary adult phases, dispersion relies on drifting larval stages. However, the dynamics and seasonal variability of seawater masses can greatly determine the connectivity pattern of these species along the same geographic gradient. For this reason, detailed information on the release moment of larvae is needed to obtain accurate estimates of connectivity. Thus, the study of individually based early life traits is fundamental to gather information to properly model larval dispersal capabilities drifting with currents. In this study, we performed backtracking Lagrangian particle dispersion simulations, based on individual early life trait data, obtained from otolith reading, for 1413 juveniles of 9 fish species belonging to 3 families (Pomacentridae, Sparidae and Labridae). For each species, individuals were sampled from 4 to 7 localities at the western Mediterranean (Fig 1). These 9 species reproduce in different times covering the four seasons and pelagic their pelagic larval durations range from 7 to 43 days. With these simulations we obtained larval potential origin maps and connectivity matrices for the 9 species studied. The hatching date and the pelagic larval duration show important effects on larval dispersal modelling both at the intraspecific and interspecific levels. These results prove the utility of using individual early life traits data in larval dispersal modelling. Our case study adds a step forward to an accurate description of larval dispersion and recruitment which is key to understand population resilience under different environmental changing scenarios. Fig 1. Map of the sampled species and their sampling locations. Red lines indicate oceanographic barriers. BF: Balearic front, IC: Ibiza channel, AOF: Almeria-Oran front.

Keywords: Western Mediterranean Sea, fish larvae, Dispersal, Lagrangian particle model, Early life traits

Eating trash: ingestion of anthropogenic fragments by fish and crabs in Brazilian waters

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Plastic pollution in ecosystems is undoubtedly a threat of global proportions. One of the biggest problems is that much of the plastic produced reaches the environment (Jambeck et al. 2015) and interacts with the biota. For more than 40 years, scientists have shown ingestion of fragments of this material by marine species (e.g., Carpenter et al., 1972). Recently, Lusher (2015) listed 140 species that eat plastic particles in marine environments; but this is only a minimum number. In our study, we investigated whether fish and crabs collected in the oceanic-estuarine-lagoon system of the Cananéia-Iguape area of São Paulo State, Brazil (25°04'43" S; 47°50'34" W), are ingesting plastic particles. This area supports such high faunal and floral biodiversity that it is a global biosphere reserve (Diegues, 197; UNESCO, 1999; UNESCO, 2005). We collected specimens by trawling with a double-rig net. Fish and crab stomachs were removed and dissected and the contents were examined under a stereoscopic microscope (Zeiss Stemi SV6) with an image capture system (Zeiss Stemi 2000-C) for recording the presence or absence to plastic. We examined 139 fish and 143 crabs, comprising respectively 16 and 4 species. Plastic particles were found in all fish species (in 47.5% of the individuals analyzed) of the following: *Menticirrhus americanus*, *Micropogonias furnieri*, *Stellifer brasiliensis*, *Eugerres brasiliensis*, *Polydactylus virginicus*, *Conodon nobilis*, *Isopisthus parvipinnisi*, *Paralichthys brasiliensis*, *Haemulopsis corvinaeformis*, *Peprilus crenulatus*, *Oligoplites saurus*, *Trichiurus lepturus*, *Chloroscombrus chrysurus*, *Genidens barbuis*, *Cathorops spixii*, *Aspistor luniscutis*. In addition, an aluminum fragment was found in one *M. saxatilis* (Fig 1). For crabs, the presence of plastic was lower, occurring in only two species, *Callinectes danae*, and *Callinectes ornatus* (3% of the individuals) (Fig 2). It is noteworthy that many of the collected species have commercial value and are exploited as human food. Of all the species listed, only five have previous records of plastic ingestion in the scientific literature: *P. virginicus*, *H. corvinaeformis*, *O. saurus*, *T. lepturus*, *Callinectes ornatus* (Vendel et al. 2017; Schmid et al. 201; Silva et al. 201; Santana et al., 2017). Ours is the first record for the others. The high

incidence of plastic particles in fish stomachs in our study is alarming because this material likely generates negative effects in live organisms. Therefore, Brazilian authorities together with the society must act to cease plastic use and pollution. However, because this material is transported by currents throughout the Atlantic Ocean (if not globally) -control efforts must be global. Figure 1. Marine fish collected in the Cananéia area, São Paulo, Brazil, showing absence and presence of particles in the entire stomachs and guts: a) aluminum inside *Menticirrhus saxatilis*; b) and c) microplastic inside *Polydactylus virginicus* and *Aspistor luniscutis*, respectively. Figure 2. Marine crabs collected in the Cananéia area, São Paulo, Brazil, showing absence and presence of particles in stomachs: a) and b) microplastic inside *Callinectes danae* and *Callinectes ornatus*, respectively. Crab photo by PhD. Daphine Ramiro Herrera.

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Keywords: Aluminum, Commercial fish, Trophic web, Anthropogenic influences, crab

Exploring anammox bacteria ecology to improve nitrogen removal in wastewater treatment

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PROBLEM The constant increase in population led to the intensification of agricultural practices, which consequently incremented the use of fertilizers, particularly nitrogen based. Over the last century, humans have substantially influenced the global nitrogen cycle by increasing both the availability and the mobility of nitrogen compounds in aquatic ecosystems (Camargo and Alonso 2006). The Directive 91/271/EEC highlighted the need of new biotechnological processes for water management solutions. The conventional wastewater treatment technology for nitrogen removal requires nitrification followed by denitrification. However, the procedure is not cost-effective (high oxygen demand, high sludge production, and requires an external electron donor supply), nor environment-friendly, since releases CO₂ and N₂O, contributing to global warming (Jetten et al. 2009; Kartal et al. 2010). **CHALLENGES** Anaerobic ammonium oxidation (anammox), an autotrophic process, has been considered as a promising alternative to convert ammonium directly to dinitrogen gas, using nitrite as an electron acceptor under anoxic conditions (van de Graaf et al. 1995). The anammox process

can be applied to wastewater treatment instead of the conventional treatment, since requires no external carbon source, produces less sludge, and is more-cost effective (60 % reduction in costs), being environmentally friendly (90 % less CO₂ emissions) (Kartal et al. 2010). Anammox bacteria are chemolithoautotrophs, belonging to a branch deeply within the Planctomycetes phylum (Oshiki et al. 2016). There are five known anammox genera within the order of Brocadiales: *Candidatus "Brocadia"*, *Candidatus "Kuenenia"*, *Candidatus "Anammoxoglobus"*, *Candidatus "Scalindua"*, and *Candidatus "Jettenia"*. Although anammox bacteria were first discovered in wastewater treatment plants and their applications studied worldwide in this context, they may account for more than 50% of N loss from natural environments playing a significant part of the biogeochemical N₂ production (Francis et al. 2007). Presently, large-scale applications of anammox are limited by the long start-up period owing to the low growth rate and doubling time of anammox bacteria described so far (Jetten et al. 2009). Additionally, several variables can affect the efficiency of anammox bacteria activity, such as the availability of nitrite and ammonium, pH, temperature, organic matter content, salinity, and toxic compounds (Jin et al. 2012). These parameters can modify the anammox bacterial community structure and inhibit partially or totally the specific anammox activity (Gonzalez-Martinez et al. 201). Nevertheless, the potential of anammox bacteria in the wastewater treatment application has attracted the scientific community attention to study the anammox ecology, phylogeny, and functional dynamics and nitrogen removal capacity (Gonzalez-Martinez et al. 201, Oshiki et al. 2016). However, the interactions among all these aspects remains relatively unexplored. OBJECTIVE In this research project, we expect to contribute with fundamental knowledge about anammox ecology by exploring the relation between community structure and process activity. We intend to enrich anammox bacteria from natural environments, and ultimately to develop microbial inocula to be used as seeding sources for reactors. Our goal is to improve current available technology, and promote a wider application of anammox process in wastewater treatment. METHODOLOGY/EXPECTED RESULTS To attain the aims of this study, four main activities were planned and structured accordingly to Figure 1. The first activity intends to explore the presence of anammox bacteria in different ecological niches, such as estuarine sediments, freshwater sediments, groundwater, and agriculture soils. Locations were selected based on environmental characteristics, and several environmental parameters were monitored, namely the temperature, conductivity, pH, dissolved oxygen, oxidation-reduction potential, nitrate, nitrite, ammonium, and organic matter. The presence, abundance, and diversity of anammox bacteria was characterized by phylogenetic analysis and quantitative PCR targeting anammox bacteria, and specific functional genes (such as hydrazine oxidoreductase, *hzo*; and hydrazine synthase, *hzs*), using previously successfully described primer sets (Harhangi et al. 2012). The use of the 16S rRNA and functional genes has become a key procedure providing insights into the ecological role of anammox bacteria and their nature and constrains in several environments (e.g. Dang et al 2013). In the scope of the second activity, anammox biomass was enriched in batch cultures as well as laboratory scale bioreactor systems, such as Up-flow Biofilter and Anaerobic Baffled Reactor. For this, environmental samples with the most anammox potential were selected as biomass sources starter for enrichment with a synthetic nutrient medium (ammonium and nitrite), under anoxic conditions (van de Graaf et al. 1996). Nitrogen compounds were periodically monitored by standards methods as previously described in Teixeira et al. (2016). Several operational parameters were screened, including a range of different temperatures, conductivity, pH, dissolved oxygen, oxidation-reduction potential, nitrate, nitrite, and ammonium, and organic matter. Afterwards, optimum growth conditions and nitrogen removal efficiency were evaluated. Quantitative PCR targeting functional genes (*hzo* and *hzs*) was used to detect and as an indicator for growth of the anammox population (van der Star et al. 2007). Anammox activity was measured using the N isotope pairing technique that involves performing incubations amended with different 15N-14N isotopic mixtures and the different N masses (2N₂, 29N₂ and 30N₂), quantified by isotope-ratio mass spectrometry (IRMS), following the procedures described in Teixeira et al. (2016). The microbial community structure was assessed under the scope of the third main activity in environmental samples (activity 1), as well as in enriched samples (activity 2), using automated rRNA intergenic spacer analysis, as described in Teixeira et al. (2014). Based on the level of the obtained diversity, bacterial community composition of selected samples was further investigated using next generation sequencing (NGS) Illumina MiSeq platforms. Bioinformatic analysis of raw reads was performed using QIIME Illumina pipeline for the metagenomic data analysis (e.g. Ribeiro et al. 201). This analysis provided insights into the phylogeny of the communities, and the presence of catabolic genes present in each environmental sample (activity 1), and in the enrichment cultures (activity 2). All collected data has been analyzed by means of several statistical methodologies, including multivariate data analysis to explore relationships between molecular microbial data, operational parameters, and nitrogen removal activities. FUTURE PERSPECTIVES Successfully enriched cultures will be maintained, and the links established between enriched cultures and function will be matched with wastewaters with specific properties. This will allow the use of enriched cultures as seed sludges for anammox reactors for wastewater treatment, as planned in the fourth activity of the projected study. FINAL REMARKS The findings of this study will improve the currently available knowledge concerning the relationships among environmental parameters, phylogeny/functional taxa, anammox ecology in order to explore microbial inocula to be used as seeding sources for anammox reactors. Therefore, these results will be useful for optimizing parameters in a rapid startup and enhanced bioreactor

efficiency, contributing to a wider and efficient application of anammox process in wastewater treatment.

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Keywords: Anammox, bioreactor, microbial ecology, nitrogen removal, wastewater treatment

Direct and indirect effects of climate change on *Cyrtodoclea nodosa* in the Canary Islands

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Human activity is increasing at the rate of 0.4% yr⁻¹, the concentration of CO₂ in the atmosphere, which is expected to double from preindustrial level by the middle of this century. Approximately 30% of CO₂ emissions are absorbed by marine waters, therefore, marine species have to cope with increasing ocean acidification in combination with rising temperatures, causing a perturbation in a ecological component or system. Seagrass has been considered the most productive ecosystems worldwide, support high diversity, refuge and ecosystem engineers. However actually are exposed to multiple perturbations and different response species specific has been observed in different regions of world. The effects of climate change (warming and/or ocean acidification) has been tested on broad variety of seagrass (Tomas et al 2015; Jiménez-Ramos et al

2017), showing the effects of climate change on seagrass communities. How these changes affect seagrass and seagrass-herbivore interactions are on the main question for future seagrass research. In Canary Islands, *Cymodocea nodosa* play an important role in shore ecosystems (Reyes et al., 1995; Tuya et al 2014a, b). Various studies have reported a declining trend of *Cymodocea* meadows at Canary Islands (Tuya et al 2014a, b; Fabbri et al 2015), however any study has evaluated the effect of climate change on *Cymodocea nodosa* in Canary Islands. In this study we assessed the direct effects of climate change (temperature and acidification) on growth, net photosynthesis, respiration, chlorophyll a concentration and the indirect effects of climate change on herbivory of *C. nodosa*. In November of 201 the seagrass *Cymodocea nodosa* was randomly collected by scuba divers in the Abades locality, Tenerife Island. Plants were selected in the field and collected from different patches between 15-17 m deep. Plants were transported to laboratory in wet conditions. Upon arrival, 3-4 plant units were selected for healthy appearance and allocated to each of 60 transparent incubations beakers (1.3 L) containing natural sandy previously. Previous to the experiment, plants were acclimated for 7 days to laboratory conditions (salinity, light, temperature, ph). The plants were subjected to two pH treatments: control (pH=.1) and low pH (pH=7.5) and two temperature treatments (control =21°C and high=26°C), following the predictions for next century in Canary Islands. The system consisted in two tanks of 300 L interconnected in each treatment; one tank was header tank where pH and temperature were adjusted although a CO2 injection system in low pH treatments and the temperature was adjusted although coolers and heaters. The other tank was the support where the beakers were placed. A total of 15 replicates were used per each treatment. Diverse variables were taken for growth, weight, maximum leaf length, total root length, number of leaves. These measures were taken prior to experiment and end to experiment in laboratory, following methodology of Hernán et al (2017). Net primary production and respiration rates were calculated following methodology used by Legrand et al (2017).The content of chlorophyll a was calculated according to Lichtenthaler and Wellburn (193).The indirect effects were tested although feeding assays with *Paracentrotus lividus* for 4 hours at the end of experiment following methodology Rodríguez et al (201). In order to assess the effects of climate change on species studied, increase in weight, maximum leaf length, total root length, number of leaves, chlorophyll-a content, net primary production and respiration the data were analyzed by means of two-way permutational ANOVA in each variable. In the case of consumption rates, the data were analysed by two-way ANCOVAs performed by permutations: In all analysis temperature (2 levels) and ph (2 levels) were used as fixed factors. Euclidean distances were used for all analysis of variance, and respective significant terms were examined posteriori pairwise comparisons by permutations (Anderson 2001). If there were not enough possible permutations for a reasonable test, corrected p-values were obtained with Monte Carlo random draws from the asymptotic permutation distribution. All statistical analyses were carried out using PRIMER 6 &PERMANOVA+ v. 1.0.1 software. Our results showed significative differences between pH treatments where in low pH we observed higher increase in weight than in pH control ($F= 6.447$; $p=0.011$), Figure 1. However in any other variable of growth we found significative differences. The net primary production varied between pH treatments ($F=11.42$; $p=0.001$) where higher concentrations of O2 were registered in low pH than control pH and between temperature treatments ($F= 16.559$; $p=0.0002$) where in the control conditions were observed the highest concentrations of O2 Nevertheless, there were no differences in respiration or in chlorophyll a content between pH or temperature treatments. In feeding assays, significative differences were observed between pH treatments, where the highest consume rates were observed on plant reared under low pH ($F=13.939$; $p= 0.0022$) Our results show the negative effects of climate change on *Cymodocea nodosa*, where high temperature decrease the net primary production rate and low pH increase the growth and net production rate but however increase their vulnerability to consumption by sea urchin *P. lividus*. Therefore, it is expected that increase in temperature decrease the photosynthesis rate and low pH (acidification) will trigger an increase in the grazing of seagrass tissues. Additional research is required to better predict and address changes in secondary metabolites and nutritional contents, as change in seagrass palatability under different climate scenarios.

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Keywords: Climate Change, *Cymodocea nodosa*, Direct effects, Indirect effects, Herbivory

Effects of ocean acidification on growth and feeding rates of spotted jellyfish *Phyllorhiza punctata* early life stage/polyps

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The increase of atmospheric carbon dioxide (CO₂) is causing the reduction of the seawater pH. It is well known that ocean acidification affects marine organisms causing impacts on its survival and growth rates. In recent years, jellyfish blooms have been increasing the interaction of humans with such organisms, leading to socio-economic negative impacts. In order to better understand jellyfish blooms dynamics, it is essential to achieve a better knowledge about the impacts on the different aspects of jellyfish life cycle, both attached polyps and free-swimming medusae. In this study, it was evaluated the effect of ocean acidification on the growth and ingestion rate of *Phyllorhiza punctata* polyps, when submitted to projected values of seawater pH for the year 2100 (a decrease of 0.3-0.4 units of pH) and extreme values (decrease of 1-2 units of pH). Three polyps of *P. punctata* with a similar size ($1027 \pm 76 \mu\text{m}$) were placed in microplates containing 5 ml of non-CO₂-enriched seawater (pH = .1) and CO₂-enriched seawater (pH = 7.5, 7.0, 6.5, 6.0). Salinity and temperature were kept constant and equal to 35 and 22, respectively. Prior feeding experiments, polyps were subjected to a 24 hours period of starvation. After that, the polyps were exposed to 50 newly hatched *Artemia* nauplii. After 1 hour the remaining nauplii were counted. Feeding rate (prey ind⁻¹ hour⁻¹) was calculated as the number of prey ingested per polyp per hour. The obtained results showed a significant linear relationship between pH and feeding rate ($F_{4,75}=52.32$ $p < 0.001$), with feeding rate decreasing with pH (Fig. 1). For growth rate, one polyp per well with similar size ($312 \pm 41 \mu\text{m}$) were transferred to 3 mL 24 wells microplates containing 2 mL of seawater at the same conditions described in previous section. Polyps were fed ad libitum every 2 days with *Brachionus* sp. and measured every 5 days. Water was changed daily. The growth rate (mm day⁻¹) was calculated from the relationship between time and size. The pH decrease showed a negative impact on growth rate, inducing a decrease from 19.34 ± 0.65 mm day⁻¹ (pH .1) to 14.2 ± 0.732 mm day⁻¹ (pH 6.5) and 14.7 ± 0.930 mm day⁻¹ (pH 6.0) (Fig. 2). Significant differences ($H_{4,50} = 20.67$, $p < 0.001$) were only observed between pH 6.5 and 6.0 (Dunn's method). The obtained results suggest that polyps of *Phyllorhiza punctata* may have some resistance to pH levels expected to the year 2100 (a decrease of 0.3 – 0.4 units of pH). Nevertheless, the exposition to extremes value of pH (6.5 and 6.0) showed a clear negative impact on polyp growth. Although an increase in prey ingestion was expected to compensate the increase cost in basal energy due to acidosis caused by a decrease in pH, in this work it was observed a reduction in ingestion rate, which may contribute to the decrease observed in growth and even affect other biological processes (e.g. reproduction). Other studies found that when organisms are exposed to both acidification and temperature increase the effect of the acidification may be ameliorated or exacerbated by the temperature increase. Future studies should evaluate the effect of pH on asexual reproduction and the combined effect of an increased temperature, in order to better understand how global changes will affect *Phyllorhiza punctata* jellyfish both on polyp and free-swimming medusae.

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Keywords: Ocean acidification (OA), jellyfish, *Phyllorhiza punctata*, Growth and ingestion rate, Early life cycle

Ecopath and Ecosim as tools in foodweb understanding - the Gulf of Gdansk example

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The understanding foodweb dynamics in coastal areas and their response to various anthropogenic pressures, including climate change and different economic scenarios, allows us to foresee their future state and their ecosystem goods and services. Therefore, Ecopath and Ecosim models for the Gulf of Gdansk (Southern Baltic Sea) have been prepared and tested as a case study for coastal areas. The Gulf of Gdansk is subjected to strong anthropogenic pressures. The Vistula River, the second largest river in the Baltic catchment, discharges more than 30 km³ year⁻¹ of nutrient loaded freshwater into the gulf, contributing to its eutrophication. During the past decades, changes in the Polish economy and the implementation of various mitigation measures resulted in declining nutrient loads to the gulf, but despite these efforts the response of its coastal ecosystems seems to be relatively weak. The Gulf of Gdansk fish assemblages are under constant, strong fishing pressure. Commercial fishing is diversified, targeting mainly sprat, herring and cod. In the coastal waters a small-boat fishery is focused on herring, flatfish, salmonids and freshwater species. Based on biomass data of individual ecosystem components, knowledge of their trophic relationships, and group specific fishing pressure, a trophic ecosystem model of the Gulf of Gdansk was constructed for the period 2004-2006 using Ecopath software. It consists of 17 groups, including detritus. The pelagic part of the model consists of fish (cod, flounder, herring, sprat, other predatory fish, other non-predatory fish, and round goby), zoo- and phytoplankton. Benthos is represented by four groups (deposit feeders, predators, filtrators and meiofauna). The model was also completed by piscivorous and benthivorous birds. A "Fisheries" module, with fishing mortality specified for different gear types, describes fishing pressure. Based on the Ecopath model, a dynamic Ecosim model was created that describes the time-development of all model groups for the period 2006-2016. Biomass time series extracted from monitoring data and environmental studies, as well as time-dependent fishing pressure derived from fisheries statistics, were used to calibrate and force the model. In addition, the model was forced by a set of environmental factors (e.g. temperature, nutrients, salinity, oxygen). Thus, apart from trophic dependencies and changes in fishing pressure, the variability of environmental factors shape the biomass development of the different groups in the model. Among the set of environmental factors represented in the model by forcing functions, the best model fit was when primary producers were forced with river nutrient loads, and selected consumer groups by water temperature and hypoxic bottom. Environmental forcing time-series were generated by the BALTSEM model, a biogeochemical model of the Baltic Sea. BALTSEM can simulate changes in nutrient state, primary production, and oxygen conditions for different Baltic Sea nutrient load and climate scenario. Forcing the Ecosim model with the biogeochemical model output will allow us to predict ecosystem change in response to climate projections and nutrient load scenarios.

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Keywords: food web, modelling, Gulf of Gdansk, Ecopath, Ecosim, Climate Change

Effects of rainfall disturbance on a sandy beach macroinvertebrate community structure and composition in southwestern Spain

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The distribution, abundance and diversity of marine invertebrate species are determined by various biological functional factors and by extrinsic factors such as ecological disturbances that modify the biotic environment. In sandy beaches, there is evidence that macrofaunal communities alter their diversity and abundance depending on climatic and structural variables that determine the structure of the sandy substrate, such as sand availability, exposure to waves in the swash zone, rain events or storms. This variation often overlaps with the seasonal fluctuation inherent in some environments to shape the invertebrate assemblage composition and diversity. We studied the macrofauna of a sandy beach of gentle slope in the SW of Spain, by selecting 2 representative zones separated from each other by 2000 meters approximately, in 5 different dates, before a climatic disturbance with heavy rains events prolonged for several weeks. (1st date), during (1 day after the disturbance) and afterwards (3 different dates, 5, 20, and 46 days after the disturbance). One of the areas is close to the urban building frontline (with a lesser breadth of the dry sand strip) and another one at a greater distance and protected by a small dune field. We divided both beach sampling sections into six transects perpendicular to the coastline. In each transect we took a sample of sand in 10 height levels between lower and upper tide limits, plus an additional one per level for sedimentary analysis. The first 15 cm of sand were collected with a shovel from a 25 x 25 cm square and filtered through a 1 mm mesh size bag in seawater. The filtered samples were transported to the laboratory where they were fixed, stained (Bengal rose) and separated by decantation and manually sorted to species or lowest possible taxonomic level. An overall 24 invertebrate species and 34 individuals were collected from both sampling zones. For both beach zones studied, there were significant differences in macrofaunal taxonomic composition, abundance and diversity in relation with the date of the disturbance. The granulometric structure of the sediment fluctuated more intensely at lower and mid beach levels (near the low tide, the swash zone) than at upper beach levels. There were also differences in granulometry and related abiotic variables (grain size groups, size selection indices, organic matter content and water content), among different dates from the disturbance. In addition, a significant effect of the time after and before the disturbance event was also observed on macrofaunal abundance and richness, although this effect seemed to be attenuated at upper beach levels. Combined with (or caused by) rainfall/storm events, other extrinsic factors, such as sediment movement from other beach sections during disturbances, proximity to urbanization, sand availability and passive protection of the macrofaunal environment provided by nearby dune fields, could interact with intrinsic specific seasonal variations of macrofaunal taxa, and life history of some species to shape macrofaunal assemblages.

Keywords: macrofauna, intertidal, Sandy beach, SW Spain, Weather disturbance

Effects of ocean warming on larvae development of *Patella tenuis crenata* in the Canary Islands

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Ocean warming is causing Sea Surface Temperature (SST) to rise, which is projected to reach +2 to +4.50C by the end of 21st century (IPCC 2014). Temperature is considered the most important factor controlling the distribution, physiology, morphology and behavior of marine invertebrates (Doney et al 2009). Early life history stages may be particularly sensitive to ocean warming. Effects of ocean warming on larvae development has been documented in a wide range of invertebrate species (Byrne et al 2013; Lester et al 2007). However, few studies have tested the effects of temperature on gastropods larvae. Impairment of survival, development and growth are early life history stages that may have particularly severe consequences for species where persistence or recovery from disturbance hinges on larval recruitment. One such sub-species is the *Patella tenuis crenata* (Class Gastropoda, Family Patellidae), which is endemic to Canary Islands. *P. tenuis crenata* has experienced severe population declines in recent decades as a consequence of intensive anthropogenic pressure (Batista, 2001; Cabrera, 2001). However, the effects of ocean warming have not been investigated as

a significant threat to *P. tenuis crenata*. Here, we investigate the effects of temperature on survival and larvae development of *P. tenuis crenata*. Larvae were reared for the entire larval duration of 2 days and investigated responses to warming in terms of larval survival and development. Larval development of this subspecies was performed for the first time. In February 2019, mature specimens of the *P. tenuis crenata* were collected by divers from natural habitat (Punta Larga, Tenerife). The specimens were transported to the laboratory under controlled conditions immediately after collection. Animals were induced to spawn by air bubbling technique (Ferranti et al., 201) and/or by injection with 1ml of KCl (0,1M) through the mantle. Three males and seven females spawned successfully. Gametes were mixed before being put in contact with gametes of the opposite sex. Sperm was collected dry and kept at 40C until usage and eggs were collected in seawater previously filtered and autoclaved. To carry out fertilization essays, eggs and sperm were added together in pots with 100 ml of filtered seawater for one hour. Afterwards, the solution was filtered (60µm) and treated at different temperatures. Three replicates were performed for all the experiments, consisting in 100 ml of filtered and autoclaved seawater with a density of 14 fertilized eggs / ml. To asses survival and early development, pots were incubated at different temperatures and different lengths of time after insemination. The short sampling times were 3, 6, 9, 19, 24, 2, 32 and 42 hours. For all combinations of temperature and time, 3 replicates were performed. The density and stage of development was observed. We estimated the number of eggs in different stages of cell division: 2 cells, 4 cells, morula, trochophore, veliger and pediveliger. To carry out this evaluation we observed 3 aliquots of 1 ml for each replicate pot, under microscope. In order to assess the effects of seawater temperature on larval survival on species studied data were analyzed by means of a two-way permutational ANOVA, where temperature (2 levels) and time (levels) were used as fixed factor. To evaluate the effects of temperature and time on larval development, in each larvae stage (2 cells, 4 cells, morula, trochophore, veliger, pediveliger), two-way permutational multivariate analysis of variance was performed. A two-way design was carried out with temperature (2 levels) and time (7 levels). Euclidean distances were used for all analysis of variance, and respective significant terms were examined posteriori pairwise comparisons by permutations (Anderson 2001). If there were not enough possible permutations for a reasonable test, corrected p-values were obtained with Monte Carlo random draws from the asymptotic permutation distribution. All statistical analyses were carried out using PRIMER 6 &PERMANOVA+ v. 1.0.1 software. When analysing survival, measured as number of larvae, significant interaction of treatment*time was observed ($F= 2.369$; $p=0.049$), posteriori pairwise test revealed that these differences appeared among temperature treatments after 42h, when larvae density in the control treatment was 0, larvae/ml, while in the high temperature treatment was 0 larvae/ml (Figure 1) However, significant differences on larval development were observed among temperature treatments ($F= 3.6$; $p=0.027$) where in the control treatment we observed all larvae stages. Despite significant differences in time were not observed, there existed trend to a higher larval development in the high temperature treatment during the first time period (Figures 1-2). Similar results were obtained by other studies focused on the larval development of marine invertebrate species (Byrne et al., 2013). Although *Patella tenuis crenata* spawning in winter months when sea water temperatures of 19-20°C are registered in Tenerife Island, in an ocean warming scenario projected by the end of the present century, our results raises questions about the future survival larval of this species .

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Keywords: global change, Global Warming, *Patella*, Conservation -, Marine Biology

Poster session 3

A biogeographical analysis using artisanal dredging fisheries discards of the northern Alboran Sea

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Discards are defined as the portion of the total catch brought on board and then returned to the sea for whatever reason (e.g. unmarketable species, individuals below minimum legal size, etc.). Its analysis is considered of importance for fisheries management, allowing to improve the knowledge on the composition of benthic communities harboring living resources that are included in several European Directives (Marine Strategy Framework Directive, EU Directive 200/56; Common Fishery Policy, EU Reg. 130/2013). Besides, it provides a baseline for biodiversity conservation purposes, with the monitoring of rare and singular species. This is of interest in the Alboran Sea as it represents a biodiversity hotspot in an Atlantic-Mediterranean marine transition area, where some marine protected areas have been recently declared for the benthic biodiversity conservation. In this context, a biogeographical analysis has been done using information of a monitoring program on discards of artisanal shellfish fisheries in the northern Alboran basin. A total of 27 discard samples (standardized weight of 5 kg) were collected between March 2013 and March 2014 from the catches obtained with nine artisanal fishing vessels with similar characteristics operating from La Línea de la Concepción to Caleta de Vélez (90 nautical miles apart) (Figure 1), and targeting the most demanded commercial bivalves in the area (the wedge clam *Donax trunculus*, the striped venus clam *Chamelea gallina* and the smooth clam *Callista chione*). In the laboratory, every specimen from each sample was separated, identified to species level (when possible), quantified (abundance and biomass [± 0.1 g wet weight]), and characterized according to their biogeographical distribution considering several geographical sectors that included the Alboran Sea; the Mediterranean Sea (except the Alboran Sea); the Gulf of Cádiz; western Europe; northern Europe; and western Africa. The discards generated by shellfish fisheries in the northern Alboran Sea showed a high biodiversity (134 spp., of which 5 are of commercial interest), with the dominant species corresponding to components of the "Sables Fins Bien Calibrés" biocoenosis (well-sorted fine sands) defined for the Mediterranean Sea. The analysis has showed that most species, including the top abundant ones, have a wide biogeographical distribution along the north and western European and Mediterranean coasts, whereas strictly Mediterranean species represent a minority group. Interestingly, a total of eight species with tropical West African affinity (at least south to Senegal) were detected and included the gastropod molluscs *Mesalia varia*, *Bivetiella cancellata*, *Cymbium olla*, *Tectonatica sagraiana*, *Sinum bifasciatum* and *Natica vittata*, as well as the decapod crustacean *Albunea carabus* and the starfish *Luidia atlantidea* (Figure 2). All of them are considered native as persistent populations have been observed during the last decades in the Alboran Sea. These species seem to be self-introduced by active dispersal (planktonic larval development) promoted by the flux of Atlantic surficial waters through the Strait of Gibraltar. The analysis of discards represents a useful complementary tool for improving the distribution range of benthic species and for monitoring benthic biodiversity components in a hotspot as the Alboran Sea, including exotic range-expanding species in the area that could be collected and assessed with the help of these coastal monitoring programs.

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Keywords: Discards, Artisanal fisheries, Biodiversity, Alboran Sea, Shellfish

ACUSQUAT Project: Acoustic behavioural monitoring of the Angelshark (*Squatina squatina*) in critical conservation areas

Diego G. Campos, David Jiménez-Alvarado, José Juan Castro-Hernández, Airam Guerra-Marrero, Jorge Cabrera-Gámez and Antonio C. Domínguez-Brito

The Angelshark (*Squatina squatina*) is a critically endangered species with a geographic distribution currently reduced to the Canary Islands and with punctual sightings along the Mediterranean and Wales (UK) coasts, primarily due to overfishing. This benthic shark uses sheltered beaches of the islands as reproduction and nursery areas, thus tourism development represents another major threat to its survival. During this study, 11 females and 4 males of Angelshark have been tagged using acoustic transmitters, in order to track their circadian routines and to study their movements around these critical areas, that are also vital for the tourism industry of the Archipelago, particularly during mating season for adults and along the first year of life, when these beaches are used as nursery areas for juveniles. Results, combined with data obtained from visual census and citizen sighting reports, are being used to promote Use and Management Plans in beaches considered as critical conservation areas for the species, and make them compatible with tourism. The tracking methodology has been based on the use of Vemco VR2W, VR2C-Mini and VMT receivers, as well as Vemco V9 coded transmitters. An initial acoustic telemetry network was implemented from October 201 with 3 fixed receivers (VR2W and VMT) installed on both ends and in the middle part of the study area, and a third receiver (VR2C-Mini) used from a boat to “comb” the area on punctual occasions. The first results show that this kind of tags are very appropriate for tracking benthic elasmobranchs like the Angelshark in restricted coastal areas, with an average detection range of 300-700m depending on the morphology and bathymetry of the area. In the Canary Islands, adults and juveniles are found very close to the shore in beaches and ports that support intense human activity, thus negative interactions are frequent and need to be addressed, particularly with this threatened species.

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Keywords: Angelshark, acoustic telemetry, critical conservation areas, Tourism development, Canary Islands

Analysis and damage assessment of discards in the hand dredge wedge clam fishery (*Donax trunculus* Linnaeus, 175) in the Gulf of Cádiz (SW Spain)

Raimundo Blanco, Sara Román, Ana Rodríguez-Rua, Miguel Coján, Luis Silva, Antonio Sánchez2, Esperanza Lisa-García and Marina Delgado

Within the framework of the research project VENUS, the aim of this communication is to provide a first insight of the discards associated with the artisanal hand dredging fishery targeting wedge clam *Donax trunculus* Linnaeus, 175 in the sandy beaches at littoral of Huelva, in the south west of Spain. Small scale fisheries represent one of the main types of fisheries occurring in the area with a high socioeconomic importance. *D. trunculus* constitutes a very important fishing resource due to its high economic value and has been exploited over the last decades using primarily either artisanal hand-operated dredges (rastros manuales) in shallow water and commercial boat-operated dredges from subtidal to deeper waters. The hand dredge wedge clam fishery has been regulated by the Andalusian autonomous government since 200. Several measures for the control of fishing and protection of this valuable economical resource were established in order to maintain its sustainability: licenses (the protect area Doñana NP has a specific license regulation), daily quota, a minimum size limit and a closed season (Silva et al., 2014). In general fishing trawling is one of the most impactful fishery activities on the seabed. The physical and biological impacts in the communities are variable. They depend

upon size and weight of the gear as well as fishing practice and ecosystem. In the area of study, there is a lack of scientific information about of some aspects of the wedge clam fishery with hand dredge: discard structure and composition, and damage caused to specimens during the fishing and the manipulations. A basic tenet of the ecosystem approach to fisheries management is that harvesting should be conducted with minimal impact on juveniles, non-target species, and marine habitats. An analysis of discards and damage will be useful for the adoption and assessment of the discard mitigation strategies for this fishery (Bellido et al., 2011). The study area comprised the low intertidal and the upper (2 - 3 m depth) infralitoral zone of the sandy beaches of the Huelva province coast. The area was divided into six units from the mouth of the Guadalquivir river to Ayamonte (mouth of the Guadiana river): Doñana National Park, Mazagón, Huelva, Punta Umbría-La Bota, Isla Cristina-El Rompido, and Ayamonte. Sampling was carried out using a hand dredge similar to those used by artisanal fishermen. It consists in a wood towing arm, dredge mouth of 44.5 cm width by 15 cm height with an even cutting edge, a mesh sieve size of 7 X 7 mm and a length of 5 cm, with a rear net bag of 200 cm length and mesh size of 5 X 5 mm. The gear digs deeply into the sediment up to 10 cm. A total of 17 hauls were performed at 17 stations, between the lower intertidal and 2 m depth of the infralitoral zone in June 201. At each station the hand dredge was towed diagonally to the shoreline for 5 min. Every transect was geo-referenced by a GPS. The samples were sorted in the laboratory. Macrofauna and megafauna were identified to the lowest taxon level (mainly species level) with the uttermost precision and their respective abundances and weights recorded. Additionally, a damage assessment was performed on the fauna, taking into account the whole fishing process. A three level damage scale was used to assign the level damage to the individuals of each taxon: D0 (no damage), D1 (low damage), D2 (intermediate damage) and D3 (high damage, not survival after their release) (Anjos et al., 2011, Urrea et al., 2017). The preliminary study of the 397 specimens yielded 25 taxa belonging to 5 Classes: Polychaeta (4), Bivalvia (1), Gastropoda (3), Scaphopoda (1), Crustacea (6), Echinoidea (2) and Actinopterygii (1). However, the number of taxa could be even higher, because Paguroidea and Polychaeta could not be identified to species level. Bivalvia was the dominant group in abundance (94.12%) and biomass (95.41), followed by Crustacea with 4.72% and 2.06%, Echinoidea 0.44% and 2.42%, Polychaeta 0.39% and 0.06%, Gastropoda 0.21% and 0.01, Scaphopoda 0.10% and 0.01% and finally Actinopterygii with 0.02% and 0.03%. The targeted species, *D. trunculus*, represented 3.34 % of the specimens and 76.5% of the biomass of the capture (324 specimens and 447.03 g); The discard fraction was composed by 24 taxa and represented the 16.65% of the collected individual and the 23.42 % of the biomass. Discards were dominated by Bivalvia in taxa richness (7), abundance (64%) and biomass (0.39%). Overall, 2.44% of the discarded specimens were undamaged, 0.92% display low damage, 0.15% display intermediate damage, 16.49% display severe damage. Bivalve was the group with more damaged individuals (66.67%), all belonging to *Macra stultorum* (Linnaeus, 175), followed by brachyuran crustaceans (25.44%), irregular sea-urchins (6.14%) and fishes (1.75%).

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Keywords: Discards, damage, Megafauna, macropfauna, Wedge clam, Small scale fisheries, Ecosystem approach

Basking shark *Cetorhinus maximus*, (Lamniformes: Cetorhinidae), at northern and western Iberian Peninsula waters (NE Atlantic)

Julio Valeiras, Pablo Covelo, Marisa Ferreira, Leire Ruiz, Pablo Cermeño, Luis Laria, Catarina Eira and Alfredo López

The basking shark *Cetorhinus maximus* is a plankton feeding shark (Lamniformes: Cetorhinidae) distributed worldwide in boreal to warm temperature waters. The presence in Spanish and Portuguese Iberian waters is poorly documented although this species occurs and it is well-known by fishermen. In this work we present data on basking shark presence in 30 years in Atlantic Iberian waters from monitoring data by involved organizations, also including records from monitoring data collected by the organizations involved, including records known by people, social networks and media. We record sightings off shore (31%), strandings (17%) and incidental fishing captures (52%). Incidental catch occurs mainly in fishing net used by artisanal fleet all along the continental shelf. Some of stranding records could be dead or injured sharks discarded at sea due to large size, low economic value and to comply with the legislation that prohibits its commercialization. The presence of basking sharks concentrates mainly at north-western coast and data suggest a seasonality of abundance in the study area. 73% of the basking sharks appeared between the months of February and April. These results indicate a temporal pattern in the abundance which coincides with the scientific data of recent years with satellite telemetry indicating migratory movements of the species. In the Bay of Biscay, most registrations take place between May and July, and in Ireland and the United Kingdom, the highest density occurs in spring and summer. The decline in records after spring may be due to the movements of the species during summer and autumn to other waters, perhaps to northern areas that are rich in zooplankton at that time of the year. This recording pattern could be influenced (1) oceanographic conditions and the abundance of food resources are a key factor in the distribution and appearance of this plankton feeder, (2) displacement of the species to far pelagic areas where the observation and capture is less frequent by the shortage of human activity and (3) spatio-temporal changes in fishing effort throughout the year, and therefore in accidental catches. Although the records of the species are mainly concentrated in Galicia, the net fisheries work throughout the coast in all the area. The knowledge of species occurrence and mentioned factors will allow clarifying the presence and working on conservation problems in the study area.

Acknowledgements

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Keywords: Basking shark, bycatch and discards, Shark ecology, Fisheries, Fish migration

Differential distribution patterns of *Balaenoptera* spp. from tropical areas

Jose C. Báez, Camino García Ramos, María L. Ramos, Pedro Pascual and Francisco Abascal

Tropical tunas (i.e. *Katsuwonus pelamis*, *Thunnus albacares*, and *Thunnus obesus*) tend to aggregate around all types of floating objects (both natural and artificial), including whales. Cetaceans, especially spotted dolphins in the eastern Pacific Ocean, are also found in association with tuna in tropical areas. Some authors suggest that records from the fishing industry is the only data source for reporting whales occurrence in the Tropical open ocean waters. Currently, 6 species of the genus *Balaenoptera* are known in the Atlantic Ocean and only three of them have been recorded in tropical regions: *B. physalus* (fin whale), *B. edeni* (Eden's whale) and *B. musculus* (blue whale). The industrial Spanish purse seine fleet targeting tropical tunas perform an important activity in the tropical Atlantic region. The main aim of present study was to understand the differential distribution patterns of *Balaenoptera* in relation to other cetaceans in the tropical Atlantic region. The Spanish Institute of Oceanography (IEO) observers' program on board commercial purse seine vessels follows a scientific programme developed under the EU Data Collection Framework. The main aim of this observer program is to obtain 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 19 cetaceans sightings observed during the fishing operation: 19 *Balaenoptera* spp., 39 dolphins, and 131 other cetaceans (including Ziphiidae, whales no *Balaenoptera*) (Figure 1). *Balaenoptera* occurrence was modelled as a function of longitude, latitude and the following environmental variables: sea surface temperature (SST), sea surface height (SSH), meridional surface velocity (v), zonal surface velocity (u), mixed layer depth (MLD), dissolved oxygen concentration (DO), and sea surface salinity (SAL). Moreover, we included the spatial variables: longitude and latitude. Model coefficients were assessed by means of an omnibus test and the goodness-of-fit between expected and observed proportions of by-catch events was 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 receiver 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 included SST and u as explanatory variables. According to the Omnibus test, the adjusted model is significant ($\chi^2 = 39.67$, 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 ($\chi^2 = 2.02$, degrees of freedom $P = 0.946$). The likelihood (-2LL) was 3.642 and R^2 of Nagelkerke = 0.395. The logit connection function is expressed as: $Y = 25.19 - 1.147 * SST - 9.44 * v$. According to the Wald test (where $\alpha = 0.05$) these parameters of the model were considered to be significant. Finally, the AUC obtained was 0.90, which reflects a good adjustment. Our results suggested that the habitat preference of the genus *Balaenoptera* from the tropical zone is related to lower SSTs and the surface current component. On the other hand, the sightings distribution evidences that the majority of the cetaceans observed, and especially of the genus *Balaenoptera*, are concentrated in areas close to the coast (figure 1). This could be due to the upwelling occurring next to the coast, which result in lower SSTs and increased productivity.

Keywords: whale, Sightings, Atlantic tropical, habitat, modelling

Disentangling the hierarchical structure of environmental effects on fish assemblages in tropical coastal ecosystems

Ellen M. Camara, Magda F. Andrade-Tubino, Taynara P. Franco, Alberto T. Correia, and Francisco G. Araújo

Understanding the processes driving the structure of coastal fish assemblages across space and time is of major relevance in the face of the increasing cumulative human impacts on food webs and ecosystem functioning. Factors in large scales, such as historical contingencies and contemporary climate changes, determine the species pool, which is gradually restricted by factors in smaller scales. Local assemblages are ultimately a result of the selection of species by local environmental conditions. The environmental effects at the site and landscape scales and spatial effects at three hierarchical levels (type of system, individual systems, and zones within each system) on the composition and abundance of species in two types of coastal ecosystems (coastal lagoons and bays) in Southeastern Brazil were investigated. Samples were obtained during the 2017 wet season (September, November, and January) in three coastal lagoons and three bays. In each ecosystem, sampling was performed at three zones with different distances from the ocean (inner, middle, and outer). Site-scale environmental measurements of water quality and substrate type were recorded concurrently with the fish sampling. Landscape-scale environmental metrics representative of land use and the number of close estuaries were obtained for each zone using a geographical information system. Species were classified in groups according to the habitat use (estuarine resident, marine migrant, marine straggle, semi-diadromous, and freshwater) and trophic guild (planktivores, benthophages, herbivores, detritivores, piscivores, and opportunists) to investigate if environmental effects differ depending on the degree of dependence from estuarine areas. The pure environmental effects of each variable set (site and landscape scales) and the fractions shared with the hierarchical levels were accessed by variance partitioning. The relevance of individual environmental variables was quantified by partial canonical correspondence analysis (pCCA) controlling for the hierarchical structure. Prior to the analyses, a forward selection procedure based on the adjusted R^2 and a nominal p -value of 0.05 to identify a parsimonious subset of environmental predictors to be included in the pCCA models using the hierarchical levels as conditioning terms were used. For all cases, as the investigation was focused on spatial patterns, the effect of the period of sampling was controlled. The variance inflation value < 4 for all variables indicated that no model was biased by collinearity between the environmental predictors. According to the variance partitioning, landscape-scale variables explained more of the variance in the composition and abundance of species (26%) than site-scale variables (16%). However, an expressive fraction was shared between both variable sets (6%) and with the hierarchical spatial structure (7%), with the later influencing a total of 19% of the species variance. Marine migrant species were primarily associated with higher number of close estuaries, distance from the ocean and mangrove cover, indicating a positive relationship with the continental influence and the availability of estuarine habitats. Marine straggle species were primarily associated with higher values of salinity, depth and transparency, evidencing the relevance of a higher marine influence for that group. Freshwater species and on a lesser extent estuarine resident and semi-diadromous species were associated with the opposite gradients, with the later groups distributed across the environmental gradients, but primarily associated with intermediate conditions. In general, these relationships did not change controlling for the influence of the hierarchical structure, but a greater relevance of the mangrove cover was observed for the semi-diadromous and estuarine resident species. Regarding to the trophic guilds, the species were, in general, distributed across all the environmental gradients. A group of benthophages, planktivores and piscivores was primarily associated with higher salinity, transparency and depth, whereas other group was more associated with a higher number of close estuaries, distance from the ocean, and mangrove cover. The opportunists were primarily associated with lower transparency, depth, and salinity, but also with higher mangrove cover. Controlling for the effect of the hierarchical spatial structure, piscivores were primarily associated with a higher marine influence, whereas a especially benthophages, detritivores and opportunists, but also planktivores were primarily associated with intermediate to higher values of the mangrove cover. Herbivores were also positively associated with the mangrove cover, but primarily with intermediate to higher number of close estuaries. These results evidenced the relevance of disentangling spatial and environmental effects for a better understanding of the major determinants of the structure of fish assemblages in different types of coastal ecosystems. The availability of estuarine habitats was of major relevance for the composition and abundance of species with different degrees of dependence from estuarine areas. Therefore, actions focusing on the conservation of the mangrove cover and the maintenance of the connectivity with close estuaries are critical for fish assemblages in different types of coastal ecosystems.

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Keywords: Hierarchical spatial structure, scale dependence, multi-scale environmental effects, land use, fish, Coastal zone

Seasonal growth variability of young of the year European hake (*Merluccius merluccius*) off NW Spanish waters

Piñeiro A. Carmen, Lorena R. Fernandez, Jose Q. Hervas, Agueda C. Rodriguez and Alberto G. Garcia

Daily growth of Atlantic juvenile hake (*Merluccius merluccius*) from Northwest Spanish Iberian coasts was estimated by counting daily increments of the transversal and sagittal sections of otoliths. The microstructural analysis of otoliths enabled to relate variations of growth rates with the seasonal variability of the upwelling regime. Juveniles were sampled in one of the most important recruitment areas of the species of NW Atlantic Iberia in surveys that were carried out during March and July, 2012. A total of 125 otoliths (sagittae) of individuals ranging from 2 and 25 cm total length was successfully aged between 59 to 443 days. Overall, the growth rates of individuals from March and July were not significantly different (ANCOVA $F(1, 9) = 2.3591$; $p > 0.05$). However, otolith microstructure analysis revealed that juvenile growth show increased growth pulses of varying intensity throughout the year related with variation of hydro-climatic conditions linked to the upwelling regime of the Galician coasts. This hypothesis is supported by the variability of increment widths, used as growth proxies. These show a declining trend from 7.2 to 5.7 μm from June 2011 to January 2012 which is temporally coincident with a similar declining upwelling index pattern (Fig. 1). Furthermore, hatch dates of juveniles were back-calculated from otolith readings and the results indicated that spawning takes place all year round with spawning peaks mainly occurring in winter and summer (Fig. 2).

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Keywords: Hake (*Merluccius merluccius*), age estimation, Daily growth, Juveniles, NW Iberian waters

Simulating trophic impacts of recreational fishing scenarios on two oceanic islands using Ecopath with Ecosim

Lorena Couce, Villy Christensen², Alberto Bilbao-Sieyro¹, Yeray Perez¹ and Jose J. Castro³

A mass-balance trophic model was built to describe the impact between 2006 and 2016 of the different fleets that operate on the marine ecosystem of the Tenerife-Gomera islands. Thirty-one functional groups were defined, corresponding to benthic, demersal, and pelagic domains, also including the deep scattering layer (DSL) and detritus. Keystone index and mixed trophic impacts matrix showed a bottom-up control in the benthic-demersal area while in the pelagic zone intermediate predators such as cephalopods play a bigger role in the ecosystem. Toothed whales, turtles, oceanic sharks and tunas were identified as a potential keystone species in the pelagic domain, and sparids, epidemersals fish, and cephalopods also were considered keystone groups in the benthic-demersal domain. The assessment of the impact that recreational fishermen exert on most groups is highly dependent of methodology used to collect information of captures and effort from anglers. When the most conservative scenario is considered, the impact of recreational fishing on the ecosystem would hardly be relevant in the short-term. However, if considering two other studies based of on-site survey, the biomass of fish groups, mainly serranids, sparids and herbivorous, will greatly decline, coinciding with the overfishing scenario described for the Canary Islands in the scientific literature.

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Keywords: Ecological Indicators, Ecosim, Fishing impact, Network analysis, Recreational fishing, Trophic model

Trophic ecology of European hake (*Merluccius merluccius*) larvae by stable isotopes analysis influenced by seasonal variability of oceanographic conditions off NW Galician waters

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A combined study of environmental variables, and stable isotope analysis (SIA) was performed to compare the trophic ecology of European hake (*Merluccius merluccius*) larvae from northwestern Iberian shelf off Galicia (NW Spain) during the two main contrasting spawning seasons, spring and summer 2012. Distinct oceanographic conditions of each seasons influenced nutrient uptake from the pelagic food web according to the differences observed in nitrogen isotopic signatures not only in larvae but also in micro- and meso-zooplankton fraction analyzed. Isotopic niche widths were estimated using SIBER package (Stable isotope Bayesian ellipses in R) from 39 and 130 larvae from spring and summer respectively. Estimated Standard Ellipse Areas (SEAc) were discussed based on contrasting spawning scenarios with different isotopic baselines. Higher isotopic niche width was estimated in the spring hake larvae according to higher productivity and food availability, suggesting a more euriphagous diet in spring condition comparing to summer season. Lower values together with high variability of $\delta^{15}\text{N}$ in both plankton and larvae were found during spring coinciding with unusually strong upwelling. Inversely, higher nitrogen isotopic signatures were observed during summer suggesting strong nitrogen limitation for primary producers during this season. Distinct carbon isotopic signature reveals differences in the origin of food sources in spring and summer. Both nitrogen and carbon stable isotopes val-

ues vs size or weight relationships observed in spring and summer suggest an ontogenetic diet shift in hake larvae.

Acknowledgements

This study was funded by CRAMER-MICINN.

Keywords: trophic ecology, European hake (*Merluccius merluccius*), Larvae, seasonal variability, NW Galician waters, Isotopes analysis

A web application as a contribution to the risk analysis of the Ciguatera in the Canary Islands

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In Spain the data of the catches by professional fishing sector are managed through the official first sale software (OFSS). This process is only carried out in authorized facilities by the competent authority (Real Decreto 41/2015). In the Canary Islands, these centres have computer equipment with access to the OFSS for the electronic transmission of data to the competent authority. The Ciguatera Fish Poisoning (CFP), commonly known as ciguatera, is the main disease caused by marine nature toxins worldwide (Friedman et al., 2017). After the first endemic cases in humans reported in the Canary Islands in 2004 (Pérez-Arellano et al., 2005), it is currently considered an emergent disease of urgent mandatory declaration (Orden de 17 de agosto de 2015). According to Sanchez-Henao et al. (2019) the Canary Islands are already an area of endemic expansion of the CFP. Since 2009, the Directorate General of Fisheries (DGP) of the Canary Islands Government monitors catches of professional fishing in accordance with an official protocol that considers certain species and weights. The objective is the control of fishing species potentially susceptible to being carriers of ciguatoxins and, depending on the analysis, decides whether the fish can be incorporated into the commercial chain or not. The communication process - between fishing operators, administration and laboratory - about the presence or absence of ciguatoxins in fresh fish is done through e-mail and paper documents. Among the legal competences of the DGP are the control and monitoring of fishing activities in relation to landings and first sale, in coordination with other competent administrations. In addition, DGP promotes and supervises studies of interest for the Autonomous Community in fisheries matters. Therefore, one of the tasks developed within the framework of the Mimar Project - <http://mimarproyecto.com> - is the development of a web application (desktop and mobile phone) to work in an integrated way with the OFSS. We intend to automate certain processes related to the evaluation and communication on the presence (or absence) of ciguatoxin in fresh fish. We specifically seek (i) to generate suitable formats for data recording, (ii) to have a traceability interface to consult in real time what stage each sample is in, (iii) configure communications between the different actors, (iv) export of records, etc. This communication shows the information flow charts that arise in the application between the fishing operators, the administration and the laboratory, all in an integrated way with the OFSS. The next steps (from May 2019) will be the presentation and use of the application in an association of professional fishermen (as a point of first sale), and after the identification of problems, its implementation as a pilot action in all authorized establishments of first sale in the Canary Islands. Then, adaptations could be made in the flow charts for each of the partner regions of the Mimar Project, that is, Madeira, Senegal, Mauritania and Cape Verde. In short, the proposed integration can lead to an efficient improvement both in the communication and in the evaluation of the CFP within the framework of the food security risk analysis (FAO/WHO, 2016; EFSA, 2017). If a satisfactory experience is achieved, it could be implemented in the current official ciguatoxin monitoring system carried out by the DGP in the Canary Islands.

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Keywords: Ciguatera fish poisoning (CFP), Risk analysis (RA), Professional fishing, Canary Islands - Spain, Web application

Age and growth of chub mackerel (*Scomber colias*) in North and Northwest Iberian waters based on direct age estimation and back-calculation in otoliths

Maria Rosario Navarro, Jorge Landa, Begoña Villamor, Carmen Hernández and Rosario Dominguez

Atlantic chub mackerel (*Scomber colias*, Gmelin, 197) is a middle-sized pelagic fish distributed in warm and temperate waters. Its landings have significantly increased in the most recent years in the Iberian Peninsula, possibly related to the rise in its abundance due to an increase of the water temperature, and resulting a new target species for both Portuguese and Spanish purse seiner fleets. This has increased the EU interest in its biological knowledge in order to launch its stock assessment in the near future. The study of this species is framed within the Spanish National Program of collection, management and use of data in the fisheries sector and support for scientific advice regarding the Common Fisheries Policy. Based in samples from commercial catches and scientific surveys between 2011 and 2017, this work studies the age and growth of chub mackerel in North and Northwest Iberian waters. The age of the specimens were estimated by interpreting the annual increments (annuli) of the otoliths based in the age estimation criteria for this species established during the first ICES Workshop on Age Reading of Chub Mackerel otoliths, WKARCM. The consistency of the age estimations was checked by international otolith exchanges among international readers of different institutions. Age-length keys were here performed by direct age estimation and length-at-age values from two consecutive years were also estimated by backcalculation, providing consistency to the age estimation. Von Bertalanffy growth parameters, based on direct otolith observation and backcalculation, were estimated and compared with those of previous studies. In addition, the annual length distributions of the catch showing interannual variability are presented. Length/weight relationship, and mean length and weight at age were also estimated. The age and growth results here obtained are now available for the analytical stock assessment.

Keywords: Age-length key, Length - weight relationship, length distribution, Growth parameters, Back-calculation
Topic: Fisheries, Aquaculture and Biotechnology

Age, growth and reproductive biology of the tub gurnard (*Chelidonichthys lucerna*) in North-East Portugal

Juliana Rodrigues, Diana Feijó, Alberto Rocha, Karim Erzini, and Alberto T. Correia,

The tub gurnard, *Chelidonichthys lucerna*, is an important commercial marine demersal fish species that belongs to Triglidae which can be commonly found in the north-eastern Atlantic, in the Mediterranean Sea and in the Black Sea. *C. lucerna* is mainly caught through bottom and beam trawls, gillnets, trammel nets and long-lines. Within the gurnards, *C. lucerna* is the most important and the one with the greatest economic value in the Portuguese coast. The aim of the study is to obtain, for the first time, data on the age, growth, sex ratio and sexual developmental stages of *C. lucerna* captured on the northern coast of Portugal (Matosinhos) bimonthly between June 201 and May 2019. Moreover, we intend to test whether these basic biological traits, at present unknown for this species in the southern area of the NE Atlantic, differ from other geographic locations. These new findings are important to a sustainable and rational fisheries management.

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Keywords: Triglidae, Fisheries, life history traits, age, Reproductive biology

An overview of small-scale fisheries in the Northern Portuguese coast

Cláudia Mendes, Sérgia C. Costa-Dias, Catarina Teixeira, António Afonso and Adriano Bordalo

Small-scale fisheries play a major role in the Northern Portuguese Coast with small vessels (<10 m) comprising 70% of the fisheries licenses in 2017. Multi-gear are common among small-scale fisheries and deploy a combination of a variety of fishing gears. Despite the socio-economic relevance of multi-gear fisheries in the Northern Portuguese Coast, data focusing on different fishing gear types and respective target species is still scarce. The project "RecBio - Contribution to the management and conservation of the biological resources of the Portuguese North coast", aims to integrate scientific data and local knowledge from fishermen communities, towards a participative management plan contributing to the conservation of marine biological resources in the Northern Portuguese coast. In a first approach, we reviewed literature data on local fisheries over an 0 km coastal stretch, from Caminha to Matosinhos. Main fishing gears and target species were identified and will provide the basis for further actions. A time-series between 1995 and 2017 (INE 2019) was used to investigate temporal trends of the nominal catch (in metric tons), in the most officially important fishing harbours: Viana do Castelo, Póvoa do Varzim, and Matosinhos. The major harbour of Matosinhos (17 042 t) represented the majority of the total landings of the Northern Portuguese Coast (20 706 t), while the smaller harbours of Viana do Castelo and Póvoa do Varzim, accounted for the remaining (2 397 t, and 1 267 t, respectively). The landings composition differed between these harbours when considering the average nominal catches from 1995 to 2017 (Figure 1). The sardine *Sardina pilchardus* was the main species in Matosinhos (15 60±6 476 t), and Póvoa do Varzim (1 056±1 499 t), and a major species landed in Viana do Castelo (334±275 t). Sardine catch has been decreasing drastically through the years, being the stock condition addressed by the European Union through fishing quotas. The octopus *Octopus vulgaris* fisheries assumed a major role in the small harbours of Póvoa do Varzim (420±223 t), and especially Viana do Castelo (565±1 t), where it represented the most landed species. The Atlantic horse mackerel *Trachurus trachurus* was also an important component of the fisheries in the Northern Portuguese Coast (Viana do Castelo: 15±129 t; Póvoa do Varzim: 207±116 t; Matosinhos: 3 023±1

934 t). In Matosinhos, the Atlantic chub mackerel *Scomber colias* (1 336±1 46 t) catch has been increasing. Considering the fishing gears, purse-seine (73%), and multi-gear (1%) were the dominant fisheries in Matosinhos in terms of total nominal catch (INE 2019). Purse-seine fisheries are mainly used for sardine fisheries, as well as the Atlantic horse-mackerel. In the other two harbours, multi-gear fisheries accounted for 1% of the total nominal catch in 2017 (INE 2019). Main fishing gears used by small-scale fisheries in the North of Portugal comprised longline fisheries for conger and pouting, trammel nets for flatfish *Solea* spp., flounder *Platichthys flesus* and rays *Rajidae*. Artisanal traps are used for octopus fisheries. Gill nets were also used for pouting, hake, and sardine fisheries (Gaspar et al. 2014). Fisheries patterns of the target species have been investigated in the Portuguese Coast (e.g. Leitão et al. 2016; Lourenço and Pereira 2006; Pita et al. 2015), although most studies have primarily focused on the Southwestern and Southern Portuguese Coast (e.g. Braga et al. 2017; Sonderblohm et al. 2017). Therefore, further studies are required to ascertain spatial and temporal trends in the use of these artisanal fishing gears in the Northern Portuguese Coast. In the future, this information could be integrated with knowledge on the biology and ecology of the main targeted species (e.g. Malta et al. 2016; Moreno et al. 2014; Santos et al. 2011), as well as valuable inputs from local fisherman communities towards the co-management of the marine biological resources of the Northern Portuguese Coast.

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Keywords: Fisheries, Artisanal, Fishing gears, Multi-gear, Fisheries policy, CatCh, landings

Analyses of the sex ratio for bullet tuna, Atlantic bonito and little tunny from Portuguese and Spanish waters

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INTRODUCTION The small tuna species *Auxis rochei* (bullet tuna), *Sarda sarda* (Atlantic bonito) and *Euthynnus alletteratus* (little tunny) are widely distributed in Atlantic and Mediterranean waters. Although these species have a high socio-economic relevance for a considerable number of local communities at the regional level, the status of these fish stocks is unknown. Information on life history traits should be considered to develop realistic models to assess fish stocks; these models could then be used as the basis for the sustainable exploitation, management, and conservation of fish species. Sex ratio can be male- or female-biased with increasing length or age. In general males of marine fish species predominate in small length classes while females predominate in the large ones. However in species of the genus *Thunnus*, which are large tunas, males are more numerous in the larger length classes. Bullet tuna and Atlantic bonito from the Iberian Peninsula are belong to the same populations (Ollé et al., 2017., Viñas et al., in press), whereas no genetic studies have been addressed for little tunny. The aim of this study is to analyse the sex ratio for the three mentioned small tuna in the western Mediterranean Sea and Atlantic margin of south Iberian Peninsula. **MATERIAL AND METHODS** Individuals of bullet tuna, Atlantic bonito and little tunny were collected from 32 localities throughout the Mediterranean Sea (Spanish coast) and Atlantic Ocean (south of the Iberian Peninsula) between 2003 and 2017 (Figure 1). The individuals of the three species were caught by both commercial and recreational fisheries. Fork length (FL) was measured either to the nearest 0.1 cm (when fish was measured in the lab or at landings) or to the nearest 0.5 cm (fish measured on board). A total of 3929 fish (bullet tuna, 120; Atlantic bonito, 13; little tunny, 1261) were sampled. The sex was identified and classified as male, female and undetermined (individuals with small gonads, which sex cannot be identified visually) (Table 1). Sex ratio was calculated as the proportion of females by 2 cm FL class. The observed female proportions by length class were modelled by means of a binomial generalised linear model (GLM). Statistical differences between length classes were assessed by means of the chi-square test. **RESULTS** A total of 16 bullet tuna were classified as undetermined. The size of males ranged between 26.0 and 4.7 cm FL (n = 540) and females ranged between 24.0 and 47.5 cm FL (n = 554). Significant differences in the proportion of females were found between length classes ($\chi^2 = 23.54$, df = 12, p = 0.02). The sex ratio was 1:1 in the length class group between 34 and 36 cm FL. Females predominated in length class less than 26 cm FL. The analysis of the predicted sex-ratio by length showed a trend of abundance of males in the larger length classes (Figure 2a). A total of 197 Atlantic bonito were classified as undetermined. The size of males ranged between 25.6 and 79.3 cm FL (n = 520) and females ranged between 23.9 and 71.0 cm FL (n = 671). Significant differences in the proportion of females were found between length classes ($\chi^2 = 39.7$, df = 23, p = 0.02). The analysis showed that females predominated in almost all of the length class groups. The sex ratio was 1:1 in the length class group between 40 and 42 cm FL. Predicted sex-ratio by length is shown in (Figure 2b). A total of 34 little tunny were classified as undetermined. Two intersex fish were sampled in the trap located in Mazarrón (for details, see Macías et al., 2014). The size of males ranged between 30.6 and 101.0 cm FL (n = 414) and females ranged between 2.5 and 99.0 cm FL (n = 461). Significant differences in the proportion of females were found between length classes ($\chi^2 = 73.49$, df = 36, p = 0.0002). The analysis showed that males were more abundant in the larger length classes (> 96 cm FL). Predicted sex-ratio by length is shown in (Figure 2c). **CONCLUSIONS** Females predominated in small length classes; however, this result should be considered with caution because for the smallest fish (i.e. tiny gonads) the ovaries are easier identified than testes. The analysis of sex ratio by length for both bullet tuna and little tunny indicated that females predominated in the smaller length classes and males in the larger length classes. Specifically, for the Atlantic bonito the trend showed that were consistently more females than males across all sizes classes.

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Keywords: Sex ratio, *Auxis rochei*, *Sarda sarda*, *Euthynnus alletteratus*, Western Mediterranean, Atlantic Ocean

Body morphology as a tool to infer about the population structure of bigtooth corvina (*Isopisthus parvipinnis*) in the SE Brazilian coast

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Information on population structure of aquatic organisms is a prerequisite for rational fisheries management and conservation strategies. Fish's morphometric characteristics are measurable variables that result from an evolutionary process and adaptation to the environment. It means that phenotypic variation between fish individuals is primarily due to heritability of genetic factors modulated by surrounding environment. This work aims to investigate if the different environments between São Paulo (North – NSP, Central – CSP, and South – SSP) and Paraná (PR) in Southeast Brazilian coast, could affect *Isopisthus parvipinnis* body morphometry, revealing important information about its population structure. Fish between 0 and 140 mm of total length were captured between September and November 2011 by local fishermen using an otter trawl. After collection, fish with selected arbitrarily landmarks were photographed and morphometric analysis were performed using tpsUtil and tpsDig softwares. Data was tested for allometric growth with ANCOVA, and analyzed through uni and multivariate analyses. Results showed a high overall re-classification rate (95%), with low overlapping between individuals from NSP and PR. The hereby data suggest that *I. parvipinnis* populations from the four regions are somewhat distinct. However, as a preliminary study, it is recommended the use of complementary information, namely other natural tags, to corroborate the observed results.

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Keywords: Fisheries, Sciaenidae, Natural tag, tpsDig, Population-Units

Catch and yield trends of the Portuguese purse seine fishery (2006-2011)

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The purse seine fishery is one of the most important fisheries in Portugal, accounting for about 50% of landings in weight. Historically, the sardine (*Sardina pilchardus*) is the target species of the purse seine fleet. However, with the decline of the sardine stock and the reduction of annual quotas, this fleet has supplemented its yields by capturing other pelagic species such as chub-mackerel (*Scomber colias*), horse mackerel (*Trachurus trachurus*) and anchovy (*Engraulis encrasicolus*). This work aims to investigate changes in the purse seine fleet activity and catch and landings compositions associated with the decline of sardine, including inter-annual variations of fishing effort, catch, landings and slipping compositions, using on-board observations from 2006 to 2011. The Portuguese mainland coast was split in three main areas: North, Centre and South (from Viana do Castelo to Olhão, as describe in Feijó et al., 2011). During the study period (2006-2011), the observers were trained to follow the same standard protocol (Feijó et al., 2012) on-board commercial vessels (Length-overall >16 m). Most trips were carried out within the framework of PNAB/EU-DCF. Since 2010 and in the ports of Peniche, Olhão and Portimão, 0 trips were performed within the scope of SAFESEA and Life+MARPRO projects (Marçalo et al., 2015). Data were collected on fishing set characteristics (number, depth, geographic position), fishing activities (trip total duration and time spent in steaming, searching, fishing and resting as described in Feijó, 2013) and catch compositions (total biomass and species composition for retained, slipped and discarded catch). Each net deployment was counted as a fishing set. Between 2006-2011, more than 400 trips and 450 sets were recorded, corresponding to 0.5% of the total fleet effort (Silva et al., 2015). On-board observation work was carried out in more than half of the national fleet. Fishing occurred throughout the year but effort decreased between November and April due to voluntary area closures (in the North and Centre areas, fishing stops in February and March), sardine ban restrictions (2012-2011) and bad weather conditions. In earlier years, most trips were around 9h long with one to two sets observed. During the study period, there was an increase in the number of sets (3-4 sets) performed and time dedicated to searching in order to obtain the same catch and yields. Sardine catches and landings declined mostly due to quota restrictions, and were compensated by the increase of chub mackerel catches and landings. Changes in the target species were also observed although sardine was still the main species captured. In the 2017 and 2011, an increase in anchovy catches and landings was also observed. The reduction of annual quotas and establishment of daily limit quotas led to the increase of slipping, creating the differences between catches and landings. We observed behavior changes in the fleet along the country, such as a search for new fishing grounds and species with more market value. Our results are of interest to stakeholders as they provide a perception of changes in the fishery at the national and regional level.

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Keywords: Purse seine, Catches and landings, Slipping, Sardine (*Sardina pilchardus*), Chub mackerel (*Scomber colias*)

Common octopus (*Octopus vulgaris*) fisheries in Andalusia Mediterranean coast: Trends in fleet dynamics and the population structure of stock

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In the last years cephalopods are increasing in economic importance and in consequence there have been a rise in their demand and their global landings (Hunsicker et al. 2010). In Andalusia (Mediterranean coast) *Octopus vulgaris* is one of the most important marketed cephalopod with a great socio-economical importance in the local fisheries being the fourth one in landings of all species (6.2% total weight of landings) and the first one in total landed value (16.63% of the total first sale value) (Official Regional statistics 201). Common octopus is mainly fished by coastal trawlers (OTB) and artisanal fishery focused in common octopus catches with pots and traps (FPO). Also a small percentage are captured by small-scaling fleet with trammel nets, hooks and other fishing gears. Trawling fleet operates deeper than 50 m only in soft, sandy or muddy bottoms and landing a wide range of commercial species. Artisanal fishery fleet targeting *O. vulgaris* operates in any type of bottoms in coastal areas. These fisheries are regulated by nationally and regionally rules, nevertheless octopus is a difficult resource to manage due to their biological characteristics (short life span, rapid growth, high natural mortality and sensitivity to environmental conditions with highly recruitment success) (Pierce et al. 2010, Vargas-Yañez et al. 2009). The aim of this study is to improve the knowledge of fishery dynamics and the population of the octopus stock in order to contribute to its better management for a sustainable exploitation. Study area comprises the Mediterranean coast of the Andalusia Region. Data of fleet and landings during 2002-2017 were obtained out of the annual official fisheries statistical. In this work, fishing effort was measured in terms of number of trips with octopus landings. Total body weight, recorded to the lower 100 g, was selected as a measure of size. Biological data were obtained from at sea and at market monthly samplings of the two main fleets targeting octopus (trawlers and pots), during 2010-2017. The sampling was located along the coast, from Estepona to Almería. To standardize the individual size with others publications, dorsal mantle length (DML) was calculated applying the length-weight relationship: $W=a*Lb$, with a (2.0427) and b (2.4204) estimated via ordinary least-squares regression (González M. Pers. Com). A statistical analysis of the data was carried out with Excel software. Operational fleet landing *O. vulgaris* in the study area was composed of 19 OTB and 90 FPO vessels in 2002 and while OTB fleet decreased to 95 vessels in 2017, FPO fleet increased to 17. Regarding the fishing effort, it decreased from 1063 trips (57 trips/vessel) in 2002 to 9320 (9 trips/vessel) in 2017 for OTB and steadily increased from 1329 (15 trip/vessel) to 9647 trips (54 trip/vessel) for FPO. Total annual landings have fluctuated widely from 716 t to more than 1400 t along the time-series (2002-2017) with a maximum of ~140 t in 2013. Overall, OTB landings decreased over time (with a maximum of ~700 t and minimum of ~220 t) while FPO landings increased significantly and were greater than OTB since 2012. The average in terms of first-sale price for landings increased in the same period from 4.5 €/Kg to more than 7 €/Kg. Octopus is fished all along the coastline although ports located in the occidental coast had the larger landings. The most representative ports in terms of total landings were Caleta de Vélez (229 ± 19 t·y⁻¹ with a contribution of 51 % for OTB and 49% of FPO), Fuengirola (154 ± 10 t·y⁻¹, 40% OTB and 60% FPO), Estepona (153 ± 15 t·y⁻¹, 50% of both OTB and FPO) and Marbella (115 ± 10 t·y⁻¹, 57 % OTB and 43 % FPO). According to the intra-annual population dynamics, *O. vulgaris* showed a visible seasonality in landings although it differs between gears. For OTB the highest landings were in the first quarter of the year, while the highest landings of FPO were in July. The lowest landings were in September-October for both gears. In general terms the yield, landing per unit of effort, was higher for FPO (annual average 64 ± 3 kg·trip⁻¹) and in both gears it started to increase up to September with a maximum in late spring. The population structure of *O. vulgaris* display gear-related differ-

ences. Commercial size range during 2010-2017 were 12-32 cm DML for trawlers and 12-29 cm for pots, while the corresponding modal size were 13-14 cm and 13-16 cm, respectively. Pots captured larger specimens (average 16.15 ± 1.25 cm) than trawlers (average 14.4 ± 1.35 cm) ($t=2.6$; $p=0.005$). Considering the average size by month the larger individuals were catch during the summer in both cases even though for pots the largest specimens were landed in July and the size decreased until October while for OTB the largest were in September and the size decreased until November. Intra-annuals landings and population structure in both gears reveal a marked seasonality reflecting the octopus' annual life cycle described by several authors (short life span, rapid growth and high sensitivity to the recruitment to environmental conditions)(Guerra 191, González et al. 2011, Quetglas et al. 2015). In the study area the spawning season takes place from late spring to the end of summer (Tirado et al. 2003, García-Martínez et al. 2017), which results in higher landings and higher average individuals weight specially in FPO. After, the adults die while juvenile-recruitment settle in September to November and incorporate to fisheries from late autumn to winter. The recruitment of young individuals to the fishery and their fast growth is reflected in the increase of catches of both fisheries from late winter to late spring. In conclusion, from 2002 the number of trips targeting octopus have been multiplied by 7 in FPO and they have been stabilized in OTB. However, after 2013, landings have not increased according to the effort, and the productivity of trips are reducing every year. In addition, the growing of their economic value makes octopus more valuable for fishermen what could increase the risk of overexploitation.

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Keywords: Mediterranean, northwest, Octopus (*Octopus vulgaris*), Otter trawl, Artisanal fleet, Fisheries

Estimating composition of mixed species landings: case study of gurnards in the Portuguese coast

Diana O. Feijó, Alberto Rocha, Rita P. Vasconcelos and Patricia J. Gonçalves

Landings of gurnards (Triglidae) are often not sorted by species and are usually classified in official sales notes and statistics under one generic commercial category (“gurnards”) not discriminated at species level. Across the NE Atlantic, the generic classification of “gurnards” can include eight species. Concurrent sampling of landings at-market under the Data Collection Framework (DCF) is a reliable source of data concerning the composition of gurnards at the species level. To estimate the annual landings of gurnards at the species level for the period 2017-201 in Division 9a, we used data from the Portuguese national sampling program (PNAB) that collects fisheries data in the context of the DCF. We applied multivariate analysis and statistical modeling approach to explore the observed species compositions of landings and the relationships with fishing métiers, temporal and spatial effects, aiming at finding means to describe gurnards landings species composition based on other variables. Results will be useful for analysis of gurnard landings time series and also for

the design of fisheries sampling programs where similar issues occur.

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Keywords: Fisheries, Concurrent sampling, Gurnards, Unsorted landings categories, Triglidae

Fishery artisanal monitoring of the communities of Jaqueiral, Barreta Island, Pescaria Island, Rio do Campo and Timbuca island (2017 - 201), Serinhaém channel, Camamu Bay, Bahia state, Brazil

Rogério C. Santos, Henry L. Spach, Juliana C. Rodrigues and Alberto T. Correia

In the Serinhaém channel of the Bay of Camamu, Bahia state, Brazil, the artisanal fisheries present a high economic and social relevance for the fishermen communities. The main objective of this work was to monitor, for the first time, the landings of the seafood caught in the communities of Jaqueiral, Barreta Island, Pescaria Island, Rio do Campo and Timbuca Island. The information was collected directly in the field with the fishermen through semi-structured questionnaires, applied in the ports of the communities, at the time of the landing of the fish. A total of 104 questionnaires were applied monthly in the communities during one full year, between October 2017 and September 201. Data showed that most fishermen perform their fishing practices individually or in pairs, using fishing gears such as a gill nets (e.g. "redes de emalhe") or traps (e.g. "armadilha tipo manzúa"), operating from small wooden boats ("canoa de madeira"). The main form of propulsion used by the small boats is a pulp engine, powered by gasoline. Some species are more frequently captured, such as mullet (*Mugil Sp*) and siri (*Callinectes spp*), but the other species are also economically important. This study presents estimates of the catch per unit effort for each community involved in the survey, evidencing the financial net income obtained with the fishing activity. The data obtained from this study is fundamental for the planning and implementation of adequate public policies in the artisanal fishing sector.

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Keywords: Fisheries monitoring, Artisanal fishery, Fishermen Communities, Camamu Bay, Bahia state

Incidence of fisheries impact and purification treatment in the survival rate of wedge clams caught in the northern Alboran Sea

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The wedge clam *Donax trunculus* Linnaeus, 175 is one of the most demanded commercial bivalve molluscs targeted by the artisanal fleet using mechanized dredges in the western Mediterranean Sea. Recently, the damage caused by this type of fishing gear in the shell and foot of wedge clams, as well as the survival of the species after treatment in a purification centre for the detoxification of specimens (in accordance with Annex II of Regulation (EC) No 54/2004), was evaluated by the Spanish Institute of Oceanography. Samples were collected on board commercial vessels using mechanized dredges in two sites (Fuengirola and Caleta de Vélez, southern Spain; Figure 1), and in the case of Fuengirola on two types of bottoms that differed in the hardness of the substrate (called as "hard bottoms" and "soft bottoms" by fishermen), and at three towing speeds: i) the usual as during normal commercial fleet operations (0.3 knots); ii) at a slower speed (0.1 knots); and iii) at a faster speed (0.5 knots). All hauls were replicated (n= 3) for a total of 36 hauls. Individuals displaying any external impact signal (e.g. dislocated or fragmented shells) and/or any damage in the foot were recorded and quantified. Additionally, a determination of the benthic fauna inhabiting fishing grounds was done from discard samples collected in the analyzed commercial hauls (n= 12). Finally, sediment samples (n= 3) were collected from the "soft" and "hard" bottoms for granulometric characterization. On the other hand, the survival of the commercial wedge clams was monitored upon arrival at port, where the number of live and dead (specimens with open, dislocated or fragmented shells, and/or that did not respond to mechanical stimulation) wedge clams was determined. Samples were then transferred to the purification centre, where they remained for 24 hours (as usual), after which they were checked once again, and kept refrigerated (4°C) for 96 hours, making survival checks every 24 hours. Kruskal-Wallis analyses were used to compare results between substrates, sites and towing speeds. The wedge clams caught in Caleta de Vélez displayed a higher number of specimens with dislocated or fragmented shells (3.6%) than in Fuengirola (0.25%), with the highest values observed for normal and fast towing speeds (between % and 3%) in hauls carried out on substrates with a high pebbles content (ca. 0% of the inert material collected by the dredge). Regarding the damage in the foot of wedge clams, a significantly higher proportion of damage was observed in individuals caught in Caleta de Vélez (n= 360; 22.03%) than in Fuengirola (n= 360; 9.93%), for hauls carried out at low and fast towing speeds (Kruskal-Wallis: p< 0,001). This damage ranged from small shallow cuts to total amputations of the foot (Figure 2). In both sites, the proportion of damaged individuals was lower in the case of slow towing speeds. In relation to the type of substrate ("hard" versus "soft"), results did not display significant differences (Kruskal-Wallis: in all cases p>0.05). The amputation of the foot can be mainly due to 1) predation by certain decapod crustaceans (crabs); and 2) self-mutilation induced by stress caused by capture. Our observations confirms the incidence of these two factors, since there are injuries compatible with an amputation caused by a fast and powerful closing of the valves (comarginal cuts at the edge of the shell), as well as deep perpendicular cuts at the margin of the foot that are characteristic of the mechanical action of a crab's chelae. In this line, a greater abundance of predatory crustaceans was found in samples from Caleta de Vélez (ca. 19% of the total biomass corresponding to *Liocarcinus vernalis* and *Portunus latipes*) in comparison to samples from Fuengirola (ca. 6%). The proportion of living individuals after treatment in the purification centre ranged between 9.% and 100% in all cases. Additionally, three subsamples of commercial wedge clams caught in this study were kept in purification for 72 hours, displaying in all cases a survival rate higher than 99%. Similarly, the survival rate ranged between 94% and 100% after three days in refrigeration (4°C). On the other hand, controls (subsamples of wedge clams with no purification treatment) that were kept in refrigeration during four days displayed survival rates between 92% and 100%. The results of this study show that (1) a higher proportion of damaged shells could be related to a higher trawling speed and higher pebbles content on the seabed; (2) the damage presented in foot, whether caused by amputation by predation or by self-mutilation by stress, does not influence the survival of most specimens within 96 hours after fishing, which is derived from the high survival rate observed in this experience (>95%); (3) the purification treatment applied to the wedge clams does not seem to affect its survival rate, with very high values that did not decrease after refrigeration at the usual temperature of storage of shellfish resources. If the cold chain is kept intact and the shipping conditions are correct, wedge clams should remain in good condition for consumption until at least five days after capture.

Acknowledgements

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Keywords: Donax, Mediterranean Sea, Survival, Fisheries Impact, Wedge clam

Influence of the change of trawling otter boards on demersal species' catches

Fabio Blanco Murillo and José L. Sánchez Lizaso

Mediterranean fisheries are characterized by being multi-specific and having several target species. In terms of the number of vessels and total catch, one of the most important fleets is demersal trawling. This fishery has a particularly complicated management due to its environmental impact and low selectivity. Along the Spanish Mediterranean, its main target species are the European hake (*Merluccius merluccius*), the red shrimp (*Aristeus antennatus*), blue whiting (*Micromesistius poutassou*), mullets (*Mullus* spp.) and Norway lobster (*Nephrops norvegicus*). Demersal fisheries in the Mediterranean Sea are heavily overfished and, the Common Fisheries Policy has tried to improve its sustainability. One of its measures are subsidies of any modification of a fishing gear which improves selectivity, reduces environmental impact or fuel consumption but in any case, this modification must increase the fishing effort on target species ((UE) 50/2014). One of the modifications which have benefited from these subsidies are pelagic otter boards. These otter boards reduce the impact on the seafloor and fuel consumption but their effect on the catches of target species has not been assessed. To this end, five vessels of three different ports of Alicante (Denia, Altea and Santa Pola) which changed their conventional trawling otter boards to pelagic ones were studied and compared with five vessels of the same characteristics which did not change their otter boards. Landings of 10 days during two years before the otter board change and two years after the change were compared to assess its influence in catch composition. The nMDS did not show differences in capture composition due to the high variability of trawling catches shadowing the possible effect of otter boards. An analysis of variance (ANOVA) was performed to assess differences in total daily catch and for the 5 main species of each port. ANOVA did not show significant differences in total landing weight in Santa Pola and Altea although, in Santa Pola, pelagic otter boards seem to increase their captures compared with conventional ones. In the case of Denia, the interaction between the Before/After and the otter board type factors was significant, which means captures increased after installing the pelagic otter boards. These results show that the effect of pelagic otter boards could depend on the target species. In Denia, the main target species was the red shrimp, which showed a marginally significant ($p < 0.1$) increase in catches in boats using pelagic otter boards, becoming a possible sensible species to the otter board modification. Probably the pelagic otter boards may expand the net opening which could increase catchability on certain species making this modification a possible negative effect on demersal species despite the benefits of fuel consumption and the lower environmental impact on the sea bottom.

Keywords: Mediterranean fisheries, Demersal trawl, Otter board, catchability, Fisheries Management

Meristic differences between juveniles of two tuna species from the Western Mediterranean Sea

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Bluefin tuna (*Thunnus thynnus* Linnaeus 175) and little tunny (*Euthynnus alletteratus* Rafinesque 110) are highly migratory species that inhabit Atlantic Ocean and Mediterranean Sea. The Mediterranean Sea is a spawning area for both species (Rodríguez-Roda, 1964; Oray and Karakulak, 2005). 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 it is expensive, time consuming and results are not immediate. The objective of this study is to identify meristic characters to discriminate between juvenile of bluefin tuna and little tunny. Juvenile of bluefin tuna and little tunny were caught between August and September from 2016 to 2017 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 5 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). 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). Eight meristic characters were recorded: first and second dorsal fin rays, anal rays, pectoral rays, dorsal and anal finlets, left and right side gill rakers (table 2). A pin and a dissection needle were used to improve the count accuracy. Gills were taken out from the head to count the gill rakers. The results of the analysis for bluefin tuna and little tunny are shown in the table 3. The number of first dorsal rays is the unique variable that does not overlap between both species (bluefin tuna 13-14 vs little tunny 15-17). The highest variability was found on the anal rays for bluefin tuna ($\text{CV} = 6.79\%$) and on the second dorsal rays for little tunny ($\text{CV} = .69\%$). The number of dorsal finlets range between 7 and 9 for both species. The counts of meristic characters are shown in table 4. Second dorsal rays on bluefin tuna has a probability of 96% of being included in the range 13-15 and little tunny has a probability of 94% of being included in the range 11-13 rays. The number of pectoral rays in bluefin tuna has a probability of 97% of being equal or more than 30 rays, and in little tunny the probability to be less than 30 rays is 100%. The anal rays in juvenile bluefin tuna has a probability of 9% of being included in the range of 12-15 rays and little tunny has a probability of 99% of being included in the range of 11-14 rays. The number of gill rakers was similar in both right and left sides. Bluefin tuna has between 35 and 43 gill rakers, and little tunny between 33 and 42; which indicates that gill rakers are not useful in identifying these two species. The number of dorsal and anal finlets are shown in table 5. When comparing the counts of dorsal and anal side we found: a) one more finlet on the dorsal side than in the anal side (9 vs , vs 7, 7 vs 6) in 73% of bluefin tuna and 93% of little tunny; b) the same number of finlets in both dorsal and anal side (9 vs 9, vs , 7 vs 7) in 24% of bluefin tuna and 2% of little tunny and, c) two more finlets on the dorsal side than on the anal side (9 vs 7) in 2% of bluefin tuna and 5% of little tunny. Our findings are in line with those of Pruzinsky (201), who reported that pectoral rays in little tunny were less than 30 rays in 100% of individuals. Regarding genus *Thunnus*, Pruzinsky (201) reported 100% of individuals with equal or greater than 30 pectoral fin rays, but our results showed 3% of bluefin tuna with less than 30 pectoral fin rays. In conclusion, the number of first dorsal fin rays is the unique meristic character analysed in this study that can be used to discriminate juvenile of bluefin tuna and little tunny.

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Keywords: Meristic characters, Taxonomy, *Thunnus thynnus*, *Euthynnus alletteratus*, Juveniles

Interacting environmental effects influence early body condition of a colonial waterbird living in complex coastal environments

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Environmental influences at early growth stages are increasingly well documented across a variety of taxa and often involve life-lasting developmental effects. Nevertheless, it is still not clear how most of the natural environmental stressors accrue, and how the organisms cope and respond to such environmental pressures in the wild. In animals, body condition indexes provide excellent metrics of how individuals respond to their environment, but their typical use tends to focus on single environmental variables that portrait an unrealistic uni-variate environment. In this study, our aim was to assess the multi-variate effects of a complex coastal environment on the condition attained by chicks of a focal colonial waterbird species: the Glossy Ibis *Plegadis falcinellus*. Specifically, we studied chicks from a growing breeding colony in the Camargue, in southern France, where the number of breeding pairs has increased from 14 in 2006, to more than 2,000 through to this day. This coastal region hosts one of the largest Mediterranean wetlands of Europe and is an important hotspot for hundreds of breeding and migratory species that profit from its high marine connectivity and coastal water dynamics. We first quantified inter-annual variation on the body condition of chicks over a 12-year long-term study period (from 2006 to 2011), while assessing the potential variation within each year owing to seasonal effects. We then tested for interacting effects of three locally relevant environmental parameters of interest in this coastal environment (the mean temperature, the rainfall and the wind) on the condition attained by the chicks of our focal species. We present and discuss our results in the light of coastal environment complexity and its relevance for coastal wetlands and waterbirds.

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Keywords: Environmental predictors, body condition, Coastal environments, wetlands, waterbirds

Rays in bottom trawling and trammel nets in south western European waters: discards, survivability and implications for the application of the landing obligation

Julio Valeiras, Blanca Álvarez-Blázquez, Eva Velasco, Esther Abad, Mateo Barreiro, Nuria Lluç, Gloria Cordeiro, Jose A. Neira, Alberto Otero3 and Maria B. Santos

We present the results of a study to investigate the survivability of rays caught by bottom trawlers and trammel nets in northern Spanish waters. Trials were carried out to evaluate the survival rates of rays that would be discarded since they are too small or not the target of the fishery. The trials consisted of: (a) an assessment of the vitality of the individuals brought on board and (b) a long-term study of survival after capture in the lab (to where the animals caught were transported). Our results indicate that ca 93.5% and 100%, respectively, of the sampled rays captured by bottom trawlers and trammel nets survive the fishing operation and handling onboard. For bottom trawls, 62% of the thornback rays (*Raja clavata*) and 0% of the cuckoo rays (*Leucoraja naevus*) were recorded as in 'Poor' health condition. Estimated survival at medium term was 5% (47.7-69.9)

and 17% (10.1-27.4) at the end of the observed period (one month). Trammel nets captured four species: the thornback ray, the spotted ray (*Raja montagui*), the small-eye skate (*Raja microocellata*) and the undulate ray (*Raja undulata*). 12.9% of rays were recorded as in 'Poor' health condition. Estimated survival at medium term was 100% and it was 73% (56.3-93.9) at the end of the observed period (one month). Thornback ray scored the lower survivability (33.3%), spotted ray intermediate (3.3%) and all undulate ray survived the trial without mortality events. Our trials represent the first study of this type in the area and by, helping to quantify the survivability of the ray species analysed, support the granting of the discard exemption to these fisheries, as included in Article 15 of the Common Fisheries Policy (the so called Landing Obligation).

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Keywords: Fisheries, Rays (Fish), landing obligation, discards management, Survivability analysis

Research and management as key drivers in the evolution of the fishery of striped venus (*Chamelea gallina*) in the Spanish waters of the Gulf of Cádiz

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The fishing of the striped venus *Chamelea gallina*, (Linnaeus 175) in the Spanish waters of the Gulf of Cadiz, called locally "chirla", has been traditionally carried out with artisanal boats that use dredges in soft and shallow bottoms mainly located in the coast of Huelva (SW Spain). However, the introduction in the early 90's of the hydraulic dredges changed radically this type of fishery. The number of dredges targeting only this bivalve increased progressively from 27 units in 1997 up to 96 units, whose number is currently maintained since 2007. Consequently, this management measure caused the catches to increase abruptly from 500 tons at the end of the 190s to the almost 4000 official tons that were reached in 2001 and 2005. Nevertheless, at the beginning of 2010 catches decreased reaching minima historical values, which caused the closure of the fishing ground. These historical events brought significant changes in the management of this fishery, with the implementation of specific Fishing Plans, quota establishments, effort regulations, minimum sizes and closures, among other measures. Under this scenario, multidisciplinary studies became essential to achieve a more sustainable fishery. Studies of gear selectivity and density/biomass survey estimations have been already initiated at the end of the 90s, but it was in 2007 when fisheries and biological studies carried out by the Spanish Institute of Oceanography in collaboration with the Regional Administration in fishing of the Junta de Andalucía provided key information that made possible the modification and adaptation of new and effective management measures. Sampling on board with observers provided relevant information on the evolution of abundance, confirming a need for change in the management of the fishery based on fishing times instead of quota control. The biological studies provided a size of first maturity estimated at 10.4 mm, an extended period of reproduction between February and July and a population composed of three age classes (12.3, 25, and 30 mm), reaching the size of first capture (25 mm) at two years of age. In relation to fecundity, its increase was confirmed with size, providing 4 times more oocytes an individual with 30 mm of size than another showing 20 mm (Delgado et al., 2013; 2015). The discards estimated of this fishery showed that around 29% of the total catch biomass corresponded with other species that were not *Chamelea gallina*. Throughout the development of the studies, there was a collapse of the population, specifically at the end of 2010, when the high fishing effort together with recruitment failures due to adverse environmental conditions had a strong negative effect on the population. New management measures by the Regional Administration with scientific bases and a serious awareness by the own fishers made it possible to recover the resource at the highest known levels since the beginning of the fishery. The yields increased from 6 kg / hour up to 100 kg / hour from

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2011 to 2013, with specific yields of 1000 kg / hour in some areas. During this period, the market started to regulate effectively the catches and their traceability, supported by the incorporation of the “Chirla del Golfo de Cádiz” as Quality Mark established by the Regional Administration. It reached an exceptional state as the result of the good cooperation between the stakeholders that can make the sustainability of a fishery can be possible: sector, managers, researchers and the market itself. Unfortunately, in 2015 new adverse environmental conditions together with the lack of control at the level of fishing effort made that the interaction between all stakeholders involved was broken, establishing new closures of the fishery due to the decrease of the yields in the fishing ground. New management changes, with the Maximum Sustainable Yield as a priority objective, were established in 201. With the scientific information obtained from the assessment models and the assessment survey initiated in 201, biological-fishery reference points have been established as well as an annual TAC, in order to achieve such sustainability in the fishery over time.

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Keywords: *Chamelea gallina*, hydraulic dredge, Management, Gulf of Cadiz, yield

SARDINHA2020: An ecosystem approach to sardine fisheries management

Cristina Nunes, Ana Moreno, Susana Garrido, Alexandra A. Silva and Manuela Azevedo

Pelagic fish are the main target of Iberian fisheries, in particular sardines (*Sardina pilchardus*), but also horse mackerel (*Trachurus trachurus*), anchovy (*Engraulis encrasicolus*), Spanish mackerel (*Scomber colias*) and mackerel (*Scomber scombrus*). Fishery management of these species is generally complicated due to the large temporal fluctuations of population size, which is mainly attributed to the variability of recruitment (e.g., the number of young sardines that join each year the stock). The abundance of sardines has declined drastically in recent years due to recruitments as low as the historical lows which, coupled with the currently small number of large fish, are alarming signs for the present and future of the species. This decline is of particular great concern in the context of the vulnerability of these resources to the above mentioned fluctuations in abundance, with several global collapses having occurred over time, and the consequent dramatic impact of these collapses not only on fisheries but also on the entire marine food chain, since these species are the main prey of a high number of predators, such as birds and marine mammals. The Project SARDINHA2020 was developed by IPMA to address this issue aiming at improving the understanding of the decline in the recruitment strength of sardine Iberian stock and our ability to forecast the response of sardine population and the other components of the pelagic ecosystem in face of different scenarios (climate change, levels of exploitation, predation, etc.), coupling the dynamics of the social and economical components of the purse-seine fishery. Although the distribution and dynamics of sardine populations and many aspects of its biology in European waters are generally well studied, the relationships between sardines and environmental conditions are poorly understood, in particular the reasons that led to the decline in recruitment of the southern stock in the last 15 years. Likewise, the knowledge on the trophic interactions between sardines and other components of the pelagic ecosystem, the biology and abundance of these other species, and the social and economic components of the purse seine fishery is still limited. Since sardine stock productivity has been changing, it is essential to develop multidisciplinary scientific research to assess how the physical environment, interactions with other species, and fisheries affect the biology and abundance of sardines. This knowledge will be incorporated into the development of a model that intends to describe and predict how the population will respond in the future to different scenarios and, in collaboration with the industry and management,

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develop a purse-seine management plan, which focus on the key aspects to be taken into account in terms of the biology of all exploited species and the social and economic components, enhancing the protection and revitalization of biodiversity through the restoration of the coastal pelagic habitat. SARDINHA2020 is a multidisciplinary and knowledge integrator project that involves researchers and stakeholders, from IPMA, Universities, the purse-seine Industry, and the Administration. It is also an innovative project in terms of methodologies, actions and analyses of the results which are intended to be unconventional and “out of the box”. This project aimed at answering the subsisting questions regarding the drivers of the Iberian sardine population dynamics, given the pressures imposed by fishing, climate change and other environmental, biological and anthropogenic factors and build the basis for a more effective fishery management, following an ecosystem approach to the pelagic habitat and its living resources. SARDINHA2020 is coordinated by IPMA, bringing together a team of scientists with extensive experience in the biology, ecology and fisheries management of coastal pelagic resources and other experts in complementary areas essential to the tasks to be developed in the project (eg., electrical and naval engineering, mathematics and statistics, ecology, physical, chemical and biological oceanography, ecotoxicology, veterinary medicine, chemistry and aquaculture). The Project also involves scientists, experts in bio-economics, sociology and oceanographic modelling from Research Centres and Universities. In addition, the project involves the entire purse-seine industry and the administration (DGRM) in specific tasks. SARDINHA2020 is organized in 4 main components: 1. Coordination and Dissemination, 2. Habitat and Life Cycle, 3. Population Dynamics and Fisheries and 4. Modeling and Management Plan of the purse-seine fishery; and include 7 work packages (WP). The WP “Physical environment and Productivity” will identify the environmental variables that are mostly responsible for the dynamics of the pelagic fish species inhabiting Portuguese continental waters, and will produce temporal series of environmental data that allow to modelling and predicting those relationships. The WP “Natural mortality” aims to obtain mortality rates of each developmental stage (from the eggs to the adults) of pelagic fish species as a function of the variability of oceanographic conditions, the feeding availability, predation and exposure to contaminants. The WP “Growth and Reproduction” is studying the effect of the variability of the environmental conditions and biological factors that are considered most relevant for the growth and reproduction of pelagic fish species. The WP “Abundance, Distribution and Connectivity” aims to improve the estimates of abundance and distribution of pelagic fish species in the monitoring scientific surveys in a multispecific and ecosystem approach, to determine the influence of the environmental factors in the distribution, abundance and structure of the pelagic community as well as the assessment of sardine populations connectivity to re-evaluate the structure and identity of the population units in the Atlantic. The WP “Fishing activity and Production” is improving the knowledge of the social, economical, technological and biological components of the purse-seine fishery and the canning industry in collaboration with stakeholders. WP “Dynamic of Populations and Ecosystems” is evaluating the stocks of the main species of the pelagic community in Portuguese continental waters, using models and approaches that are appropriate to the biology of each species and the availability of data, integrating the environmental effects and the spatial dynamics of the populations and the fishery. Finally, the WP “Modelling and Management Plans” will develop a Management Plan for the purse-seine fishery to improve the assessment and recommendations for the management of the main target species captured by this fishery, particularly those for which there is no knowledge on the current exploitation status. The management Plan will allow incorporating the biological interactions between the main species of the purse-seine fishery, as well as the economic and technological issues. The expected outcome of SARDINHA2020 will be the development of a Management Plan for the purse-seine fishery, aligned with the Common Fisheries Policy and Marine Strategy Framework Directive, based on the principles of sustainability of fisheries resources, recovery of the pelagic habitat biodiversity and the fisheries industry profitability. SARDINHA2020 will be running until the end of year 2020 and its findings already started to be published in the scientific international journals (e.g. Garcia-Seoane et al. 2019; Silva et al. 201).

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Keywords: Iberian sardine, Ecosystem modelling approach, Natural mortality, Population Dynamics, Fisheries management advice, Multidisciplinary research

Spatio-temporal distribution of demersal fish species in the northern Alboran sea and Gulf of Vera

Cristina García-Ruiz, Manuel Hidalgo, Cristina Ciercoles, José M. Serna, Pedro Torres and María Gonzalez

Fish species play an important role in ecosystems, influencing the flow of energy and sustaining a diversity of ecosystem functions. In the Mediterranean sea, demersal resources consist of a large variety of fish species, many of them considered target species of economic interest for fisheries. Thus, understanding the distribution pattern of most relevant species is essential to improve spatial management and ensure a long-term sustainable conservation of the species. In this study, interannual and spatial distribution (bathymetric and geographic) of 9 relevant fish species (*Serranus hepatus*, *Boops boops*, *Pagellus acarne*, *Capros aper*, *Merluccius merluccius*, *Coleorhinchus caelorhinchus*, *Phycis blennoides*, *Nezumia aequalis* and *Galeus melastomus*) of the Alboran sea and the Gulf of Vera was evaluated. These species constitute some of the most representative in the soft bottoms of the study area from 30-00 m depth. In addition, fishing effort was analysed and compared with the standardized abundance for the above mentioned species. The study area ranged from Gibraltar to Cabo de Palos and corresponds to Geographic Sub Area 1 (GSA 1), according to the General Fisheries Commission for the Mediterranean Sea (GFCM). Data of fish fauna were collected from 1994, during 24 spring trawl surveys, as part of the MEDITS (International bottom trawl survey in the Mediterranean) project. The project use a randomised and stratified sampling design based on depth (five bathymetric strata: 10–50, 51–100, 101–200, 201–500 and 501–00 m). Catches were standardised to swept area (individuals·km⁻²) and data were pooled to obtain a matrix of species abundance by station. Bray–Curtis dissimilarity matrix was constructed to perform a non-metric multidimensional scaling (nMDS) analysis in order to identify species associations. Species abundance (x) were log (x+1) transformed to reduce the influence of dominant species. Analyses of similitude percentage (SIMPER) were used to identify those species that contributed to the similarity between groups of samples. The multivariate analyses were carried out using the PRIMER v6 (Plymouth Routines in Multivariate Ecological Research). Generalized additive models (GAM) were applied to assess the bathymetric, geographic and temporal effects on abundance using information at species level by haul. Year was considered as a factor. A one-dimensional smoother was used to investigate the bathymetric effect, while a two-dimensional smoother was used to account for the geographic effect, combining latitude and longitude. For the selection of the best model for each response variable, minimization of the Akaike information criterion (AIC) was applied. Fishing effort data, in terms of trips of trawlers per year, collected from 2003 to 2017, was analyzed. Correlation between fishing effort and abundance values of analyzed species was calculated using Pearson correlation coefficient. Fishing effort in the study area was compiled in the framework of the EU Data Collection Regulation (DCR). We identified four assemblages being depth the main factor that determines the distribution of species: inner continental shelf (30-100 m), outer continental shelf (101-200 m), upper continental slope (201-500 m) and middle continental slope (501-00 m). According to the SIMPER analysis the most representative species (contribute more than 30% to the similarity in each assemblage) were: *S. hepatus*, *B. boops* and *P. acarne* for inner shelf; *C. aper* and *M. merluccius* for outer shelf; *C. caelorhinchus* and *P. blennoides* for upper slope; *G. melastomus* and *N. aequalis* for middle slope. In every species (except *B. boops*), GAM analysis showed a statistically significant effect of depth and latitude-longitude on log-transformed data of abundance ($p < 0.01$) (*B. boops* only showed for depth). The deviance explained ranged between 61.5 for *M. merluccius* and 91.4 for *N. aequalis*. The bathymetric effect showed a non-linear pattern in all species analyzed, with abundance peaking between 30 to 100 m depth in *S. hepatus*, *B. boops* and *P. acarne* with decreasing trend below this depth; *C. aper* and *M. merluccius* showed maximum values at 200 m while *C. caelorhinchus* highest values were between 300-400 m. *G. melastomus* abundance increased from 200 to 600 m and *N. aequalis* from 300 to 600 m. Species were distributed heterogeneously throughout the entire study area. *G. melastomus* and *N. aequalis* abundance values were higher in Alboran Sea than in Gulf of Vera while opposite trends was showed by *P. blennoides*. The other species did not show a clear geographic pattern rather than a patchy distribution. Interannual variations in abundance values were highly heterogeneous and species-dependent. *B. boops* showed an increase of abundance from the first years to the rest of the time series. *P. acarne* abundance values decreased from 2013 to 2017. The other species showed high inter-annual

fluctuation between 1994 and 2017 but no clear trend for the abundance index. The examination of fishing effort showed that number of trips has declined with a statistically significant negative trend since 2003 ($r = 0.92$; $p < 0.01$). *C. aper* was the only analyzed species whose mean annual abundance values were negatively and significantly correlated with fishing effort ($r = 0.60$; $p < 0.05$). Regarding the other species, it has not been found increase or decrease on abundance values with reduction of fishing effect. Possibly a longer time series is required to reveal changes of these species. In conclusion, it has been identified four assemblages in the study area being depth the main factor that determines the distribution of species. Results of GAM on abundance indices of 9 species selected based of contribution to the similarity in each of the identified assemblage showed that depth and location explain much of the variance explained. No increasing or decreasing trend has been identified but high inter-annual and independent variation was observed for most of species.

Acknowledgements

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Keywords: Demersal, Mediterranean sea, Alboran sea, Gulf of Vera, distribution

Standardization of catch-per-unit-effort in a multi-gear multi-species fishery

Lorena Couce, Angelo Santana, Alberto Bilbao-Sieyro, Yeray Perez and Jose J. Castro

Catch-per-unit-effort (CPUE) data are commonly standardized taking into account factors that influence catch rates that are not related to resource abundance. In this work we analyze catch rates and effort data of a small-scale fishery targeting multiple species in the Canary Islands. We developed a method that combines principal component scores derived from a Principal Component Analysis (PCA) of the effort data within a Generalized Additive Model (GAM) framework. From the PCA analysis we also estimated the relative fishing power of each vessel with reference to a standard vessel for which the principal components are equal to zero, and based on the results we define two categories; the first category includes the benthic and demersal species and the second category includes the oceanic and pelagic species. The GAM model described 66.1% of the total deviance and includes seasonal variables, fishing grounds, climate index, oceanographic and biological parameters and dummies for tuna season and medium-sized coastal pelagics. The variables with the highest contribution in order of importance were the tuna dummy, the first principal component, the medium-sized coastal pelagics dummy and the fishing ground. The standardized CPUE time series showed a slight positive trend throughout the analyzed period as well as a marked seasonal component due to the tuna harvest. However, when the CPUE is analyzed according to the target groups, a negative trend is observed in the herbivorous and invertebrate feeders group, reef-associated fishes, shallow-water demersals and molluscs from 2010, and the benthic cephalopods show an appreciable downward trend from 2012. We consider the approach presented here has potentially useful for stock assessment in similar multi-species fisheries.

Acknowledgements

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Keywords: Small-scale fishery, GAM (generalized additive model), PCA principal component analysis, CPUE, standardization, Multi-gear fisheries, Multi-species fisheries

The fishery of the striped venus *Chamelea gallina* (Linnaeus, 175) in the Spanish waters of the Gulf of Cadiz (SW Spain): distribution and abundance of target and commercial discarded species.

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Chamelea gallina (common name "chirla") fishing grounds extend between 4 and 12 m depth in soft and shallow bottom in the Gulf of Cádiz (SW Spain). Until the mid-90s the fleet targeting this species was composed by towed dredgers. Later on, the hydraulic dredge was introduced as a new fishing gear with a greater fishing capacity, being "chirla" the only species that can be caught and landed by this fleet. The number of dredges increased progressively from 27 units in 1997 to 96 in 2007, a number that is currently maintained. Consequently, the catches experienced a great increase from 500 tons in the late '0s, to 3000-4000 tons that are officially caught since 2000, which placed the chirla as the main species landed by the demersal fleet of the Gulf of Cádiz. This work presents the results obtained in the CHIRLA 071 survey carried out at the end of July 201 by the Spanish Institute of Oceanography in collaboration with the Agrarian and Fisheries Agency of the Junta de Andalucía, in order to assess the state of the resource. Three commercial hydraulic dredgers with similar technical characteristics were used for the survey, covering all the production areas of the fishing ground. The distribution and abundance of the target species are shown, as well as the main commercial studied species, which were weighed and measured in each haul. The production zones, located between the mouths of the Guadalquivir River and the Guadiana River, were divided into 1 square nautical mile grids and a systematic sampling was applied. The starting point of fishing operations was located in the centroid of each cell and the duration of the haul was established in 10 minutes. cells of a total of 200 sampled cells had presence of the target species, not having been caught in the remaining 112. The biomass estimates were obtained by the swept area method using a GPS to measure the distance covered by the gear in each haul. The results showed a heterogeneous distribution (patches) of the target species throughout the fishing ground, having been estimated a biomass of 12,3 tons. The highest biomass were recorded in the shallowest range between 4 and m depth, and in the areas close to the Guadalquivir Fishing Reserve, where its capture is prohibited. However, the reserve effect is much more evident when analyzing the information in number of individuals than in terms of biomass. The number of different commercial species captured was 15, of which the group of fish stood out with 73,3% (Conger conger, *Dicologlossa cuneata*, *Diplodus vulgaris*, *Lithognathus mormyrus*, *Pegusa lascaris*, *Raja Linnaeus*, *Solea senegalensis*, *Solea solea*, *Synaptura lusitánica*, *Torpedo marmorata*, *Trachinus draco*). The mollusks (*Octopus vulgaris*, *Sepia officinalis*) and the crustaceans (*Penaeus kerathurus*, *Squilla mantis*) contributed with 13.3% each one. Species with higher estimated biomass were *S. officinalis*, *O. vulgaris*, *P. kerathurus*, *T. draco* and *L. mormyrus*, respectively, and in terms of abundance, the more significant species were *P. kerathurus*, *T. draco*, *S. officinalis* and *L. mormyrus*. The areas with the greatest abundances of commercial species were detected close to the mouths of major rivers such as the Guadalquivir Tinto-Odiel and Guadiana, since these areas are used by most of species as breeding and growing area. At average yield level (grams per hour), *S. officinalis* stood out with 335, followed by *P. kerathurus* with 114, *L. mormyrus* 107 and *T. draco* with 97, also in areas close to river mouths. The maximum values of the yield indicated considerable catches as registered for *S. officinalis* and *P. kerathurus* with 610 and 1365 grams / hour respectively. All these data point to an important accidental capture of these species that should be considered and taken into account when estimating the catches of these species, which are target species of other demersal fisheries in the Gulf of Cádiz.

Acknowledgements

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Keywords: *Chamelea gallina*, Hydraulic dredge, Discarded, biomass, abundance, Gulf of Cádiz

Topic: Fisheries, Aquaculture and Biotechnology

The prevalence of parasitic pea crab *Afropinnotheres monodi* in mussels depending on the degree of exposure of habitats: implications for mussel culture

Pilar Perez-Miguel, Jose Cuesta1, Enrique González-Ortegón, David Roque and Pilar Drake

Introduction As many other Pinnotherid crabs, the African pea crab *Afropinnotheres monodi* live in association with the mantle cavity of different bivalve species. This pea crab causes a significant reduction of the condition index of the mussel *Mytilus galloprovincialis*. The greatest effects brought about for the infestation with soft females (larger size and obligatory parasitism) and/or multiple infestation (two or three pea crab inside the same host); whereas erosion of the gills and mantle of hosts have been also observed in mussels and cockles (*Cerastoderma edule*) infested by hard females and males which have smaller size than soft females and are facultative parasites (Perez-Miguel et al. 2011). To test the hypothesis that the prevalence of this pea crab in subtidal mussels depends on the degree of exposure/shalowness of their habitats and consequently of the coexistence of hosts of all crab demographic categories in a reduced area, in this study we assess the prevalence and demographic structure of *A. monodi* in the subtidal mussels attached to the submerged chains of the navigational buoys (lateral marks) of the Port Authority located along the navigable channel of the bay of Cadiz. **Material and Methods** Mussels were collected 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 sampled buoys were selected along a gradient of exposure to tidal currents and with different distance to the intertidal populations of bivalves that we know are currently hosting the different demographic categories of *A. monodi* in this area. Buoys were seasonally sampled from each of the buoys number 2 (B2), 4 (B4), (B), 10 (B10) and 12 (B12) in August 2017 (summer), November 2017 (autumn), February 201 (winter) and May 201 (spring). The buoy most external to the bay is number 2 and the innermost one is number 12. **Results and Discussion** The size of mussels sampled in this study was very homogeneous regardless of the buoy considered and of if they were hosting or not the pea crab. On average, 43.3% of mussels examined during this study hosted the African pea crab *A. monodi*. When the whole period is considered, the maximal average prevalence corresponded to B10, with 74.5% of mussels hosting crabs and the minimal to B2 where only 17.0% of mussels hosting crabs. Results of χ^2 tests indicated that these inter-buoy differences in the annual pea crab prevalence were statistically significant ($p < 0.01$) among the three inner buoys (B12, B10 and B) and the two outer buoys (B4 and B2), as well as among the two inner buoys (B12, B10) and the intermediate buoy (B; Figure 1). Fig. 1. - Prevalence of parasitic pea crab *Afropinnotheres monodi* inside the mussels (*Mytilus galloprovincialis*) at the 5 different submerged chains of the navigational buoys in the bay of Cádiz. The letters indicate the differences in the annual pea crab prevalence were statistically significant ($p < 0.01$). At the top of the error bars, it shows the percentage of mussels who were infested by just 1 crab. When the whole period of study was considered, the significant differences in prevalence were observed among the inner and outer buoys (especially B2 and B4 [inner ones] versus B10 and B12 [outer ones]). Furthermore, with the exception of a very small crab found inside one mussel of B2, the relatively large size of male and hard female crabs hosted by subtidal mussels indicates that they have been previously using other hosts, as expected from the asymmetrical use that the different demographic categories of this species make of their different hosts. All these features suggest that, as in the case of intertidal mussels of Bay of Cadiz, the infestation of subtidal mussels has as main source the males and hard females of *A. monodi* hosted primarily by the intertidal populations of cockles and clams. Thus, the lower infestation of mussels from the outer buoys (B2 and B4) and the higher infestation of mussels from the inner buoys (B10 and B12) could be reflecting the mayor and minor distance, respectively, to the Rio San Pedro Inlet, the area of the bay in which are located the largest natural beds of cockles (*Cerastoderma edule* and *C. glaucum*) and the clam *Scrobicularia plana*; in fact, the higher prevalence of *A. monodi* was just found in mussels from B10 (74.5%, on average) which is the buoy located the closest to this main source of the pea crabs that infest subtidal mussels of the Bay of Cadiz. The similar environmental conditions between the bay of Cádiz and Galician habitats (almost in summer), where the farms' mussels coexist with the neighboring natural bed of the cockle *Cerastoderma edule*, suggest that this crab could cause economic problems in the Galician mussel industry, one of the most important in the world. The deleterious effects of the pea crabs on the commercial production of mussels can be direct by a condition loss and/or growth reduction of mussels or indirect, because its presence in the shell cavity of fresh mussels may cause consumer complaints. Also, the removal of crabs can considerably increase the duration of the mussels canning process, increasing in a 33% the time in the scallop industrial process; a more recent and extreme case, the high infestation (up to 100%

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sometimes) of *Cerastoderma* spp. by *A. monodi* in some areas of the Algarve (South of Portugal) has made that a canning company has closed the canning line of cockles due to economic losses (Cuesta, pers. comm.). Conclusion In this work, it is revealed for the first time the possibility that *A. monodi* infects subtidal mussels, in the similar conditions as the cultured mussels in rafts. This shows up the risk of an infestation by the parasitic pea crab *Afropinnotheres monodi* in the economically relevant for the culture of mussels. According to our results, for reducing at least partially the negative effects of these parasites, moving mussel farms offshore, when possible, has the potential to reduce the prevalence and the deleterious effects of pea crabs in mussels.

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Keywords: Parasitism, Mussel farm, *Afropinnotheres monodi*, *Mytilus galloprovincialis*, Prevalence

Valorisation of marine products from Macaronesia: MACAROFOOD Grey triggerfish and wahoo

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The main objectives of the MACAROFOOD project (Valorisation of marine products from Macaronesia: tourism, gastronomy and professional training) are to promote reliable fishery resources management, promoting good practices and sustainable exploitation, to develop synergies between social/marine science and gastronomy, and to establish a dynamic flow of information/knowledge to propel excellence in tourism and thus enrich local marine products. One of the activities of the project is the determination of oceanographic, biological and genetic parameters of target and by-catch species with commercial importance; and to ensure the consolidation of Macaronesian reference collections and biobanks. To determine the biological parameters of traditional or alternative fish resources (grey triggerfish and wahoo) which are common in Macaronesia, i.e. NE Atlantic archipelagos of Madeira, Canary Islands and Cabo Verde, between 2017 and 2019 grey triggerfish *Balistes caprisicus* (n=700 individuals) (Balistidae) and wahoo *Acanthocybium solandri* (n=350) (Scombridae) were obtained from local fish markets, and then sampled at laboratory. Standard biometric measurements were taken. Gonads were fixed for further histological analyses and muscle samples stored for genetic analyses. Despite the high quality and taste of their flesh, these species does not represent an important fisheries resource in Madeira Island, being captured mostly by recreational fishery (both species) and spear game fishing (*A. solandri*). In the Canary Islands, both species are chiefly caught by the local artisanal fleet. In the Cabo Verde Islands, the wahoo is one of the most traditional target species and very appreciated in the national cuisine, while grey triggerfish is an emergent fish product mainly at the northern-occidental islands.

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Keywords: marine products, Macaronesia islands, Macarofood project, Grey Triggerfish, Wahoo, Madeira, Canary Islands and Cabo Verde Islands

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Coastal Biology Laboratory (UMinho): Multilevel approach for research studies in nearshore communities in northern Iberia

Marisa Gomes, Catarina Alves, Fábio Faria, Susana Pereira, Diego Carreira-Flores, Regina Neto, Edgar Fernandes, Hugo Ferreira and Pedro T. Gomes

The northern Iberian coast is a perfect area to monitor changes in the biological communities related to global changes. For that purpose, a set of common techniques, applicable all over the study areas, have been implemented by the Coastal Biology Laboratory team since 2012. These techniques include side scan imagery for habitat cartography, artificial substrates for quantitative sampling of benthic organisms, baited video devices for fish populations evaluation, hand sampling by scuba diving, underwater linear transects, habitat classification, rocky shore monitoring and marine plankton surveys. We are involved in projects with partners from the engineering areas, electronic and civil, aiming the creation of a next generation marine management system, based on knowledge and innovation in the areas of electronics, materials, taxonomy, ecology, conservation and metagenomics (Next Sea project). We work in close association with our colleagues from Galicia, in the scope of the Campus do Mar consortium and specially with ECIMAT (U. Vigo) and Estación de Biología Mariña da Graña (U. Santiago Compostela). The Coastal Biology Laboratory team is also committed in extension and scientific dissemination activities, namely in the field of environmental education, technical training (e.g. teachers, general public, scuba diving, scientific diving) as well as in scientific dissemination actions. As a dedicated science platform, we recognize the importance of connecting the communities with the current understanding of science. Hence, through this integrated approach we are able to witness the evolution of these unique ecosystems and register the changes on-going in the nearshore communities in the northern Iberia.

Keywords: scientific dissemination activities, marine science, Nearshore communities, northern Iberia, marine monitoring

“Scientists of the Sea”: an environmental education project promoting scientific literacy

Soraia L. Castro and Leonor Cruz

Environmental Education (EE) is the process of recognising values and clarifying concepts in order to develop skills and attitudes necessary to understand and appreciate the inter-relatedness among man, his culture, and his biophysical surroundings. EE also entails practice in decision-making and self-formulation of a code of behaviour about issues concerning environmental quality (IUCN 1970). Fundamental instrument for a process of changing values, mentalities and attitudes, EE creates a deep and lasting awareness of problems associated with environmental issues (Morgado et al, 2000). For its purposes, it essentially aims to modify knowledge, values and attitudes, given priority to case analysis, critical reflection, debate and acquisition of skills. It does not consist in “Learning and admitting passively” but in “Understanding to act” (Giordan and Souchon, 1997). In fact, we can only protect what we know. Among the paths available for dissemination of EE, schools are priority ones, becoming EE projects essential for continuous learning through the school year. EE at the high school level reveals itself to be of huge importance given the intermediate level between basic and university education. At high school level, environmental and science studies should bring some utility to the educational process to the minds of students, acting as a complement to the existing school programme. Students should clearly see why they must be concerned about the environment. It is necessary to relate long term ecological-environmental goals to the short term interests of individuals (Eichler, 1977). EE in high school should develop ecological thinkers and promote scientific experiences, acting as the introduction of environmental studies. From the need to create a project focused wholly on high school students, the environmental education project “Scientists of the Sea” was created in 2017. Organized by Viana do Castelo Sea Center, an entity managed by Viana do Castelo City Council, “Scientists of the Sea” aims to promote and initiate the wish for scientific research in high school students and valorise natural ecosystems, both coastal and estuarine areas of the county. Viana do Castelo integrate two Natura 2000 Sites, Litoral Norte (PTCON00017) and Rio

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Lima (PTCON00020), therefore it is extremely important to involve the school community in conservation, leading to the understanding and knowledge of these ecosystems with high ecological and economic values (Barbier et al, 2011). During the academic year, students involved in "Scientists of the Sea" developed an original research project with technical and scientific support from the Sea Center and researchers working on Portuguese's research institutions like Interdisciplinary Center of Marine and Environmental Research (University of Porto), Center for Environmental and Marine Studies (University of Aveiro), Research Center in Biodiversity and Genetic Resources (University of Porto), Molecular and Environmental Biology Center (University of Braga) and Marine and Environmental Sciences Center (University of Coimbra group). This environmental education project begins with an opening meeting between students and researchers to discuss ideas and choose work projects. Scientific method is fully developed and applied by classes through the academic year, with the aim to develop their scientific project. At the end, we carry out a final meeting to exhibit class's works and awards the top three. Also each group create a scientific poster, which is evaluated at the final meeting by external jury members. To complement the scientific method each class do field trips to study area and visits to research institutions. In its two academic editions, "Scientists of the Sea" counted on eight enrolled classes, with a total of 131 students coming from three high schools. In 2017/201 academic year, the themes developed were: "Testing the memory of fishes", "Study of the potential of *Cakile maritima* halophyte in soil decontamination", "Influence of climate change on calcareous algae on the Norte beach" and "The sea: a sense of health and well-being." In the academic year 201/2019, the following themes were developed: "Discovering the zooplankton of the Norte beach", "Analysis of the stomach content of aquaculture and deep sea bass", "Environmental factors influencing the fixation of *Mytilus edulis* to the rocky substratum" and "What is the influence of salinity on *Ulva lactuca* of the Norte beach?". "Scientists of the Sea" has been developed with students, by working and improving knowledge about natural coastal and estuarine ecosystems of Viana do Castelo. From the final surveys sent to the involved teachers the most positive aspects were the promotion of scientific literacy and the possibility of experimental practical work. The overall analysis of this project proves itself to be a very rewarding one. Students, teachers and technicians consider these type of environmental education projects of major importance for everyone involved.

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Keywords: Scientists of the Sea, environmental education (EE), Scientific literacy, ecosystems, Scientific research activities, environmental education project , high school

Fishing the "ghosts" of our seas: awareness activities for the youngest to promote fisheries without litter

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Ghost fishing is the term given to the continued fishing by fishing gear that has been lost or abandoned in the aquatic habitat. This problem often comes to us through graphic images of various marine animals, such as fish, turtles, marine mammals, etc., trapped in these nets in the ocean. These images represent some of the impacts and destructive potential of the loss and abandonment of fishing gear at sea. At a time when marine litter is one of the biggest world's concerns, NetTag project aims to reduce and prevent marine litter derived from fisheries by working directly with fishers. To achieve this objective NetTag will use an integrative preventive approach to: i) reduce lost gear by using new technologies to localize and recover lost gears; and

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ii) promote better practices on-board regarding management of fishing waste. Complementing NetTag activities, awareness activities for the younger community, including children and young relatives of fishers will be developed. These activities will take place in schools of Vila do Conde (Portugal), an area with an important background in fisheries that has, even today, a large active fishing community. The activities to be developed in schools will include: 1) lectures on the problematic of Ocean pollution, namely pollution due to marine litter and particularly plastics; 2) several experimental activities related to the presence of plastics in the marine environment (in sediments, water or even in animals), and identification of different types of plastic (bioplastic, compostable plastic, etc.) as alternatives to common plastic, to perceive and understand its properties and advantages; 3) didactic games addressing topics related to fisheries, as impacts of overfishing, illegal fisheries and marine litter, fisheries management and governance, and the necessity of preserving healthy and productive oceans. These students will also be given the opportunity to visit a research center (Interdisciplinary Center of Marine and Environmental Research (CIIMAR)) to learn about the research developed in the area of Marine and Environmental Sciences and to contact directly with researchers and scientists, promoting the interest of the youngest by these scientific areas while learning about the importance of the work developed by these researchers. The actions proposed will engage children and young students, increasing their awareness on the marine litter and Ocean pollution problematics, raise Ocean Literacy and reinforce the engagement of young generations of fishers to adopt better practices. The success of these actions will contribute to increase the number of responsible citizens with the power to make informed decisions on important subjects as Ocean Health and Conservation.

Keywords: marine litter, Ghost fishing, Awareness Activities, Ocean literacy, children and young students

Seafood from Madeira Island: MARISCOMAC Taking fisheries science to society

João Delgado, Luisa Costa, Ana R. Gois, Grace Faria, Adriana Alves, Ricardo Sousa, José A. González, Manuel J. Biscoito and Mafalda Freitas

The project MARISCOMAC aims the development of technical and scientific conditions, training, transfer of technology and knowledge, aiming to promote the sustainable exploitation and safe consumption of seafood in the Macaronesia (Madeira, Canary and Cabo Verde Islands). In this project the target species are limpets (*Patella aspera* and *Patella candeii*), sea snail (*Phorcus sauciatius*), and big deep-sea crabs (*Cancer bellianus*, *Chaceon affinis* and *Paromola cuvieri*), among others. In 2017 the project was presented and promoted in all the geographic areas with all partners involved, and from there one informative article and seven scientific articles were published. In 201 the dissemination of the project activities started with agro-fishing and restaurant and tourism industry events, and also the "ULPGC Canary Gastronomy Center" was designed and created at the University of Las Palmas. In Madeira Island the Project was presented in several events such as: "Rota do Atum", "III Feira do Mar e do Pescador", "XIV Festa da Lapa", "Conferências do Mar" and "Ciência no Mercado", among others. Lectures were given for different target publics and workshops and dissemination activities were developed involving around 2,500 participants. The MARISCOMAC project has the ambitious purpose of carrying out its activities, not only addressed to the stakeholders of the fishing sector but to the whole society, thus raising awareness about the safe consumption of local fish obtained in a sustainable way.

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Keywords: Seafood, Madeira Island, Mariscomac project, limpets, Sea snails, big deep-sea crabs, Macaronesia islands, Dissemination activities

Traineeship at the Minho estuary lab: merging citizen science and environmental education

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In this work we document the experience of a four-year project in which young citizens were engaged in the scientific activities of a research laboratory for two weeks. The project converged science and environmental education, aiming at improving environmental literacy on estuarine ecosystems, using Citizen Science - i.e. involving the citizens in data collection, treatment and discussion for later publication (Bonney et al 2009) - as a pedagogic tool to appraise relevant environmental issues. The complex nature of current environmental issues such as climate change and biodiversity loss is widely discussed by both scientists and society. While (environmental) science education focuses mainly on teaching knowledge and skills which are essential for improving environmental literacy, environmental education also incorporates values and behavioral change towards more sustainable actions (Wals et al 2014). Although raising environmental knowledge and awareness have not always a simple and direct relationship with attitude changing, both take advantage of scientific evidence, as some understanding of biological diversity and ecology, and problem solving skills are a requisite for conservation literacy (Berkowitz et al 2005). The scientific community, therefore, has a key role in the process of environmental education of the society. The project 'from Minho Estuary to the lab' was designed to engage Portuguese young citizens in the scientific activities of an ecology laboratory from the Interdisciplinary Center of Marine and Environmental Research (CIIMAR/CIMAR, Portugal). Objectives were threefold: (1) to improve knowledge on an important natural habitat and awareness of its complexity and ecological functioning; (2) to develop the capacity to think critically, ethically and creatively in appraising ecological issues in a scientific context; and (3) to promote a participatory action in environmental data collection for the undergoing scientific projects. In this way, young citizens were engaged in scientific tasks, collaborating with the scientific staff and students in the laboratory milieu. In the four annual editions of the project, a total of 30 young citizens (Table 1) were involved. Ages ranged from 14 to 19 years old and academic interests were diverse, though Biology oriented students dominated (Figure 1). Each year, up to three scientific trainee-ships were organized for three students each, lasting for one to two weeks, during school summer holidays. In each trainee-ship, after a short theoretical introduction to the objectives, and to the scientific background and methods, the young trainees participated in all the procedures, from field trip planning, which was part of the monthly sampling program of the lab, and development for rigorous data collection, to the data treatment and discussion. Tasks were developed in a logical chronological sequence as follows: 1. Planning the field trip: area recognition, checking the local tide's schedule, organize all sampling equipment, checking equipment for proper functioning, discussion of sampling methods; 2. Field work: sampling, i.e. operating sampling gears (fishing gears, multi-parameter probes), samples transportation and storing; 3. Sorting and species identification in the lab; 4. Biometry and other laboratory work (biochemical analysis e.g. lipids and protein determination); 5. Monitoring of aquarium experiments (on growth, starvation, sex change of estuarine organisms such as the brown shrimp *Crangon crangon* and the shore crab *Carcinus maenas*); 6. Entering data in the laboratory database; 7. Data treatment and discussion. With such a plan, the two-week duration was important to assure that all field and laboratory tasks were developed in a coherent way. Because the participants did not participate in the problem definition, the project can be classified as 'Level 2 Distributed intelligence - citizens as basic interpreters' according to Haklay (2013) classification of the typology of citizen participation, where the trainee-ship correspond well with a basic training. Yet, all data collection, treatment and interpretation were done under the scientific staff supervision or in a collaborative way. The project had an increasing demand over the 4 years (Table 1). The variety of academic interests of the candidates revealed that the experience is beneficial not only in the process of decision-making about the academic route of the students, but also contributes to the scientific literacy of the participants and sensitize them to environmental conservation. The local community at Minho Estuary surroundings also revealed interest during the field trips and expressed natural curiosity. Besides representing a first contact with the 'scientific method' and the reality of scientific research, in a professional context, the project also contributed to the awareness of ecological issues. The field work took place in the Minho Estuary (north of Portugal), which has several environmental protection statuses: it is a Natura 2000 site, an Important Bird and Biodiversity Area (IBA), a Special Area for Birds Protection (ZEP), and was classified as a Corine Biotope. Field sampling focused on ecological aspects that were under investigation at the time of the internship. It consisted in measuring environmental parameters such as temperature, salinity, oxygen concentration, and other relevant water quality parameters, along with sampling for living organisms for later biometry analysis, aquaria experiments under controlled conditions and laboratory work. Food availability, predation pressure, and species responses to some of the environmental parameters (e.g. growth responses to temperature), species nutritional condition, to estuarine biodiversity, population dynamics, and trophic webs were within the subjects covered. Participants had the opportunity to explore,

analyze, and interpret conditions, reasons, and expressions of natural situations, getting in personal contact with scientific researchers and discussing possible developments. In this way participants were able to generate knowledge on complex environmental issues. Data gathered is now part of the laboratory databases, covering 10 years of field sampling at Minho Estuary. This dataset has already been partly considered for publication (theses, seminars, scientific papers) [e.g. Moreira et al 2015, Neves et al 2017, Mendes et al 2015] and is available for future publications as well. However, the trainee-ship was not conceived in such a way that these publications would reach the trainee-ship participants. Future trainee-ships at scientific institutions should be regarded as Citizen Science projects that can bring together scientists and society and increase science literacy. This way, projects engaging young citizens in scientific activities related with ecological subjects can be a pedagogical instrument to improve environmental literacy and can be incorporated in environmental education projects dealing with complex conservation concerns. For the success of such projects, guidelines for collaboration among scientists and the public must be prepared (Tweddle et al 2012) and more emphasis on the follow-up and on the evaluation of the project's impact is advised, in order to investigate the extent to which these citizen-science initiatives are efficient in changing behaviors.

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Promotion and environmental citizenship: challenges and opportunities for the Environmental Monitoring and Interpretation Center and the Sea Center of Viana do Castelo.

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1. INTRODUCTION The Municipality of Viana do Castelo has had, since 1995, a strategy based on the quality of the urban environment and sustainability of the natural ecosystems of the municipality, with the City Strategic

Plan. Through the municipal “Eco library”, this strategy was extended to the school community, enabling us to take the first steps towards guidance on emerging environmental themes. However, there was a need to provide the community with an environmental educational service in an integrated way. It means, provide a Resource Center and other activities in different areas that allows achieving the demands of general population. Integrated in the Polis Program strategy, it was possible to develop a vast urban and environmental rehabilitation operation (around 157 hectares), through which the Municipality proposed the creation of an Center for Environmental Monitoring and Interpretation endowed with physical and technical skills capable of developing awareness-raising work integrated with the community. The Center for Environmental Monitoring and Interpretation of Viana do Castelo (CMIA VC) was inaugurated on June 21st 2007, under the management and coordination of the Viana do Castelo City Council. After seven years, the Municipality of Viana do Castelo presents another project of citizen intervention directed to the theme oceans and its diverse areas - environment, culture, heritage, nautical and economy - designated Sea Center. This Center, hosted on Gil Eannes Ship-Hospital, is composed of an Environmental Interpretation Center with a strong expository and public intervention component and a Sea Documentation Center essentially aimed at preserving the memory of “sea life” in general and marine ecosystems in particular. The mission and objectives of both projects, although similar, have some specificities. The CMIA of Viana do Castelo central objective is being “A space of discovery”. It aims to (i) promote a space for debate and awareness on environmental issues; (ii) promote environmental formation and information initiatives for different publics; (iii) develop an accessible, diversified and versatile Resource Center on diverse environmental issues; (iv) provide information on environmental indicators and environmental sustainability. The Sea Center central objective is to convert Viana do Castelo in “The Nautical City of the Atlantic”. It is characterized by the following issues: (i) development of a museological area with an interactive character; (ii) improvement of sports and nautical tourism, given by the innumerable possibilities of the territory; (iii) promotion of marine biology research, with the possibility of partnerships between several universities; (iv) development of an accessible, diversified and versatile resource center in different maritime issues.

2. **ACTIVITIES PLAN TEMPORARY EXHIBITION:** this is a vehicle to promote thematic issues during a certain period to different publics in free access; this are converted into itinerant format at the end. **ITINERANT EXHIBITIONS:** developed in a practical and easy way to carry out; it always have also informative materials to support de exploration of the themes. This are available free of charge to others environmental centers, schools, museums, or other entities that carry out activities in environmental issues to a large number of persons. It is an important way to disseminate de knowledge thought other territories. **ACTIVITIES FOR ORGANIZED GROUPS:** theoretical and practical activities that can be performed indoor and outdoor. There are five main themes - water, forest, waste, sea and sustainability – wich are prepared for different age groups. This is also a great vehicle to achieve different publics in concern of the natural values of the territory, the role of all citizens in the biodiversity conservation, and the environmental threatened realities. **EDUCATIONAL PROJECTS:** this is an offer targeted specifically at schools. Themes and methodology of work are defined taking regard the current environmental affairs and the schools contents in different subjects. Each classroom choose one of the four educational project and work with CMIA or Sea Center during all the school year. The themes of the educational projects are: “From Land to Earth” in the area of the circular economy, “Nature School” in the area of conservation of biodiversity, “Scientists of the Sea” in the specific area of marine research and finally the “Beyond-Sea” project in the area of culture and maritime experience. **PEDAGOGICAL MATERIALS:** beyond the library available to the all citizens in CMIA Viana do Castelo and Sea Center, there is a wide variety of other educational resources of free request. The list of resources is available in the website of CMIA Viana do Castelo – www.cmia-viana-castelo.pt, such as provision of equipment for field trips, protocols and fact sheets for teachers, teaching catalogues. **ENVIRONMENTAL VOLUNTEERING:** this is a free initiative focused in the approach of citizens to a set of environmental issues that aren’t a priority in the daily lives of the population. Conservation of wader’s species (in the breeding season), cleansing of natural habitats (of garbage and invasive species) and identification of cetaceans’s stranding are some of the activities included in the environmental volunteering. **WORKSHOPS AND TRAINING ACTIONS:** this are short-term actions most of which intend to captivate the general public for a closer look at the territory, how we depend on it and how use it. With regard to teachers, topics are selected to enhance the use of practical classes and field trips, and whenever possible, in an interdisciplinary way. **PUBLICATIONS:** over the years there are several publications edited by this two Centers (CMIA Viana do Castelo and Sea Center). Not only thematic catalogs were prepared do to the exhibitions, but also to some thematic publications about natural resources and biodiversity of the territory, as well as educational and practical educational materials available in the website of CMIA Viana do Castelo. **CITIZEN PARTICIPATION:** beyond the environmental volunteering, there are two other different areas in which all the citizens are call to collaborate. In the Sea Center we promote the collecting of photos, documents, real testimonials and objects to enrich history of “The Nautical City of the Atlantic”. Another recent project concerns to the public participation in “Citizen Science”. In the website of CMIA there is available a platform called BIOREGISTO in which every citizen can collaborate in the registration of biodiversity.

3. **ACTIVITY INDICATORS AND METHODS OF EVALUATION AND ANALYSIS** The compilation and analysis of data

is an important work tool that allows analyzing the work developed with different audiences in different formats. The results obtained in the various evaluation instruments allows us to define the typology of activities to be developed and the great public to reach. The records of users of the services, by age group and type of public, allow us to verify that in the Sea Center 62% of the public have more than 19 years old, while in the CMIA Viana do Castelo it corresponds to 2%. This means that the main public in the Sea Center (6%) are general public, while in CMIA Viana do Castelo the schools and other organized groups correspond to the main users of the services (3%). Regarding to the evaluation and analysis methods, there are two types of questioners. One to the students integrated in the educational projects to aim if the works of the all school year in one specific subject bring some changes in the knowledge of the students. Teachers also have a satisfaction survey about the educational project in which were involved. On the other hand, all activities that have a trainer to guide the activity of group, the workshop or training actions are evaluated through an anonymous questionnaire. 4. **FINAL CONSIDERATIONS** All the work developed is consolidating over time, but it is important to mention the structural importance that partnerships with universities and research centers that allow improving and consolidating scientific knowledge that will have to be transmitted, by this kind of equipment, such us CMIA Viana do Castelo and Sea Center, in a way adapted to different publics. It is also worth mentioning the importance of submitting the projects and programs of activities to competitions and external entities, wich will have a critical and external opinion about it. Of note are three significant moments for the CMIA: attribution of “Honorable Mention in the category of Educational Service” by the Portuguese Association of Museology (APOM) in 2011, the “Natura 2000 Award” for the educational project “School of Nature”, awarded by the European Commission Union in 201 and a Vote of Praise from the municipal executive itself in the year of 201. The Sea Center has been recognized as a Blue Center by the Blue Flag Association of Europe since 2016.

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Emission of global warming gases from northern Portuguese estuaries: the contribution of CH₄ and N₂O

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BACKGROUND

The continuous increase of emissions of greenhouse gases to the environment, such as carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O), and chlorofluorocarbons, leads to the increase of their concentration in the atmosphere with commensurable impact on the climate (Sturm et al., 2017). In the last 100 years, the global average temperature has increased by 0.6 °C, and a larger increase in the next 100 years is forecasted, if no corrective action is taken (Root et al., 2003). Our research focused on two major gases responsible for global warming, CH₄ and N₂O, due to their high capacity to absorb infrared radiation (Sturm et al., 2017). Estuaries are one of the natural sources of emissions of those gases to the atmosphere (Bange et al., 1996). As such, the objectives of this work were to estimate the potential emissions of CH₄ and N₂O to the atmosphere from the Douro, Ave, and Cávado estuaries.

METHODOLOGY

Water and sediment samples were collected seasonally at each estuary. The emissions of CH₄ and N₂O by sediments were estimated using slurry incubations. In 50 mL serum bottles with 10 mL of incubation water and 5 mL of homogenized sediment sealed with butyl stopper and aluminum crimp and purged 20 min with nitrogen to remove oxygen. A separate set of time zero samples was sacrificed immediately after purge, to quantify CH₄ and N₂O levels (time 0). All samples were incubated for 4h at constant stirring (100 rpm). CH₄ and N₂O were determined by Gas Chromatography with Flame Ionization Detector (GC-FID), and by Gas Chromatography with Electron Capture Detector (GC-ECD), respectively. The GC was calibrated using a certified gas standard mixture of N₂O and CH₄.

RESULTS & DISCUSSION

Sediments of the three studied estuaries presented higher potential rates of CH₄ production in the winter, reaching 26.4 nmol h⁻¹ g⁻¹ wet sed, 1.97 nmol h⁻¹ g⁻¹ wet sed, and 0.12 nmol h⁻¹ g⁻¹ wet sed, for the Douro, Ave, and Cávado estuaries, respectively (Figure 1). The higher production values were measured in fine-

grained sediments rich in organic matter in the middle estuarine stretch of Douro River suitable for methanogens. Regarding N₂O, sediments from Douro estuary acted as a sink rather than source of this gas during the fall and winter (Figure 2). On the other hand, sediments from the Cávado and lower Ave estuary, were active places of N₂O production, especially in the summer, with the maximum value found in Ave estuary (5.1x10⁻² nmol h⁻¹ g⁻¹ wet sed). Under anaerobic conditions, N₂O is likely to be produced by denitrification (Bange, 2000). In agreement, being temperature a well-known denitrification regulator, higher potential emissions were measured during summer.

The obtained results were made comparable with other studies by, estimating the fluxes in square meters. The Douro estuary reached 57.0 μmol h⁻¹ m⁻² of CH₄ in winter survey, while Ave and Cávado estuaries presented maximum values one to two order of magnitude lower (5.9 μmol h⁻¹ m⁻², 0.32 μmol h⁻¹ m⁻², respectively). Methane production in the Douro estuary was higher than previously reported in Australian estuaries (23.4 μmol h⁻¹ m⁻² (Allen et al., 2011). Emissions of N₂O reached 27. nmol h⁻¹ m⁻², 153.7 nmol h⁻¹ m⁻², and 15.5 nmol h⁻¹ m⁻² in the Douro, Ave and Cávado estuaries, respectively. The measured N₂O values were in the lower range of those reported for other estuaries (Li et al., 2019; Middelburg et al., 1995).

FINAL REMARKS

The Douro estuary was an active CH₄ producer, while N₂O presented higher production rates in the Ave estuary. The different range of emissions of both CH₄ and N₂O from the three estuaries reflected the specific environmental conditions. Future research will explore the environmental controls and dissolved CH₄ and N₂O in the water column.

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Keywords: Global Warming, Methane, Nitrous Oxide, sediments, Estuaries

Do artificial structures affect the feed of *Patella caerulea* limpets?

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Artificial structures introduced into the marine environment are known for being a risk to local biodiversity. However, it is not rare to find endangered species established in these anthropogenic habitats, as it is the case of the limpets *Cymbula safiana* and *Patella ferruginea*, whose populations are numerous on coastal protection structures of southern Spain and northern Africa. It has been previously stated that limpets inhabiting arti-

cial structures present different gut content than those living on natural ones, possibly affecting their optimal development. Therefore, the aim of our study was to evaluate if different kind of artificial structures could affect the food consumption by limpets using carbon and nitrogen isotopes analysis. Four different coastal defence structures (cubes, rip-raps, tetrapods and sea-walls) were studied along the coast of the Alboran Sea (western Mediterranean Sea). Each structure was replicated three times in scattered localities and in each one, five limpets (*Patella caerulea*) of similar sizes were sampled in the artificial structure and five in the nearest natural rocky shore. Limpets were immediately stored at -20 °C until stable isotope analyses. A section of the foot muscle was used for δ¹³C and δ¹⁵N determinations. The tissues were freeze dried and milled. Powdered material was weighted to the nearest 0.300 mg with an error of ±0.002 mg and placed into tin capsules. All samples were combusted at 1020 °C using a continuous-flow isotope-ratio mass spectrometry system by means of Flash HT Plus elemental analyser coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface. The differences in δ¹³C and δ¹⁵N ratios between substrates and localities were explored using non-metric multi-dimensional scaling (MDS), permutational multivariate analyses of variance (PERMANOVA) and δ¹³C and δ¹⁵N biplot. PERMANOVA did not show significant differences between artificial and natural substrates. MDS (Fig. 1) and biplot (Fig. 2) results did not portray clear differences among different substrates and only the factor locality was significant in the PERMANOVA test, suggesting that the effect of local conditions is overwriting the possible impact of different artificial structures. However, pooling the locality factor, PERMANOVA did show significant differences among substrates. Rip-raps were different to every artificial substrate and to natural ones. Cubes also differed from natural substrates. In conclusion, the effect of different kinds of artificial structures was not constant across a geographical gradient and *Patella caerulea* living on rip-raps seemed to consume different diets. These results are important for the management of limpets population inhabiting artificial structures and the creation of marine artificial micro reserves.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

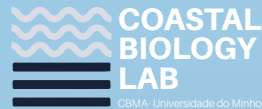
Keywords: Isotopes, Artificial structures, limpets, Alboran Sea, Breakwater armor blocks

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