The impact of Alqueva dam on the ecological networks of Guadiana estuary salt marsh areas

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Salt marshes are known to play a trophic and nursery role for many aquatic species which depend, for more a less long periods of their life cycle, on the intense primary productivity and refuge provided by those areas. Ecotonal zone at the interface between salt marsh and open water has long been suspected of harboring high concentrations of aquatic organisms. Accordingly, the lower Guadiana is recognized as an important ecosystem, providing habitat for many species, which find conditions within salt marshes suitable for their development. Unfortunately, salt marshes have been subject to high pressures, both from natural and anthropogenic origin. Dams are listed as one of the main anthropogenic pressures in Portuguese estuaries and the Guadiana is no exception. Dams are mainly regulated for economics purposes (i.e. hydroelectric power generation, irrigation, freshwater supply) causing decrease in the volumes of freshwater discharged into estuaries. Seasonal patterns of freshwater input of dammed rivers may differ considerably from the expected natural pattern. Consequently, floodplains that are essential to many aquatic organisms may be detached from the river, and marsh environments can dry out. As a result, the vegetation structure of the natural vegetation may change from water-tolerant species to more arid varieties. Similarly, many of the organisms that are characteristic of seasonally flooded environments may disappear.

The objective of this work is to develop an efficient method for assessing the changes on the lower pelagic food web structure and function in the salt marsh area of the Guadiana estuary before and after Alqueva dam construction (largest artificial lake in Europe). The comparative work was based on collections of nutrients, phytoplankton, zooplankton, fish larvae and jellyfish. Sampling campaigns were performed on a season basis (every three months) during two distinct hydrological years (pre- dam, 2000-2001 and post- dam, 2010-2011). Each time we sampled two distinct points, one inside a salt marsh creek and the other on the main channel of the estuary. A hybrid modeling approach (integrating Machine Learning and Mechanistic modeling approaches) is used to investigate the ecological network of lower pelagic food web.

Results revealed a decline in nutrients concentration, phytoplankton, zooplankton and fish larvae abundances for all seasons except spring in 2010-2011. Inversely, jellyfish species were more abundant during the sampled year after dam construction. Ecological network analysis showed that decrease on nutrient availability and increase of coastal waters influence on the estuary shifted the estuarine food web from one that was phytoplankton-based to one that is detritus based. This trophic shift produced a decline in population structure complexity and abundance of some of the resident fishes and those using the estuary as nursery. It is evident that the regulated freshwater release resulted in significant changes on the pelagic communities structure of the Guadiana estuary salt marshes.

Results from the achieved food web model are expected to provide relevant information to decision-makers, particularly those responsible for dam discharges regulation, in determining future management actions to efficiently protect and improve the quality of downstream Guadiana ecosystems, essential for the long term conservation of many estuarine-dependent organisms, namely important commercial fish species.

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Differences on population structure, dynamics and production of E. marinus populations from 3 different estuaries presenting a gradient of environmental pressure: Minho (low-impacted), Mondego (medium-impacted) and Ave (highly-impacted)

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Introduction

Echinogammarus marinus (Leach, 1815), a strongly euryhaline gammarid amphipod, able to support long emersion periods (Pinkster and Broodbakker, 1980), is extremely well adapted to life in estuaries. The species distribution extends from Norway to Portugal, having the Mondego estuary as the southernmost limit (Maren, 1975; Marques and Nogueira, 1991).

E. marinus occurs in close association with *Fucus vesiculosus* (Phaeophyta), a brown macroalgae, which constitutes an eulittoral belt on hard substrates. *E. marinus* is typically found underneath *Fucus*, in association with muddy deposits and also with the presence of green macroalgae, being less common on clean sands (Maren, 1975).

For example, in the Mondego estuary *E. marinus* is the most abundant species in hard substrate intertidal communities (Marques *et al*., 1993). It constitutes an important prey for upper trophic levels, namely for birds (Múrias *et al*., 1996, 1997), therefore playing an important role in the trophic web energy transfers.

Objectives

The present study aims at detecting the differences on population structure, dynamics and production of *Echinogammarus marinus* populations from 3 different estuaries presenting a gradient of environmental pressure: Minho (low-impacted), Mondego (medium-impacted) and Ave (highly-impacted).

3) Material and Methods

Study sites:

Mondego River:

Located between 40º08'10''N, 8º50'51''W, the Mondego River estuary includes as major sources of contamination a mercantile harbor, urban and industrial wastewaters, and discharges from aquaculture farms and intensive agriculture areas, responsible for an ongoing eutrophication process (Ferreira *et al*., 2003; Guerreiro and Pereira, 2002).

The Mondego estuary consists of two arms, north and south, that become separated by an island at the estuarine upstream area, at about 7 km from the sea, joining again near the mouth. The two arms of the estuary present very different hydrographical characteristics. The north arm is deeper (5 to 10 m during high tide, tidal range about 2 to 3 m), while the south arm (2 to 4 m deep, during high tide) is almost silted up in the upstream areas, which causes the freshwater of the river to flow essentially by the north arm. The water circulation in the south arm is mostly due to tides and to the relatively small freshwater input of a tributary, the Pranto River, which is artificially controlled by a sluice, located at 3 Km from the confluence with the south arm of the estuary.

Ave River:

Located between 41º20'49''N, 8º4'34''W, the Ave River estuary has like major sources of contamination products related to the textile sector, followed by leather tanning, rubber manufacture and plastic production (Guerreiro and Pereira, 2002; Soares *et al*., 1999). The textile sector is the most important activity and absorbs more than 70% of all people employed in industry. Leather tanning, rubber manufacture and plastic production are also significant.

Minho River:

Located between 41º53'27''N, 8º49'29''W, the Minho River estuary has about 35 Km long and comprises an area of 2 Km^2 of salt marshes (Ferreira *et al*., 2003). The Minho estuary is a very shallow system (Moreno *et al*., 2005), with a mean depth of 2.6 m (Freitas *et al*., 2009) and characterized as a mesotidal and partially mixed system, although during periods of high river flow, it tends towards a salt wedge estuary (Sousa *et al*., 2005).

Field program

Because of the existence of different environmental conditions at the 3 different estuaries, *E. marinus* individuals were sampled at three sampling stations in each one of the studied estuaries. Sampling was performed during low tide, which facilitated the access to the sampling sites. Three replicates, corresponding to areas occupied by *Fucus* sp were taken randomly each time at each sampling station.

Each replicate was obtained by scraping the rocky surface where the *Fucus* sp. (Phaeophyta) was attached. *Fucus* sp. was collected into labelled plastic bags and immediately washed after

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arrival at laboratory At the field, , the superficial 1 cm sediment layer around the rocks, under the fronds of algae, was also taken to ensure that all amphipods on the sampled area were collected. Since the sampled surface was irregular the area was estimated by photo. The sediment parts of the samples were preserved in 4 % buffered formalin, and later sieved in the laboratory through a 0.5 mm mesh size bag. Given the size of newly hatched juveniles, this mesh size retains all individuals. After separation, amphipods were kept in 70% ethanol for posterior analysis.

At each sampling date and at retention pools in each site, salinity, temperature, dissolved oxygen, pH were determined, whereas water samples were collected for posterior determination of dissolved nitrites, nitrates, and phosphates in the water column.. Sediment samples were also collected to estimate the organic matter content of the sediment.

Laboratory procedures

After initial selection, all *E. marinus* individuals were counted sexed, and the cephalic length (CL) was measured to the nearest 0.02 mm. The determination of sex was based on the presence or absence of oostegites and/or broods (females), and of genital papillae (males). Animals without these features were considered to be juveniles. Nonbrooding females were examined for the presence or absence of setae on the oostegites and classified as mature or resting females. If broods were present, eggs were counted, to estimate fecundity, measured and examined to determine the development stage. Five stages were considered, taking into account the criteria described in Skadsheim (1982) adapted by Marques and Nogueira (1991), respectively: A, newly laid eggs, spherical grouped and resembling a gelatinous mass; B, eggs well separated, oblong internally homogeneous; C, embryo comma-shape with vestigial pereopods already visible; D, constriction of the comma clearly visible, appendages segmented and looking slender, eyes visible, cephalotorax orange-red; E, hatched and free juveniles (these may stay some days in the brood pouch). Due to some difficulties in distinguishing stages A and B, data regarding these two stages were pooled in an AB stage. Since eggs become oval in shape during development, an average of their length and width was taken as measurement of their size.

Results

Results indicate that *E. marinus* density and biomass show higher values in the Mondego estuary, followed by the Minho estuary and finally the Ave estuary.

The low values of density and biomass presented by *E. marinus* populations at the Ave estuary must be related with the strong environmental impacts of this system, particularly, at the most disturbed sampling station (Estaleiro), where frequent fuel residuals are observed at *Fucus* sp. fronds and sediment. Interestingly, the sampling station that shows higher abundance of *E. marinus* at the Ave estuary is a station located at the outer most part of the estuary, with strong marine influence and characterized by beach-type sediment with very low organic matter content.

At Minho estuary, *E. marinus* individuals are found at all sampling stations from the estuary mouth until a saltmarsh upstream area. Nevertheless, during autumn and winter months, the sampling station located closest to the estuary mouth (Embocadura) tends to present the highest densities of *E. marinus* individuals.

In accordance with previous studies (Marques and Nogueira, 1991; Maranhão, 2001) all sampling stations from the Mondego estuary exhibit high densities and biomass of *E. marinus*, including the station with potential high environmental impacts, situated at a deactivated shipyard (Doca).

Discussion

Our results indicate that *E. marinus* populations are able to live in close association with *Fucus* sp. associated to diverse types of substrates, from fine sediments organic matter rich to coarser substrates with low organic matter content.

In addition, our results also suggest that the abundance, dynamics and production of this amphipod species is influenced by environmental contamination, which seems to be related with the low density and biomass values of *E. marinus* populations from the Ave estuary. However, besides contamination and pollution, other environmental stressors seem to control the abundance of *E. marinus* at estuarine areas. This is in accordance with the finding that *E.marinus* populations present clearly higher density and biomass values in Mondego compared to Minho, although the latter is considered a more preserved system than the former. However, on a year basis, the average salinity in the Mondego estuary is higher than in Minho estuary, which will favor *E. marinus*, a truly euryhaline species, strongly affected by salinity decrease (Maranhão and Marques, 2003).

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Exploring patterns of variation of amphipod assemblages at multiple spatial scales: natural variability versus coastal aquaculture effect

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Abstract

Monitoring programs and studies of environmental impact are established to evaluate and quantify environmental changes that occur in the marine environment due to human driven disturbances. Their objectives are to identify the effects and establish corrective actions to minimize them. A relevant point in this context is to know ecological processes that determine distributional patterns of the organisms, so that natural spatial and temporal variation can be estimated and scales in which they vary can be detected. In aquaculture, the development of sampling protocols and environmental monitoring plans must be based on appropriate spatial and temporal scales allowing a correct interpretation of the differences of disturbed and control areas.

A 5-factor design survey was carried out to examine the spatial distribution at different spatial scales of amphipods assemblages and sedimentary variables in soft bottoms adjacent to coastal aquaculture installations. The design included a distance gradient from fish farm cages to a reference area, in two different seasons at three spatial scales of regions (North, Central and South of Western Mediterranean, separated 100´s km), localities (separated by several km) and sites (separated 10´s meters).

Results obtained with multivariate PERMANOVA detected significant differences in the main factor Distance (p=0.0108), however, significant interactions between smaller scales, represented by random factors, and main factors were also detected. Natural variability of sediment variables showed the highest values at the scales of sites and locality, while the greatest component of variation of amphipod assemblage occurred among replicates. Regarding the influence of coastal aquaculture, the highest variability of the environmental variables was observed at locality scale, among fish farms. However, the variability of amphipod assemblages at this level was lower than reference values. The dbRDA analysis did not detect any gradient of aquaculture influence, in contrast there was a separation of the localities based on the grain size distribution of the sediment. The results of muddy, sandy and mixed localities analyzed separately revealed an aquaculture gradient related to different variables depending on the sediment type. Thus, the separation of each gradient was related to total free sulphides in sandy localities or to total organic carbon and δN15 in muddy bottoms.

Aquaculture monitoring programs should consider that the degree of influence of each installation depends on management features, (total fish production, feeding method and feed used), and local environmental characteristics (i.e. water depth, hydrodynamism and sediment type). Moreover, the magnitude of any effect differs among fish farms, making it difficult to generalize the patterns of affection by coastal aquaculture. Additionally, the remarkable small and medium scale variability of amphipod assemblages and sediment features make it necessary the use of robust design of sampling protocols with high replication at several spatial scales for a better understanding of ecological processes and for assessing impact or restoration plans.

This kind of protocols would enhance the sustainability of aquaculture industry through a proper detection of its influence over the environment.

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Transitional and freshwater bioassessments: one site - two perspectives

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Although a consistent advance is evident for scientific knowledge on benthic macroinvertebrate communities living in marine, estuarine and freshwater areas, the existing data are not exactly the same for these aquatic zones. Even with similar scientific objectives, the research teams working on these different areas use different sampling strategies and so collect different data, which result also in different perceptions of the macrobenthic communities. It doesn't mean the communities are different, they are looking like that also due to differences found along the sampling and analytical process they undergo. The lower part of big rivers and the upper part of transitional waters constitute the contact study zone between two important research areas (rivers and coastal areas). Due to the used protocols, differences found for macrobenthic communities inhabiting the contact zone may be considered as artifacts, and the apparent discontinuity observed in there by the different research teams should be considered with care. The knowledge provided by both scientific fields should be merged for a better understanding of structure and functions found in the contact area community.

To clarify the uncertainty about those areas, macrobenthic samples were collected in the contact zone of the Mondego (central Portugal), following simultaneously the protocols used by river and coastal benthologists. About 77% of the total identified taxa were present in samples treated by the river team, meanwhile 55% were present in samples analyzed by the coastal and transitional waters team. Only 32% of the taxa were found in common by both teams. From the common taxa, about 33% of them were present in more than 1/3 of the samples, for both teams. The difference found on taxonomic composition (Ephemeroptera & Trichoptera, and Gastropoda contributed each with 22 % for total diversity in river samples but were not collected by coastal water methods; Crustacea contributed more to total diversity in samples from coastal teams than for rivers) may be due to the habitats surveyed by each team and differences on identification procedures. These results are in line with recent concerns created by the WFD. Harmonization along aquatic systems should be ensured, namely for the same BQE (e.g. macroinvertebrates) in different water categories.

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Assessing the health status under European directives in the Basque Country: from estuaries to offshore waters

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Taking into account the increasing human pressures on aquatic systems (Halpern *et al.,* 2008), the European Commission wants to reduce their impacts. Hence, two European directives (the Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/58/EC) establish a framework, within which Member States shall take the necessary measures to achieve or maintain Good Ecological Status (GES) and Good Environmental Status (GEnS), in estuarine/coastal waters and offshore waters, respectively. This should be achieved by the years 2015 and 2020, respectively.

Both MSFD and the WFD rely on our ability to determine what is 'good status', described in narrative terms in both directives (i.e. Annex 5 normative definitions in the WFD and Annex 1 descriptors in MSFD). These definitions then have to be supported by a scientific justification (Mee *et al*., 2008). The concept of environmental status is more global than the ecological status and takes into account the structure and functioning (where the latter by definition includes processes) of the marine ecosystems together with natural physiographic, geographic and climatic factors, as well as physical and chemical conditions including those resulting from human activities in the area concerned (Borja *et al.,* 2010).

Considering the experience gained through the implementation of the WFD, over the past decade (Hering *et al*., 2010), we discuss here how that experience could benefit the implementation of the MSFD; in particular, in the context of setting the GEnS criteria. The implementation of these directives should be aimed at the conservation of the marine ecosystems. Such an approach should include the declaration of marine protected areas and should address all human activities that have an impact on the marine environment, throughout an integrated ecosystem-based approach (EBA), in the case of GEnS (Browman *et al*., 2004).

In the WFD the assessment of ecological status is based upon five biological quality elements (BQE: phytoplankton, macroalgae, angiosperms, macroinvertebrates and fishes), together with hydromorphological and physico-chemical quality elements, with the BQE being especially important (Table 1). In turn, the MSFD uses 11 qualitative descriptors (Table 1), and several task groups have worked during 2009 and 2010 to select adequate indicators to be used in the assessment of each descriptor. Subsequently, the European Commission has selected a set of indicators which could be taken into account in the final environmental status assessment (Cardoso *et al.,* 2010; European Commission, 2010).

A complete monitoring of marine waters and an adequate set of data are needed to assess the status under these two directives. In the Basque Country (Bay of Biscay), work has been undertaken during the past 25-30 years, monitoring estuarine, coastal and offshore marine waters within the framework of European, national and regional projects. A synthesis of methods and components sampled, which have included human pressures, oceanographic conditions, pollutants, phyto- and zooplankton, benthos, fishes, mammals and seabirds, can be consulted in Borja and Collins (2004). The WFD monitoring network, from the Basque Water Agency, started in 1995 within Basque estuaries and along the coast (Borja *et al.,* 2004, 2009); in addition, one offshore site is sampled since 2002, and two more since 2006 (see locations in Figure 1, and a detailed description in http://www.uragentzia.euskadi.net/u81-0003/es/contenidos/informe_estudio/red_costa/es_red_agua/2010.html). Samples for the determination of physico-chemical variables, pollutants (in waters and sediments), together with phytoplankton and benthic communities, are collected regularly and on a long-term basis (for details on the methods, see Borja *et al.,* 2004, 2009; Tueros *et al.,* 2009). This information permits the assessment of most of the indicators and BQEs in both directives.

In the case of the WFD an assessment method was proposed for the Basque Country by Borja *et al*. (2004, 2009). Recently, Borja *et al.* (2011) have proposed an approach to assess the status under the MSFD. This contribution presents an integration of the assessment done in estuaries, coasts and offshore waters, within the framework of the WFD and the MSFD, discussing the relationships between the human pressures and the corresponding status.

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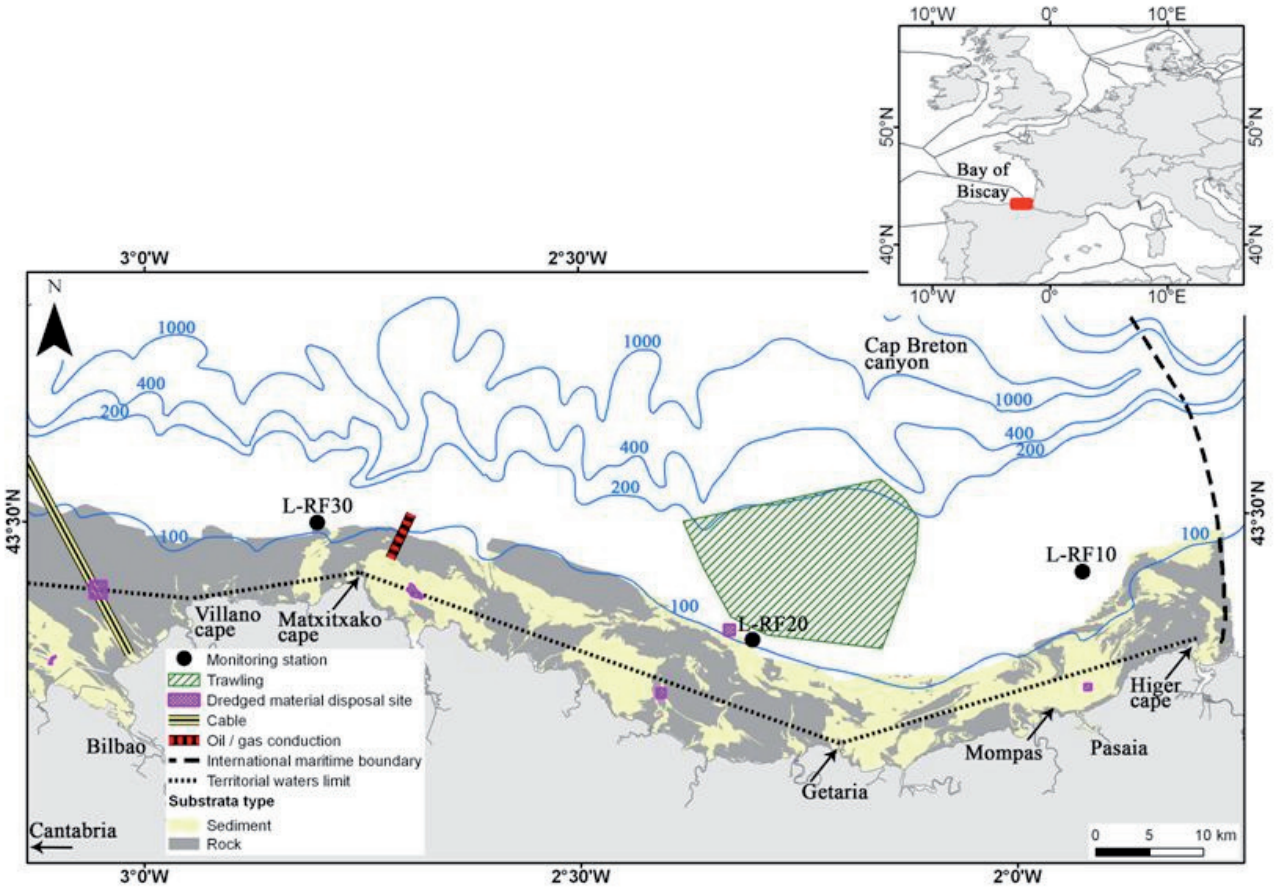

Figure 1. Area of study, within the southeastern part of the Bay of Biscay. The territorial waters limit and the international maritime boundary are highlighted, together with 3 sampling locations on the continental shelf and the main human pressures within the area.

Table 1. Main issues addressed by the Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD) (Modified from Borja *et al.,* 2010).

Main issues	WFD	MSFD
Spatial application	Estuaries & coasts (baseline + 1 nm)	From baseline to 200 nm
Main definitions	Ecological status	Environmental status
Levels of status	High, Good, Mod, Poor, Bad	Good, Not Good
Good status achieved by	2015	2020
Elements (WFD) or qualitative descriptors (MSFD) to be used in assessing the ecological or environmental status	Chemical Physico-chemical Hydromorphological Phytoplankton Macroalgae Phanerogams Macroinvertebrates Fishes (only in transitional waters)	1- Biodiversity 2- Non-indigenous species 3- Exploited fish and shellfish 4- Food webs 5- Human-induced eutrophication 6- Sea-floor integrity 7- Hydrographical conditions 8- Contaminants 9- Contaminants in fish 10- Marine litter 11- Introduction of energy/noise
Ecological differences	Integration of each element 'One out, all out' principle Bottom-up approach	Ecological integrity Ecosystem-based approach Top-down approach

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Morirás en Chafarinas: el drama de la gorgonia gigante *Ellisella paraplexauroides*

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La gorgonia gigante *Ellisella paraplexauroides* es probablemente el invertebrado marino más grande del Mediterráneo y el único representante de su género en aguas europeas. Se trata de una especie de aguas profundas, raramente presente en el infralitoral superior, cuya biología y ecología permanecen prácticamente desconocidas, a pesar de ser un organismo altamente emblemático. En este estudio se investiga una población singular, establecida en aguas relativamente someras de las Islas Chafarinas, en una zona catalogada como Lugar de Interés Comunitario (LIC) y, por tanto, con actividad pesquera restringida. Nuestro estudio proporciona los primeros datos poblacionales sobre aspectos de ecología, demografía y estado de conservación. La densidad poblacional promedio es de 0,5 colonias m^{-2} (con un máximo de 5 col. m^{-2}), el volumen promedio de una colonia es 48 ml (máximo 511 ml), la biomasa en peso seco 75 g (máximo 804 g), su altura 76 cm (máximo 167 cm) y el número de ramas terminales de 16 (máximo 72). Los estudios morfométricos revelan un rápido crecimiento inicial en altura con escasa formación de ramas, incrementándose la tasa de ramificación y engrosamiento de las ramas sólo cuando las colonias alcanzan 60-80 cm de altura. La distribución poblacional de tamaños indica que el reclutamiento de juveniles es entre moderado y bajo. En contraste, se ha detectado una elevada mortalidad que afecta a adultos y juveniles. Dentro del área de distribución de la población, se identificaron tres zonas de actividad pesquera artesanal furtiva, a pesar de que se trata de una zona LIC: zona A, con intensa actividad furtiva mediante trasmallo, currícan y, ocasionalmente, arrastre; zona B, con un nivel intermedio de presión pesquera; zona C, con moderada actividad pesquera. Las gorgonias de las zona A y B (con nivel de furtivismo intenso e intermedio, respectivamente) muestran gran cantidad de restos de artes de pesca enredados en sus ramas, causando numerosas lesiones y un elevado grado de epibiosis en las colonias. La incidencia de la mortalidad se incrementa de modo directamente proporcional a la intensidad de la pesca furtiva. En la zona con mayor presión pesquera, alrededor de la mitad (44,6%) del total de las colonias contabilizadas han muerto y aproximadamente el 82% de las que sobreviven, muestran importantes lesiones y notables cargas de epibiontes, que amenazan la viabilidad de las colonias a medio plazo. En la zona C, de mínimo furtivismo, se contabilizó un impacto de la mortalidad de sólo el 10% por unidad de área, es decir, una gorgonia muerta por cada nueve vivas. El grueso de la mortalidad detectada deriva claramente de la actividad pesquera (particularmente del uso de trasmallos), porque durante los tres años de estudio no se han detectado signos de enfermedades epidémicas en la población. Se ha documentado depredación sobre la gorgonia por parte de una población local de ejemplares de gran tamaño (> 50 cm) del poliqueto *Hermodice carunculata*, pero las lesiones causadas sobre las colonias afectan sólo al cenosarco de las puntas de las ramas y se regeneran con gran facilidad, sin afectar a la viabilidad de las colonias. Se sospecha que la población de *Ellisella paraplexauroides* estudiada es una reliquia Mediterránea única, fruto de colonizaciones de origen mauritano-senegalense durante periodos interglaciales del Pleistoceno. La actual falta de efectividad en la regulación de la actividad pesquera en la zona está causando un dramático declive en esta singular población, con serio riesgo de exterminio a medio plazo.

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Variability in the Stress-On-Stress (SOS) response in native mussels (*M. galloprovincialis*) from Biscay Bay

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Monitoring tools have been developed for the assessment of contaminated coastal areas, including the use of sentinel species in which biomarkers have been applied. Particularly, mussels are used as bioindicators and as sentinels; they accumulate pollutants and undergo biological responses reflecting exposure and biological effects of pollutants (molecular, cell and tissue level biomarkers; bioassays). The individual level biomarker Stress-On-Stress (SOS) response has been used since 1991 as a general indicator of the health status in mussels. Presently, the natural variability in SOS response (temperature, individual size, tidal regime) and the variability in the responsiveness to cadmium exposure were investigated under laboratory conditions. First, mussels SOS response was determined at different chamber (room) temperatures (18, 22, 26 and 20 °C). Second, the SOS response of mussels of 6 different sizes was determined at 18°C. Finally, SOS response was determined in mussels from several sites and intertidal levels in localities with different levels of pollution in the Basque Country (Mundaka: relatively unpolluted; and Arriluze: polluted). LT50 and LT90 (lethal times with a 50 % and a 90 % mortality, respectively) values were calculated. The LT50 values decreased at increasing temperatures (LT50 (30 °C) = 1 d; vs. LT50 (18 ºC) = 7 d). Thus, the efficiency of the SOS response assay could be improved by increasing the testing temperature-in-air (enhanced mortality); which might contribute to reduce the time needed for experimentation. On the other hand, it is conceivable that the time to survive in air of mussels also depends on their size (age). However, we did not find differences in LT50 values between mussels of different size classes. In contrast, LT90 was higher in the smallest mussels from Arriluze than in the large ones. Previous studies by other authors also stated that small mussels exhibit higher tolerance to air exposure than large individuals. There were not differences in LT50 between mussels collected from different intertidal zones. Unexpectedly, regardless of their size, mussels from Arriluze survived for longer than those from Mundaka. This aspect deserves future research efforts since it was expected that mussels from the polluted site would survive less than those from the less polluted one. However, it is likely that Arriluze mussels, which are subjected to chronic pollution, might present some adaptive strategy that renders them more resistant than expected to additional sources of stress. The results obtained after the SOS response assay were compared with cell and tissue-level biomarkers (e.g. Lysosomal Membrane Stability) after exposure of mussels from Mundaka to Cd (0.005, 0.05 and 0.5 mg Cd/l seawater) under controlled laboratory conditions. In conclusion, regardless the mussel size and the intertidal level where the mussels are collected, the cost-efficiency of the SOS response assay can be improved by conducting the assay at high chamber temperatures (e.g., 26-30ºC) and by using LT90 values instead of LT50 values.

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Impactos del desarrollo del litoral asociado al turismo en los arrecifes de coral de Bonaire

Los arrecifes de coral son los ecosistemas marinos más biodiversos, que además ofrecen numerosos bienes y servicios. Sin embargo, la alta vulnerabilidad de estos ecosistemas a perturbaciones antropogénicas y naturales han dado lugar a su degradación. En este estudio se analizan los impactos a largo plazo del desarrollo del litoral asociado al turismo en los arrecifes de coral de Bonaire (Caribe), y sus implicaciones para la industria del buceo.

Con el fin de caracterizar las condiciones de los arrecifes, se realizaron muestreos del bentos en 48 puntos de buceo. Por otra parte, se identificaron y caracterizaron las variables asociadas al desarrollo del litoral. Mediante análisis estadístico se estudiaron las relaciones entre las condiciones de los arrecifes y las variables relacionadas con el desarrollo.

Los arrecifes de coral próximos a hoteles y zonas de mayor intensidad de desarrollo, y con exposición más larga a dicho desarrollo, presentan condiciones significativamente inferiores a las de los arrecifes alejados de las zonas del litoral desarrolladas. Los buceadores responden a estas condiciones, realizando inmersiones en lugares alejados del desarrollo turístico.

Los resultados de este estudio resaltan la estrecha relación entre lo que sucede en tierra y el mar, poniendo en evidencia la necesidad de un cambio radical en la planificación y desarrollo del litoral.

The use of easy measurable variables in the sediment to assess impact in marine sediments affected by organic wastes (1978-2010)

F. Xavier Niell, Raquel Carmona and Miriam Ruiz.

The assessment of impact in sediments is feasible fitting easy measurable variables like organic matter content, chlorophyllic pool pigments and elementary analysis of C and N. From these data several indexes reveal clearly the quality of the sediment in terms of potential primary production and flow of energy and organic matter degradability in sedimentary microecosystems. The sampling campaigns carried out since 1978 in Placeres (Pontevedra, Spain) allow to compare data after several improvements in the environmental management, and to compare the current state of the system with values obtained in other sites non affected by pulp-mill factories wastes. Data have been used to perform an useful model to assess impact of the organic wastes on sediments, to be applied easily and expressed as percentage of disruption related to the control reference system.

Keywords: Sedimentary ecosystems, Chlorophyll *a,* C/N, organic matter, dilution-diffusion functions, impact assessment

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The background metal levels determination in bivalves - quality assessment of the European Water Framework

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The term "background level" has received several definitions; in environmental studies it is used to refer to pre-industrial levels or natural levels, from pristine areas (see review of Reimann and Garrett (2005)). At a European scale, within the context of the Water Framework Directive (WFD; Directive 2000/60/EC (EC, 2000)) and the Marine Strategy Framework Directive (MSFD; Directive 2008/56/EC (EC, 2008)), the determination of natural background levels in marine habitats, as a relative measure to distinguish between natural concentrations and anthropogenically-influenced concentrations, is highly relevant. These background levels have to be determined regionally since previous studies have found natural regional variability due to the underlying geological differences (e.g., Carballeira *et al.*, 2000).

For the coast of the Basque Country (northern Spain, SE Bay of Biscay), regional background levels of metals in waters (Tueros *et al.*, 2008) and sediments (Belzunce *et al.*, 2004; Cearreta *et al.*, 2000; Rodriguez *et al.*, 2006) have been previously determined. Moreover, 'high/good' status boundaries for ecological status assessment, according to the WFD, have been established. Although a previous study focused upon background determination in biota, (e.g., molluscs (Borja *et al.*, 2004)), neither status boundaries nor seasonal variation of metal concentrations mentioned by Franco *et al*. (2002) were considered.

Thus, the main objective of this contribution is to determine background levels of metals in molluscs of the Basque Country coast, considering seasonal variability, to use them in the quality status assessment, within the WFD.

The basis for this study was the statistical analysis of data of metals in mussels (*Mytilus galloprovincialis*) and oysters (*Crassostrea gigas*) compiled from monitoring programmes undertaken along the Basque coast, between 1990 and 2008 (Figure 1). For this analysis, a heuristic method described by Reimann and Garrett (2005), based upon graphical inspection and using statistical and graphical representations, was adapted and used.

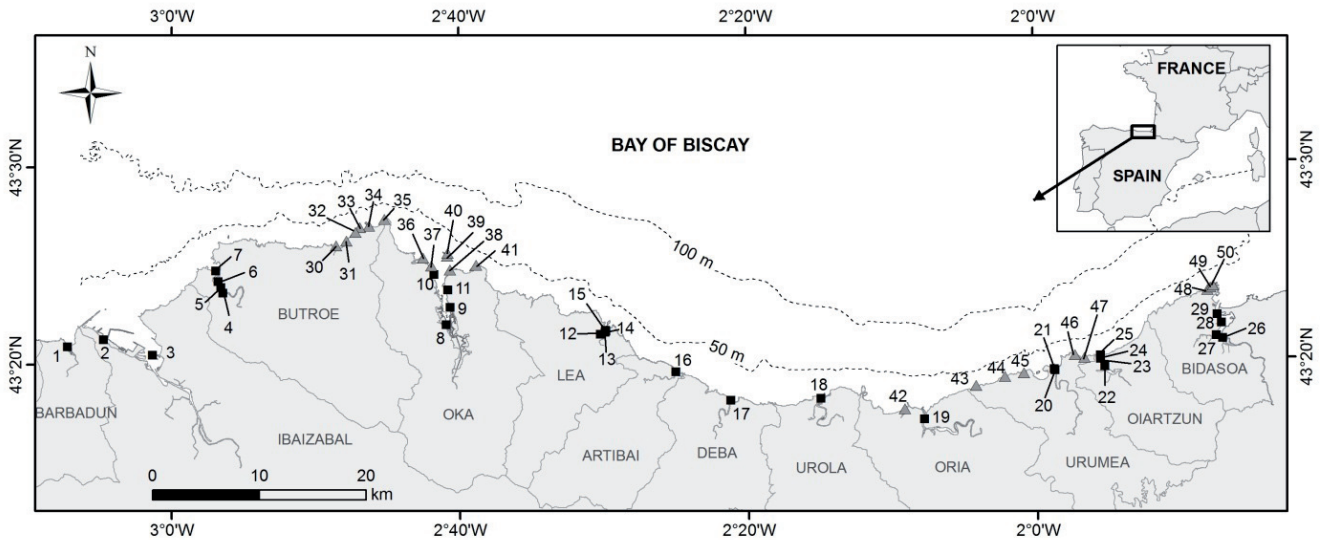

Figure 1. Location of estuarine (squares) and coastal (triangles) sampling sites, within the Basque Country.

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The approach adopted permits the removal of data from polluted areas (Reimann *et al.*, 2005). For background levels determination, data from almost all estuarine sampling sites have been rejected for, at least, one of the considered metals in one of the considered species. However, only coastal sites close to areas that have received, during many years, untreated urban and industrial wastewater, have been removed. It has to borne in mind that Basque estuaries are liable to be subjected to significant anthropogenic pressures, such as water or sediment pollution, while human pressure is lower in the coastal area (Borja *et al.*, 2006).

Results showed different metal background ranges throughout the year for both, mussels and oysters (Table 1). The main reason for this seasonal variation could be related to the physiological cycle of the molluscs. On the other hand, higher background levels were determined for Ag, Cu and Zn in oyster, relative to mussel. This is due to the fact that metal bioaccumulation in mollusc differs between metals and species (e.g., Kimbrough *et al.*, 2008; Luoma and Rainbow, 2005).

Values corresponding to the upper limit of the background range (i.e., value of the upper whisker of the box-plot) are proposed as the 'high/good' status boundary, according to the WFD, for ecological status assessment.

Table 1. Statistical descriptors of the metal background concentrations (mg kg^{-1}, dry weight) in mussels and oysters from the Basque coast.

Species	Metal	Data range	Lower whisker	Median	Upper whisker	P95
Mussel	Ag	**30/05 - 6/09**	0.04	0.30	0.75	1.75
		20/09 - 12/12	0.03	0.06	0.12	0.20
	Cd	**14/12 - 21/05**	0.03	0.62	1.69	1.65
		22/05 - 15/06	0.36	0.87	2.00	1.96
		18/06 - 29/09	0.02	0.60	1.67	1.53
		10/10 - 11/12	0.03	0.78	2.15	2.29
	Cr	**2/01 - 16/12**	0.02	2.21	6.40	5.33
	Cu	**1/12 - 23/06**	1.56	12.25	24.80	24.80
		6/07 - 29/11	2.07	9.33	18.19	21.14
	Hg	**19/11 - 25/08**	0.02	0.19	1.13	0.86
		27/08 - 18/11	0.02	0.13	0.42	0.63
	Ni	**5/12 - 15/01**	0.01	7.53	17.20	17.20
		30/01 - 4/12	0.11	1.84	7.53	6.93
	Pb	**19/11 - 6/07**	0.04	4.47	15.16	13.32
		8/08 - 18/11	0.03	2.47	9.01	9.01
	Zn	**25/05 - 6/05**	24	364	893	866
		8/05 - 23/05	50	142	1037	836
Oyster	Ag	**9/01 - 21/12**	0.01	3.71	16.26	15.74
	Cd	**9/01 - 29/12**	0.01	0.90	2.06	1.87
	Cr	**3/01 - 20/12**	0.01	1.70	4.73	4.03
	Cu	**11/12 - 30/10**	0.19	262	590	571
		2/11 - 4/12	17.6	391	1253	1055
	Hg	**19/11 - 28/02**	0.10	0.35	1.65	1.25
		25/04 - 16/11	0.02	0.15	0.53	0.82
	Ni	**15/01 - 29/11**	0.01	1.51	5.81	5.81
	Pb	**9/01 - 20/12**	0.03	2.51	7.85	7.23
	Zn	**30/05 - 3/05**	52	2536	6077	6672
		5/05 - 29/05	46	357	5072	4271

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Analysis of morphological characteristics of rhodoliths as indicator of habitat complexity and fishing effects

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Rhodoliths are unattached coralline red algae that roll on the seafloor and adopt spherical and/or branching morphologies. At high densities, these free-living algae form rhodolith or maërl beds, which constitute communities of high diversity in Mediterranean Sea which can be severally damaged by fishing activities. In the present study, we analyzed the morphological characteristics of rhodoliths as potential indicators of habitat complexity and fishing disturbance caused by bottom trawling.

The study was carrying out in Menorca Channel, where maërl beds cover more than 50% of continental platform between 50-100 m depth. Changes in rhodoliths morphology and size structure were tested analyzing the relationships with habitat complexity, comparing habitats with different structure (sand, maërl beds and soft algae beds) and comparing locations with different level of fishing pressure. Epibenthonic algae were sampled using a beam trawl of 2m width. A subsample of approximately 100 randomly chosen rhodoliths was photographed, identified and classified in different morphotypes. Measures of these rhodoliths (length of axes, diameter, perimeter, area, aspect and roundness) were obtained applying image analysis. Fishing pressure in maërl beds was defined from satellite-based Vessel Monitoring System data, defined as percentage of trawled area per year in the selected locations.

Morphotypes composition was notably different between habitats but was not significantly different between the different levels of trawling intensity in maërl beds. Size and morphological parameters were different between habitats and locations with different trawling effort, but only for the most abundant species, *Spongites fruticulosus* and *Lithotamnium coralloides.* Diverse factors may affect the growth rate and morphology of the maërl species: light, nutrients, temperature, hydrodynamic, and species composition. The results of the present study showed that changes in size and morphology of deep-water rhodoliths in this area are better explained by the current velocity, depth and rhodoliths coverage more than the trawling intensity. However, the results also suggest that the reduction of the complexity of size and morphologic structure could be related with the incipient effects of fishing intensity in an area where the fishing impact were not detected by changes in algal biomass and mega-faunal species composition.

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Development of a Bayesian Networks based method for assessing the status of hard bottom substrata biota

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The responses of hard-bottom biota to a plethora of pressures have been studied world-wide. This has allowed the development of methodologies and indices that allow evaluating the ecological status of hard-bottom benthic communities, e.g.: Rhodophyta/phaeophyta mean ratio index (Cormaci *et al*., 1985); Ecological Evaluation Index (EEI) (Orfanidis *et al*., 2001; Orfanidis *et al*., 2003); CAR-LIT (Ballesteros *et al*., 2007); BENTHOS (Pinedo *et al*., 2007); TWo-stage INdex (TWIN) (Marchini and Occhipinti-Ambrogi, 2007); Reduced species list (RSL) index (Wells *et al*., 2007); Quality of rocky bottoms (CFR) index (Juanes *et al*., 2008); Ecological Evaluation Index continuous (EEI-c) (Orfanidis *et al*., 2011); Benthic Index for Rocky Shore (BIRS) (Orlando-Bonaca *et al*., 2012) or Rocky Intertidal Community Quality Index (RICQI) (Diez *et al*., 2012). Most of these indices/ methodologies are based upon the macroalgae, without taking into account macrofaunal communities. Hence, few attempts have been made to develop an index for assessing the quality of hard substratum fauna (see Hiscock *et al*., 2005 and Diez *et al*., 2012). However, rocky invertebrate assemblages are capable of detecting environmental effects,.although their validity as marine ecosystem indicators is extensively acknowledged (Clarke and Warwick, 1994; Hiscock *et al*., 2005; 2006; Rogers and Greenaway, 2005; Hiscock and Tyler-Walters, 2006). In spite of this observation, gathering the evidence necessary to distinguish between various degrees of community alteration, to establish an ecological status classification compliant with the Water Framework Directive (WFD), is a challenging task. In this way, the simultaneous use of both flora and fauna may be more appropriate, in determining the ecological status of hard substrata; which could better correlate with a disturbance gradient (Hiscock *et al*., 2005; Goodsell *et al*., 2009).

Most of the above mentioned methodologies are based upon the knowledge of the relationships between biota and pressures (both natural and anthropogenic). However, the interactions between biota and different pressures are complex and often non-linear, making it difficult to produce robust predictions (e.g., Fernandes *et al*., 2010). 'Machine-learning' techniques (in particular, supervised classification methods) have been proposed as useful tools, to overcome such difficulties (e.g, Fernandes *et al*., 2010). The novelty of the present research is based upon the well-known fact that structural parameters of biota are related to the degree of pressures and, therefore they can predict the quality, but taking into account that the relationships between biota and pressures are complex. Here, the 'machine-learning' techniques (in particular, supervised classification methods) have been applied for identifying a set of biological elements that can predict the variability in the status of the biota, trying to develop a new approach in assessing the ecological status, within the WFD, in hard-bottom substrata.

A total of 288 samples were taken into account (Figure 1). Data were classified in the following shore levels: (i) Supralittoral fringe (intertidal area, only covered by water in extreme high water at spring tides); (ii) Midlittoral zone (intertidal area, subjected to daily tides); (iii) Infralittoral fringe (intertidal area, only discovered in extreme low water at spring tides); (iv) Subtidal zone, 5-15 m water depth; and (v) Subtidal zone, >15m water depth.

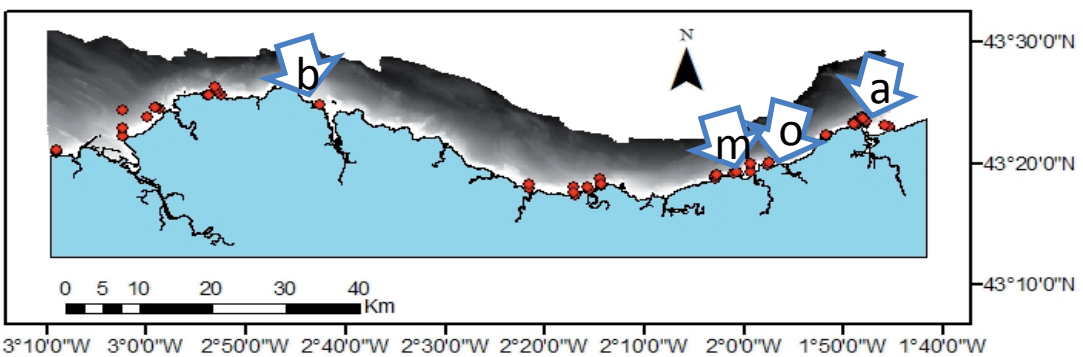

Figure 1. Study sites, showing all locations sampled. Red points indicate sampling sites. Key for sewage outfalls: a - Atalerreka, o - Oiartzun, m -Mompas-Pasaia, b -Bermeo.

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The Bayesian Network models were fitted in three steps: 1) The predictor variables were discretized into levels (less than x, more than x) using the Fayyad and Irani's Multi-Interval Discretization (MID) method (Fayyad and Irani, 1993). It searches, in each predictor, for a set of cut-off points that reduces the response variable uncertainty measured in terms of entropy and conditional entropy (Information Theory based). 2) Hall's multivariate Correlation-based Feature subset Selection (CFS) method (Hall and Smith, 1997) was used to select the predictive factors. CFS is based upon an intuitive formulation, the assumption that a good subset of factors is one that is highly correlated with the response variable and, at the same time, the predictive factors have low correlation between them. 3) A naive Bayes model was fitted (Minsky, 1961; Duda *et al*., 2001). Naive Bayes is one of the simplest Bayesian Networks Classifiers (Larrañaga *et al*., 2005). The Naive Bayes is a probabilistic model, which assumes that, given the class variable, all of the factors are independent. This assumption implies that a naive Bayes classifier requires the specification of a small number of parameters. This leads to robust models and parameter estimation when sparse training is data available.

Bayesian models were built for predicting the status of macroalgae, macrofauna, and macroalgae+macrofauna, within hard bottom areas of the Basque coast from the parameters described in Table 1. The number of statuses that can be predicted varied from two ('high-good' and 'moderate-poor') to four ('high', 'good', 'moderate' and 'poor') depending upon the shore level and the biota included (i.e. macroalgae, macrofauna or macroalgae+macrofauna). The accuracy of the models differs between shore levels and the biota included.

Table 1. Parameters derived from biotic data (based upon biomass). Key: S - number of taxa, d - richness (Margalef), Jbis - Pielou's eveness, Brillouin - H(log2) - Shannon-Wiener diversity index (H'), Lambda - Simpson's Lambda, 1-Lambda - Simpson's 1-Lambda, Lambda Bis - Simpson's Lambda', 1-Lambda Bis - Simpson's 1-Lambda', N10 - Hill's N10, N10Bis - Hill's N10', N21 - Hill's N21, N21Bis - Hill's N21', Delta - Taxonomic diversity, Delta* - Taxonomic distinctness, Delta+ - Average Taxonomic distinctness (Presence/Absence), sDelta+ - Total taxonomic distinctness (Presence/Absence), Lambda+ - Variation in taxonomic distinctness (Presence/Absence), Phi+ - Average Phylogenetic diversity averaged over number of species in sample, sPhi+ - Total Phylogenetic diversity.

	S	d	JBis	Brillouin	H(log2)	Lambda	1-Lambda	Lambda Bis	1-Lambda Bis	N10	N10Bis	N21	N21Bis	Delta	Delta*	Delta+	sDelta+	Lambda+	Phi+	sPhi+
All taxa	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX
All taxa without Nematoda, Platyhelminthes, Foraminifera and Copepoda	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX
Animalia	YX	N	YX	N	YX	YX	YX	N	N	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX
Animalia without Nematoda, Platyhelminthes, Foraminifera and Copepoda	YX	N	YX	N	YX	YX	YX	N	N	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX	YX
Arthropoda without Copepoda	N	N	N	N	YX	N	N	N	N	N	N	N	N	YX	YX	YX	YX	YX	YX	YX
Porifera	YX	N	N	N	YX	N	N	N	N	N	N	N	N	YX	YX	YX	YX	YX	YX	YX
Mollusca	YX	N	N	N	YX	YX	YX	N	N	YX	N	YX	N	YX	YX	YX	YX	YX	YX	YX
Cnidaria	YX	N	N	N	YX	N	N	N	N	N	N	N	N	YX	YX	YX	YX	YX	YX	YX
Bryozoa	YX	N	N	N	YX	N	N	N	N	N	N	N	N	YX	YX	YX	YX	YX	YX	YX
Polychaeta	YX	N	N	N	YX	N	N	N	N	N	N	N	N	YX	YX	YX	YX	YX	YX	YX
Plantae, Bacteria and Chromista	YX	N	N	N	YX	N	N	N	N	N	N	N	N	YX	YX	YX	YX	YX	YX	YX
Plantae	YX	N	N	N	YX	N	N	N	N	N	N	N	N	YX	YX	YX	YX	YX	YX	YX
Chromista	YX	N	N	N	YX	N	N	N	N	N	N	N	N	YX	YX	YX	YX	YX	YX	YX
Bacteria	YX	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	YX	YX
Chlorophyta	YX	N	N	N	YX	N	N	N	N	N	N	N	N	YX	YX	YX	YX	YX	YX	YX
Rhodophyta	YX	N	N	N	YX	N	N	N	N	N	N	N	N	YX	YX	YX	YX	YX	YX	YX
Florideophyceae	YX	N	N	N	YX	N	N	N	N	N	N	N	N	YX	YX	YX	YX	YX	YX	YX

The models were validated in areas from Portugal and Denmark, but coherent results were not find. This suggests that the Bayesian models developed in this study can be useful only for the Basque Country area. This can be due to the fact that the pressure taken into account in the Basque coast is related mainly to outfalls. Additionally, biogeographical differences in taxonomic composition (or differential responses to pollution) can be also responsible for the lack of response in Portugal and Denmark. Finally, differences in sampling strategy can also have influence on the approach.

Nevertheless, we have found that it is possible to build Bayesian models which are able to predict the ecological status in hard-bottom substrata. Therefore, in those countries with a high number of data, obtained with homogenous sampling methods, Bayesian models could be applied. However, this approach can imply difficulties in the intercalibration procedures required within the WFD, since it is possible that the Bayesian models can be useful only for a specific area and not applicable to other. This can be due to the lack of sufficient heterogeneity within a specific area to make it general enough. The methodology will benefit when more heterogeneous data is available (*i.e.*, data from different areas).

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Pinna Nobilis as a proxy to good environmental status: stable isotope signatures under a gradient of eutrophication and protection

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Pinna nobilis is an endangered large bivalve mollusc endemic to the Mediterranean. It is a fast growing organism: during the first two years it has a high growth rate and can measure up to 65 cm long in years 12-15. This bivalve lives one-third buried in soft substrata, usually in seagrass meadows, and is an efficient filter feeder, assimilating both carbon and nitrogen derived compounds in its tissues, especially in muscle tissues, from its food sources. The fan mussel is therefore a good bioindicator of water quality and hence of the inhabiting area. ^{13}C and ^{15}N isotopic signatures of muscle tissues were used to demonstrate isotopic variation amongst *P.nobilis* individuals sampled inside three marine protected areas (Santa María, Dragonera and Espardell) and outside protected areas (Magaluf, Andratx and Esponja) of the Balearic Islands, western Mediterranean. Samples of *P.nobilis* were obtained throughout 2011 from Santa María, Magaluf and Andratx sites and published data of isotopic signatures of *P.nobilis* muscles tissues from Dragonera, Espardell and Esponja sites (Cabanellas-Reboredo . 2009) were incorporated into this study. Further on, nutrients concentration values from the sampling sites were also quantified. A statistical analysis based on a permutational analysis of variance (PERMANOVA) was carried out to study significant differences between isotopic signatures values of individuals from the six studied sites. Individuals sampled outside marine protected areas have higher ^{13}C and ^{15}N isotopic signals in their muscle tissues than those inside these areas. *P.nobilis* individuals from Magaluf had the highest mean carbon and nitrogen isotopic values, -18,63 ± 0,17‰ and 5,77 ± 0,23‰ respectively while individuals sampled inside the National Park of Cabrera, Santa Maria site, had the lowest mean carbon and nitrogen isotopic values, -19,35 ± 0,44‰ and 3,01 ± 0,25‰ respectively. Environmental differences such as light intensity, nutrient concentrations and species composition affect $\delta^{13}C$ and $\delta^{15}N$ values of primary producers in a region, so populations from different geographic locations show dissimilar isotopic signatures, even if they have similar diets. Organisms living in areas with high nutrient loads and no fisheries protection present higher isotopic signatures reflecting a more anthropogenic environment. This study can be used to reflect the importance of filter feeder bivalves such as *P.nobilis* as good bioaccumulators, giving us information about the surrounding environmental conditions and therefore helping to asses the good environmental status of littoral ecosystems.

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Development of innovative tools for understanding marine biodiversity and assessing good environmental status: the EU project DEVOTES

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The Marine Strategy Framework Directive (MSFD) was issued in 2008, by the European Parliament and the European Council, for "establishing a framework for community action in the field of marine environmental policy" (Marine Strategy Framework Directive; MSFD, 2008/56/EC). Member States (MSs) therefore join together in their commitment to protect, preserve and, where practicable, restore the quality of the marine environment across Europe. The MSFD requires MSs to take the necessary measures to achieve or maintain Good Environmental Status (GES) in the marine environment, by the year 2020 at the latest.

The MSFD states that "in view of the dynamic nature of marine ecosystems and their natural variability, and given that the pressures and impacts on them may vary with the evolvement of different patterns of human activity and the impact of climate change, it is essential to recognise that the determination of good environmental status may have to be adapted over time". Further, the same section states: "Accordingly, it is appropriate that programmes of measures for the protection and management of the marine environment be flexible and adaptive and take account of scientific and technological developments".

To assess GES, the MSFD requires the study of 11 descriptors (D), of which biological diversity is the first (Figure 1; Cochrane *et al.,* 2010). GES is defined as regarding the structure, functioning and processes of marine ecosystems, together with natural physiographic, geographic and climatic factors, and physical and chemical conditions including those derived from human activities. However, it is recognised that the dynamic interactions of human activities within ecosystems, which also are undergoing climate-driven changes, still are only partly understood. GES is further described in narrative terms (Borja *et al.,* 2010; Cardoso *et al.,* 2010) and its scientific basis outlined in Mee *et al.* (2008). This concept of environmental status, within the MSFD, therefore is a challenging issue and provides multiple opportunities for European marine research, in order to support the ecosystem-based approach to environmental management, which the MSs have undertaken to implement (Borja, 2006).

Biodiversity (D1), as a central MSFD descriptor, is one of the key factors which support ecosystem resilience and the sustainable provisioning of goods and services, which in turn contribute to the performance of economic systems and human well-being (Holmlund and Hammer 1999; Worm *et al.,* 2006; Costanza *et al.,* 2007). Many indicators of biodiversity exist, most are mutually complementary, and representing different aspects of the multidimensional concept of biodiversity. However, to be informative at a societal level, biodiversity indicators need to be combined within integrated monitoring, modeling and decision-making frameworks (Vačkář *et al.,* 2012). Indicators of biodiversity should be used to translate biodiversity loss and degradation directly into policy responses. Regulatory policies based on such integrated knowledge-based mechanisms directly empower those who implement them to manage the risks of human exploitation of biodiversity and the environment in general, and to minimise the degradation of available ecosystem services.

A set of assessment criteria has been appointed for each of the MSFD descriptors for GES, and a further set of indicators has been developed for each of the criteria. In order for this assessment system to be applied to the issues outlined above, a conceptualisation of how the MSFD descriptors, criteria and indicators operate, at the various scales, and how they feed into management, is essential.

The implementation of the MSFD must benefit from experience gained via the RSCs and Water Framework Directive (Hering *et al.,* 2010). Each of the four European Regional Seas has some unique characteristics and is governed by their own RSC (i.e. OSPAR, HELCOM, Barcelona and Bucharest). These RSCs have started to implement ecosystem health assessments, based upon the principles of the MSFD (HELCOM, 2010a; OSPAR Commission, 2010). Additionally, international institutions have also implemented working groups on some descriptors (ICES, 2011) and different biodiversity and sustainability indicators are being developed by international networks (i.e. Canada Healthy Oceans Network, CHONe: http://chone.marinebiodiversity.ca/). Furthermore, EU member states, following the guidelines of the MSFD, are also conducting an initial assessment of the environmental status of the marine waters, which should be ready to the EC by July 2012. Nonetheless, a common issue has been raised throughout this process: the need for consistent harmonized approaches and principles in setting the GES criteria and targets across the four European Regional Seas.

For a better understanding of these concepts, recently a FP7 project, called DEVOTES (DEVelopment Of innovative Tools for understanding marine biodiversity and assessing good Environmental Status) has been approved by the European Commission. The overall goal of DEVOTES is to better understand the relationships between pressures from human activities and climatic influences and their effects on marine

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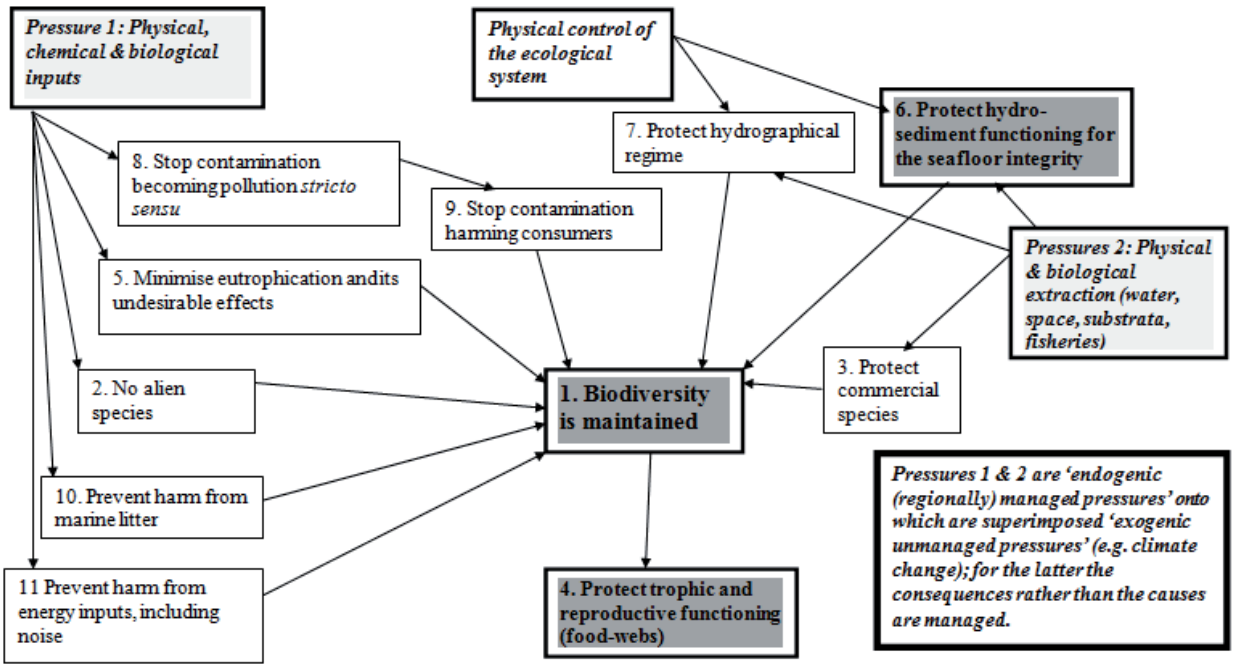

Figure 1. A conceptual model showing interlinks between managed and unmanaged pressures and the 11 qualitative descriptors covered by the MSFD (Borja *et al.,* 2010). Boxes with light grey shading are pressures, and boxes with dark grey shading are the descriptors investigated in DEVOTES (1, 4 and 6), indicators of marine biodiversity or relate closely to impacts of human activities and climatic influences on biodiversity.

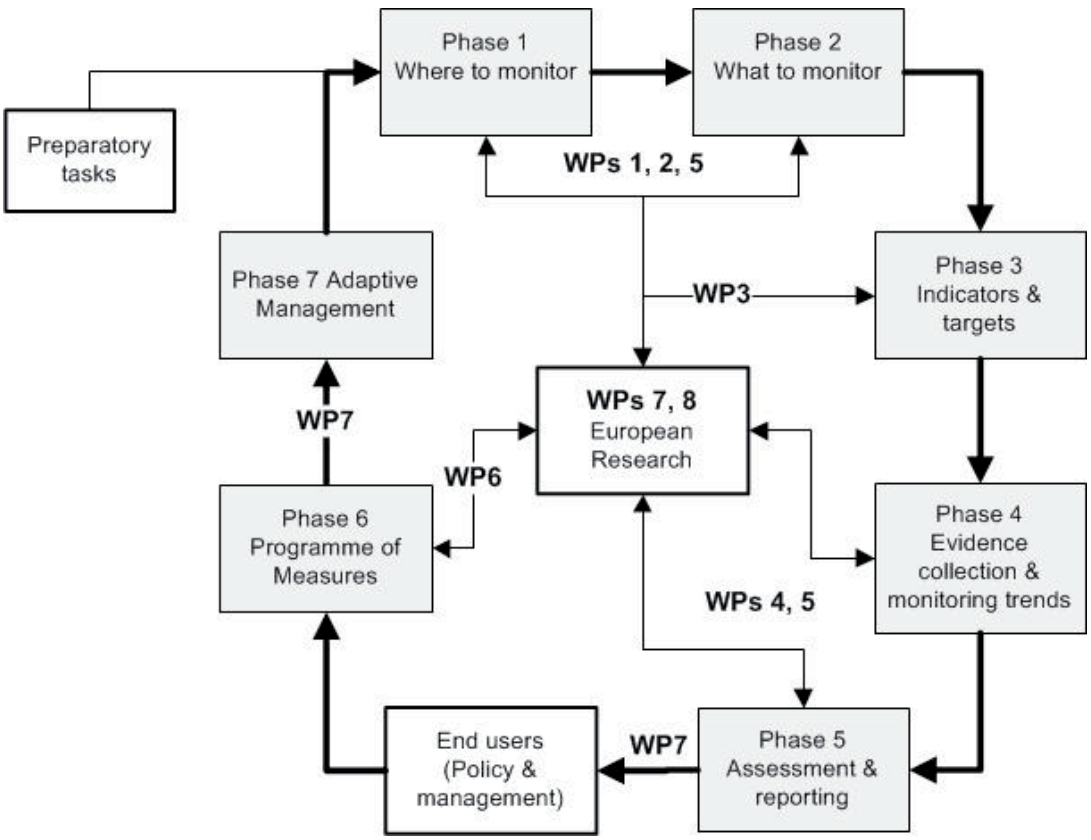

Figure 2. A conceptual model showing interlinks between the phases to be covered when implementing the MSFD and the European research, together with the Work Packages (WP) proposed in DEVOTES (Modified from Descriptor 1 report, Cochrane *et al.,* 2010).

ecosystems, including biological diversity, in order to support the ecosystem based management and fully achieve GES of marine waters. This will be undertaken in a harmonized way for the four regions identified in the MSFD, by testing and validating approved indicators, developing new ones when necessary, using modelling tools for the assessment of biodiversity, developing new monitoring tools and establishing an integrated way of assessing the environmental status of biodiversity and related descriptors (i.e. food-webs and seafloor integrity).

To build the DEVOTES concept and its system of Work Packages (WP) described below, we considered the cyclical phases of a monitoring and adaptive management programme, such as that conceptualised during the development of D1 for Biodiversity (Figure 2). Hence, considering the abovementioned framework the following five key objectives for DEVOTES have been established:

(i) Improve our understanding of the impact of human activities (cumulative, synergistic, antagonistic) and climate change on marine biodiversity, using long-term biodiversity and human pressures data from the 4 EU Regional Seas, both in pelagic and benthic environments (in WP1: 'Human pressures and climate change'). This will identify the barriers and bottlenecks (socio-economic and legislative) that prevent GES from being achieved (in WP2: 'Socio-economic implications of GES') (for links with the MSFD implementation, see Figure 2).

(ii) Test the relevant indicators listed in EC (2010) and currently being compiled by the RSCs, and develop new, innovative ones to assess biodiversity at several ecological scales (species, habitats, ecosystems), including functional diversity, metagenomic and metagenetic analyses and approaches; test the relevant level of integration of the indicators to produce a holistic assessment of biodiversity (in WP3: 'Indicator testing and development') (for links with the MSFD implementation, see Figure 2), in a harmonized way throughout the 4 regional seas.

(iii) Develop, test and validate innovative integrative modelling (e.g. statistical, conceptual) and monitoring tools to further improve our understanding of ecosystem and biodiversity changes in space and time, applying both traditional sampling and autonomous data acquisition devices (in WP 4: 'Innovative modelling tools' and WP5: 'Innovative monitoring techniques'). Such tools are required by statutory bodies, Small and Medium Enterprises (SMEs) and marine research institutes to monitor and assess biodiversity. These tools will improve our capacity to provide assessments and where possible predictive advice and strengthen the knowledge base necessary to effect sustainable management of seas and oceans resources. We will propose and demonstrate the pros and cons of innovative monitoring systems capable of efficiently and effectively providing data for many biodiversity parameters (including those used in non-EU countries), to be used as indicators of GES, and, finally, for integration into a unique and holistic assessment (in WP6: 'Integrative assessment'; for links with the MSFD implementation, see Figure 2). It is important that, as far as possible, the methodologies developed are "platform independent", i.e. they are sufficiently robust to be applied to data from multiple sources and still produce consistent and comparable results. This will prevent assessments from neighbouring areas having very different outcomes due mainly to different methods of assessment.

(iv) Implement cost-effective indicators, monitoring and assessment strategies, which should enable uniform applicability across EU Regional Seas (in WP5) (for links with the MSFD implementation, see Figure 2), which will serve as operational tools for managers and policy makers, with a view to reduce pressures through actions.

(v) Propose and disseminate strategies and measures for ecosystems' adaptive management (related to EA), with consultation of MSs. These strategies will include the active role of industry and relevant stakeholders (including non-EU states) and an economic assessment of the consequences of the management practices proposed by DEVOTES (in WPs 2 and 6, but also in WP7: 'Outreach, stakeholder engagement and product dissemination') (for links with the MSFD implementation, see Figure 2). These actions will promote EU-wide harmonisation in the environmental status classification of the marine waters in the four regions for a coherent implementation by all MSs.

This contribution will present the main tasks to be developed within the DEVOTES project.

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Comparison of the RSL and CARLIT indices for macroalgae in the Gibraltar Strait and western Alboran Sea (Southern Spain)

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According to the Water Framework Directive (WFD) macrophytes are one of the Biological Quality Elements (BQE) proposed to assess the ecological status of coastal water bodies. Several indices based on composition and abundance of aquatic flora have been developed to assess the ecological status. To develop these indices within the WFD, biogeographical and ecological differences that appear along European coast have been considered. In this sense, European coasts were divided into different eco-regions. Moreover, the coasts in each region were classified according to environmental characteristics to delimit different types. Intercalibration exercises has been implemented for each region and type to ensure comparability among different methodologies and to guarantee that all methods provide equivalent quality assessments for the same level of anthropogenic pressure. In this sense, intercalibration exercises have been carried out between all indices of a particular eco-region and type. In this work we aimed to compare and intercalibrate two indices based on macrophytes of coastal waters that has been proposed for two different and adjoining eco-regions: the RSL (Reduced Species List) in the Atlantic eco-region and the CARLIT (Cartography of littoral and upper sublittoral communities) in the Mediterranean. Both indices have been applied in the Gibraltar Strait and western Alboran Sea, a coastal zone that is a transitional region between the Atlantic Ocean and the Mediterranean Sea. In addition, very different conditions can be found in this zone, from high (Algeciras Bay) to almost negligible anthropogenic pressures ("El Estrecho" Natural Park). Both methodologies were applied in nine sites. Anthropogenic pressures were estimated according to the methodologies proposed by North Atlantic and Mediterranean Geographic Inter-calibration Groups. Both indices were sensitive to anthropogenic pressures, yielding significant correlations to anthropogenic pressure score systems. Furthermore, EQR (Ecological Quality Ratio) rendered by the two indices were significantly correlated. However RSL index showed systematically lower EQR values than CARLIT. These results are in accordance to the recommendations given by the intercalibration group in the North Atlantic Geographical ecoregion, which proposed to modify the boundaries of the RSL index in Andalusia to avoid an underestimation of the final EQR values. Nevertheless, some concerns about a possible overestimation of the CARLIT index must be considered in advance.

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Aproximación a las actividades humanas en la zona costera de la isla de Gran Canaria (España)

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Las zonas costeras constituyen áreas de transición entre el medio terrestre y el marino. Éstas se consideran territorios únicos por sus valores ecológicos, económicos, sociales y culturales (MMA, 2005). Los humanos, atraídos por estos valores, han escogido dichas zonas para residir. Así, por ejemplo, la población europea es de media un 10 % superior en las zonas costeras, llegando incluso al 50 % en algunos países (EEA, 2006). En este sentido, la accesibilidad de las zonas costeras al hombre las ha hecho susceptibles a una amplia variedad de impactos desde tiempos prehistóricos (Thompson *et al*., 2002). La mayoría de los impactos son localizados, asociados a áreas densamente pobladas que muestran una alta diversidad de actividades de recreo y de recolección de alimentos. En las Islas Canarias, los humanos, tanto los residentes como los turistas, usan intensamente las zonas costeras para actividades recreativas tales como el paseo, el baño, la práctica de deportes acuáticos… y actividades extractivas como la pesca o el marisqueo. En este último caso, se sabe que la recolección de organismos en la zona costera ocurre desde épocas prehispánicas (p. ej. Cabrera 2001). Conocer qué actividades se llevan a cabo en la zona costera y cuántas personas están involucradas en ellas es de vital importancia para una correcta gestión de dicha zona, así como para la conservación de las especies explotadas. No existen datos previos sobre estos aspectos para el Archipiélago Canario ni, en concreto, para la isla de Gran Canaria. Por tanto, el principal objetivo de este trabajo fue identificar las actividades realizadas en la zona costera de la citada isla y el número de personas que participaban en ellas.

Este estudio fue llevado a cabo en la isla de Gran Canaria, donde se seleccionaron dos tramos de costa (Fig. 1); el primer tramo (La Isleta) estaba situado en la costa de Las Palmas de Gran Canaria y el segundo (Telde) estaba situado en la costa de Telde (Fig. 1). Dichos tramos se seleccionaron por ofrecer diferentes tipos de costa (p. ej. costa baja, acantilados, playas, etc.) lo que, *a priori,* favorecía la realización de diferentes actividades por parte de los humanos. Sus características ambientales de nubosidad, viento y oleaje fueron diferentes debido a su posición y orientación. En el tramo de La Isleta fue mayor la nubosidad pero menor la exposición al viento y a las olas, mientras que en el tramo de Telde se mostró el patrón contrario. La longitud aproximada de ambos tramos de costa fue de 2.500 m en la Isleta y de 3.300 m en Telde. Para la recolección de los datos un muestreador realizó cada tramo a pie y utilizó prismáticos cuando fue necesario. Se realizaron un total 12 muestreos (6 en cada tramo de costa) entre en los meses de julio y octubre de 2011. Los muestreos se llevaron a cabo durante la marea baja, seleccionando tanto mareas de mañana y de tarde, como días normales de semana (de lunes a viernes) y días de fin de semana (sábado y domingo). Se anotaron datos ambientales como la nubosidad (Despejado = 0-10 %; Parcialmente nublado = 10-50 %; Mayormente nublado = 50-90 %; Cubierto = > 90 %), el oleaje (según escala Douglas) y el viento (según escala Beaufort). Para el viento, también se consultaron los datos de la Agencia Estatal de Meteorología (http://www.aemet.es). Las actividades humanas fueron clasificadas en 4 categorías: (1) Pesca, (2) Marisqueo, (3) Ocio y (4) Actividades acuáticas. Cada categoría se subdividió a su vez en subcategorías, que fueron respectivamente las siguientes: técnicas de pesca como (1) boya, (2) plomo y (3) otros (p. ej. rapala); organismos recolectados por los humanos como (1) lapas (*Patella* spp.; incluye la recolección de burgados ─ *Osilinus* spp. y la cañadilla ─ *Stramonita haemastoma*), (2) pulpo (*Octopus vulgaris*), (3) carnada de vieja (*Xantho* spp.), (4) otros (p. ej. ermitaños─ *Clibanarius* spp., camarones de charco ─ *Palaemon* spp., etc.); actividades de ocio como (1) pasear, (2) tomar el sol, (3) fotografía y (4) otros (p. ej. baño); actividades realizadas en el mar como (1) pesca submarina, (2) snorkel, (3) surf-bodyboard, (4) pesca de barco y (5) otros (p.ej. fondeo, moto de agua, etc.). Se anotó el número de personas (o embarcaciones) para cada categoría y subcategoría. Se utilizó la prueba de chi-cuadrado (X^2) para contrastar si existía alguna relación entre la distribución de frecuencias de personas para las categorías de actividad humana y las siguientes combinaciones de comparación: mañana-tarde, día normal-fin de semana y La Isleta-Telde. Igualmente se contrastó dicha relación pero para las subcategorías consideradas. Para comprobar si existía alguna asociación entre las condiciones ambientales estudiadas y la frecuencia de personas para las categorías de actividad humana se utilizó el coeficiente de correlación no paramétrico de Spearman.

La distribución de frecuencias de personas para las categorías de actividad humana fue significativamente diferente según el momento del día ($X^2 = 13.89$, g.l.= 3, $p < 0.01$) y el lugar ($X^2 = 16.33$, g.l.= 3, $p < 0.01$). Así, la frecuencia de personas fue mayor por la tarde y en Telde, pero los incrementos variaron de una actividad a otra (Fig. 2). Del mismo modo, la distribución de frecuencias de personas varió significativamente entre un día normal de la semana y un día de fin de semana, aunque con un

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Figura 1. Imágenes en donde se indica la posición y se muestran las localidades analizadas (1: La Isleta, 2: Telde) en la isla de Gran Canaria.

nivel de significación más bajo (X^2 = 8.13, g.l.= 3, $p < 0.05$); el número de personas aumentó notablemente durante el fin de semana, pero no lo hizo en todas las actividades (Fig. 2). No se encontraron diferencias significativas para la distribución de frecuencias de personas para las subcategorías consideradas entre el momento del día, el día de la semana o el lugar. Por tanto, los resultados obtenidos se presentaron de manera conjunta para las dos localidades en la figura 3. Se registró un valor medio para la relación nº de cañas/nº de personas algo inferior a uno (0.88). Por otro lado, las condiciones ambientales y la frecuencia humana por actividad humana mostraron una notable asociación. En Telde, el oleaje y el viento influyeron negativamente en la realización de la pesca, el marisqueo y el ocio, mientras que lo hizo positivamente en el desarrollo de actividades acuáticas; la nubosidad mostró el patrón contrario (Tabla 1). Mayoritariamente, el oleaje y el viento también afectaron negativamente a la pesca, el marisqueo y el ocio en la Isleta (Tabla 1); dichos factores mostraron una asociación positiva con el desarrollo de actividades acuáticas. La nubosidad se relacionó de manera positiva con todas las categorías (Tabla 1). Son mayoría las asociaciones de magnitud moderada (0.3-0.7), pero destacan las de magnitud intensa (> 0.7) entre el oleaje y el marisqueo en ambas localidades y el viento y el marisqueo en Telde (Tabla 1). De manera concreta, la nubosidad se relacionó negativamente con el viento y el oleaje (r = -0.5) en Telde, y sólo con el oleaje (r = -0.44) en La Isleta.

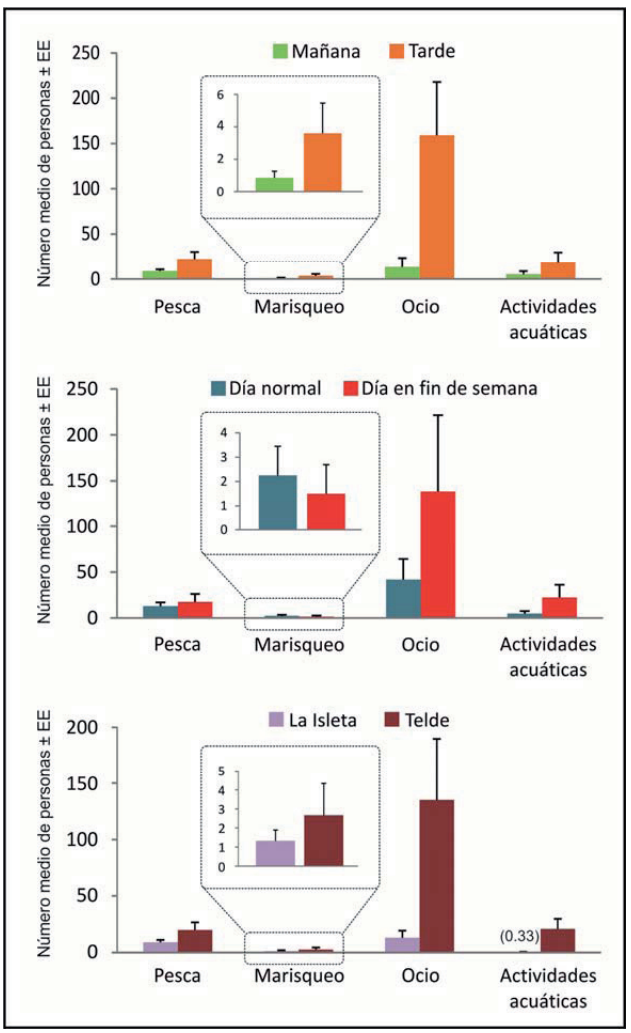

Figura 2. Número medio de personas por categoría de actividad humana para las tres comparaciones realizadas en el estudio (mañana-tarde, día normal-día de fin de semana y La Isleta-Telde).

Localidad	Actividad humana	Nubosidad	Oleaje	Viento
Telde	Pesca	0.62	-0.61	-0.63
	Marisqueo	0.59	-0.93	-0.89
	Ocio	0.35	-0.26	-0.31
	Actividades acuáticas	-0.24	0.44	0.34
La Isleta	Pesca	0.13	-0.19	-0.58
	Marisqueo	0.54	-0.81	0.19
	Ocio	0.40	0	-0.74
	Actividades acuáticas	0.20	0.44	0.28

Tabla 1. Resultados del coeficiente de correlación de Spearman entre las condiciones ambientales y la frecuencia de personas para cada una de las categorías de actividad humana.

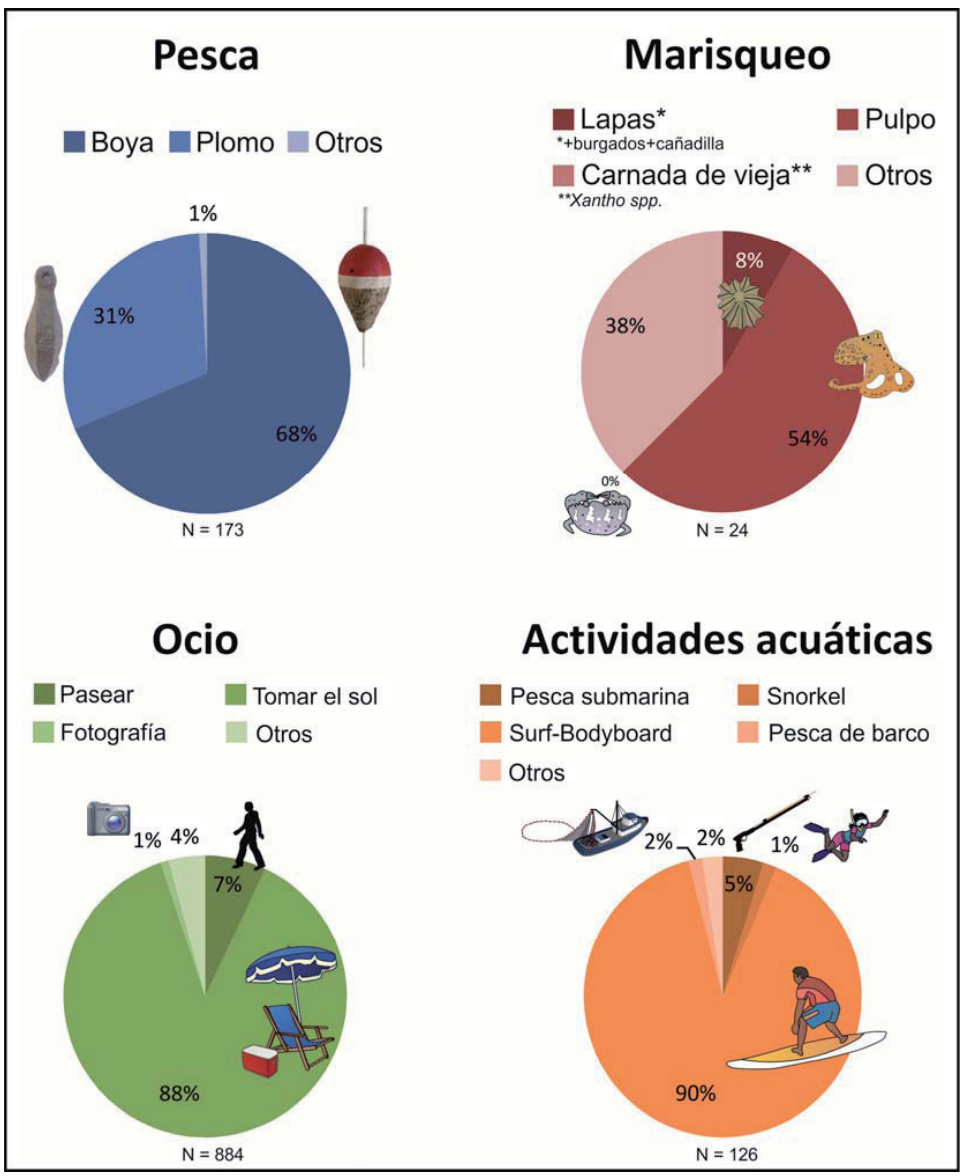

Figura 3. Esquema ilustrativo del porcentaje de personas para las diferentes subcategorías consideradas. N indica el número total de personas consideradas.

Los resultados de este estudio muestran que los humanos realizan actividades en la zona costera de Gran Canaria continuamente, pero lo hacen mayoritariamente en determinados momentos del día, días de la semana y localidades. Las actividades se llevaron a cabo preferentemente por la tarde en vez de por la mañana, lo que pudo estar relacionado con dos factores: (1) horario de las mareas y (2) horario laboral. Los muestreos se realizaron en función de las mareas, las cuales fueron en su mayoría antes de las 9.00 a.m. Además, de manera general, la población trabaja y/o estudia en horario de mañana, realizando otro tipo de actividades por la tarde. En cuanto al día de la semana en el cual se desarrollan las actividades, se registró una mayor frecuencia de personas durante los fines de semana, excepto para la realización del marisqueo. Nuevamente, la jornada laboral parece estar relacionada con este resultado. Los días de fin de semana son, principalmente, los días de descanso de la población de sus actividades cotidianas y, por tanto, cuando realizan otro tipo de actividades. La obtención de una mayor proporción de personas realizando marisqueo durante los días normales de la semana pudo responder a diversos factores, tales como: (1) que el marisqueo sea ya una actividad más lucrativa que de ocio o (2) evitar la competencia con otros mariscadores y/o pasar desapercibido ante los agentes de la autoridad. Por otro lado, las diferencias que se han encontrado entre Telde y La Isleta pudieron deberse a: (1) accesibilidad a la zona costera, (2) disponibilidad de playas y (3) diferencias en las condiciones climáticas. A la costa de Telde se puede acceder fácilmente a pie o en vehículo, mientras que ambos métodos están reducidos para acceder a La Isleta. Por ejemplo, el acceso para vehículos en esta última localidad consiste en un camino de tierra en mal estado de conservación, limitado principalmente a coches todoterrreno. Asimismo, el tramo del litoral analizado en Telde incluía 3 playas de arena, las cuales atrajeron a un mayor número de usuarios. En la Isleta, en cambio, no existieron playas de arena. Más aún, la diferencia en las condiciones climáticas como la nubosidad (mayor en La Isleta), el oleaje y el viento (ambas mayores en Telde) favorecieron o perjudicaron la práctica de algunas actividades. En este sentido, los resultados obtenidos demuestran que existe una estrecha relación entre las condiciones climáticas y la realización de actividades en la zona costera. No obstante, éstas no afectaron por igual en ambas localidades. La práctica de la pesca, por ejemplo, se vio limitada con una mayor intensidad en Telde debido al oleaje y al viento. El efecto del oleaje sobre el marisqueo fue bastante claro en ambas localidades, ya que esta actividad se realiza en la zona baja de la costa. El viento, sin embargo, no fue un factor que impidió el desarrollo del marisqueo en La Isleta. El efecto positivo del oleaje y el viento sobre las actividades acuáticas pudo estar favorecido por la dominancia del surf-bodyboard dentro de dicha categoría. La presencia de nubosidad estaba relacionada con un tiempo en el que el viento y el oleaje eran de menor intensidad (mayor evidencia en Telde); esto explicaría su relación positiva con casi todas las categorías de actividades (p. ej. pesca, marisqueo, etc.) en ambas localidades, excepto, concretamente, con las actividades acuáticas en Telde.

Existieron otros resultados sobre las diferentes subcategorías consideradas que son importantes discutir. En relación a la pesca, destaca la preferencia por el uso de la boya como técnica de pesca, lo cual nos puede orientar acerca de qué especies de peces o tipo de ellos están siendo preferentemente capturados. Se registró, además, una presión pesquera menor a una caña por pescador; las personas que no pescaban realizaban otras actividades como preparar el engodo o actividades de ocio (p. ej. tomar el sol). En cuanto al marisqueo, resalta que la recolección de pulpos es mucho más frecuente que la de lapas, a pesar de la importancia que posee esta última (p. ej. Ramírez *et al. 2009*). Sin embargo, no se observó recolección de carnada de vieja (*Xantho* spp.) ya que los pedregales (lugar donde se recolecta dicha carnada) no existieron en Telde y fueron muy reducidos en la Isleta. En particular, el número de datos sobre marisqueo fueron escasos, por lo que habría que llevar a cabo trabajos más extensos sobre la materia. Por último, entre las actividades acuáticas, se observó el desarrollo de la pesca submarina en Telde, en donde no está permitida dicha actividad.

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Evaluation of marine phytoplankton toxicity by application of sea urchin (*Paracentrotus lividus*) and *Artemia* sp. bioassays

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Over the last decades, incidence of toxic blooms of marine phytoplankton has apparently been increasing along the coastal waters worldwide (GEOHAB 2001). From an ecological point of view, the toxin production by the microalgae represents the causative agent of mass mortalities of marine vertebrates or invertebrates (Simonini *et al*. 2011). The threaten of natural marine toxins is represented by bioaccumulation along the food chains with cascading effects through the whole ecosystem (Faimali *et al*. 2012) and also by the accumulation in shellfish or finfish causing a variety of illnesses in humans (GEOHAB 2001).

Alexandrium minutum is a dinoflagellate agent of harmul algal bloom that produces paralytic shellfish poisons and can affect copepod, shellfish and other marine organisms (Zhenxing *et al*. 2006) . *Prymnesium parvum* is an haptophyte frequently reported from coastal waters around the world. Its blooms have been associated with massive fish mortalities resulting in negative impacts on the marine ecosystem and large economic loses for commercial aquaculture (Johansson and Graneli 1999).

This work aims at contributing to improve our knowledge about the toxicity of *A. minutum* and *P. parvum* by using the 48-h embryo–larval toxicity test of the sea urchin *Paracentrotus lividus* (Garmendia *et al*. 2009) and the 24-hours test with the brine shrimp *Artemia salina* (Artoxkit, 1990). Embryos of sea urchin and nauplii larvae of the brine shrimps were incubated with cultured algae and algae extracts in order to verify endotoxins and exotoxins presence. Following Beiras *et al.* (2001), EC_{50} probit analysis and NOEC/LOEC values were calculated in order to compare the sensitivity of both invertebrate marine organisms. *Isochrysis galbana* was used as a negative control and Sodium Dodecyl Sulfate as a toxic reference.

Based on our preliminary results, EC_{50} comparison analysis suggests sensitivity differences between sea-urchin embryo development and brine shrimp survival toxicity test. Therefore, the use of different organizational biological levels seems suitable tool for *A. minutum* and *P. parvum* toxicity assessment.

The present work is part of a Master Thesis in Biodiversity and Ecosystem´s Management Master from the University of the Basque Country (UPV/EHU) and it is a collaboration between AZTI-Tecnalia Foundation and the Department of Plant Biology and Ecology of the UPV/EHU.

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Monitoring of subtidal mollusc assemblages in a highly urbanized environment: the Ría de Ferrol (Galicia, NW Iberian Peninsula)

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Benthic assemblages show seasonal patterns in the composition and abundance of taxa. Those depend on such factors as hydrodynamism, granulometric composition and food availability, and biotic interactions (e.g. predation, competition) as well. Anthropogenic activities also affect benthic assemblages, often in a negative way. For example, dredging, fishing, sewage disposal and urbanization of the shoreline result in many cases in loss and degradation of habitats, reducing biodiversity and facilitating the spread of invasive species. Construction of artificial structures mostly related to harbours, shipyards or industrial facilities (e.g. seawalls, breakwaters, jetties) are responsible for alterations in local hydrodynamics, which in many cases result in changes of sedimentation rates, siltation and organic enrichment, and, finally, in perturbations of the benthic assemblage (e.g. impoverishment in diversity, dominance of opportunistic species).

The Galician rías (NW Iberian Peninsula) are highly productive environments due to their peculiar geography and hydrodynamic regimes, which result in strong upwellings and therefore in a high primary production. Because of that and the variety of benthic habitats present there, the rías support a high biodiversity. This is, however, threatened by the growing concentration of human population on their shorelines, which are getting more and more urbanized. Among the Galician rías, the Ría de Ferrol is one of the most altered by human activities, particularly due the construction of the extensive jetty of the outer harbour. This jetty extends along half of the ría's mouth and was expected to affect significantly its hydrodynamic patterns. In fact, this ría was characterized by strong tidal currents that resulted in a high water renewal. Many areas of the outer part of the ría corresponded to bottoms mostly composed by coarse sandy fractions; nowadays, those sediments sheltered by the aforementioned jetty turned to muddy sand or mud.

Starting in 2007, several subtidal sites corresponding to sandy sediments located nearby the harbour jetty are being monitored by the Marine Biological Station of A Graña (Univ. of Santiago de Compostela). This was done to test the hypothesis of whether changes in sedimentary conditions (i.e. siltation) related to alteration of currents would affect the composition of the benthic assemblage. Special attention was paid to the study of the molluscan fauna because of their utility as bioindicators of the conditions of the benthic environment.

Sampling was done monthly at two subtidal sites (8-12 m depth) corresponding to medium-fine sand and fine sand sediment respectively. Five replicates samples were taken by means of a van Veen grab thus sampling a surface of 0.28 m^2 each month. Granulometric features and organic matter content of the sediment was also analyzed and related to the seasonal variations of the mollusc assemblage.

From November 2007 to November 2008, there were no significant changes in median grain-size and no increase in the silt/clay content was detected; values of the latter were, however, slightly higher in the site closer to the jetty. Organic matter content was usually moderate (<3%). Bivalves were the most diverse and numerically dominant taxa, constituting usually 75-90% of the total mollusc abundance. *Thracia papyracea*, *Moerella donacina* and *Mysella bidentata* were present during all the sampling period and constituted more than 80% of the abundance. Gastropods were mostly represented by carnivorous taxa, such as *Euspira pulchella*, *Bela nebula* and some retusid species. The temporal dynamics of the assemblage showed a strong seasonal pattern; samples from late summer and early autumn were significantly different from those of winter-spring and were characterized by high abundance of the dominant bivalve species.

In general, results have shown that the benthic fauna of the studied sites has not been affected to date by the construction of the harbour jetty; this contrasts with that observed in the nearest sediments to the jetty. Composition and temporal patterns of the molluscan fauna were similar to those observed in non-altered areas of other Galician rias, such as the Ensenada de Baiona. Nevertheless, long-term studies are needed to assess whether other alterations in the ria ecosystem related to the jetty presence may affect directly or indirectly the benthic assemblage in the near future.

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Increased antioxidant response and capability to produce ROS in hemocytes of *Pinna nobilis* L. under anthropogenic pressure

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The exposure of bivalves to pollutants results in oxidative stress throughout the formation of reactive oxygen species (ROS), which can produce deleterious effects on biomolecules and cell damage. Organisms have developed a complex antioxidant mechanism in order to detoxify ROS and to protect molecular targets against oxidative injury. The measurement of the antioxidant response and/or the presence of oxidative damage -DNA and protein oxidation, lipid peroxidation- are potential biomarkers to evidence the effects associated to contaminants and also to eutrophication in marine organisms. The fan mussel *Pinna nobilis* L. is the largest endemic bivalve in the Mediterranean Sea under strict protection. *P. nobilis* appears in coastal areas between 0.5 and 60 m depths, mostly in soft-sediments overgrown by seagrass meadows of *Posidonia oceanica* or *Cymodocea nodosa*. The population of *P. nobilis* has been greatly reduced during the last decades as a result of recreational and commercial fishing for food, the use of its shell for decorative purposes, and incidental killing by trawling and anchoring. Nowadays, *P. nobilis* is under strict protection and all forms of deliberate capture or killing them are prohibited (Council directive 92/43/EEC). Hemocytes play an essential role in cellular immune defense and detoxification processes of bivalves. Hemocytes were suggested to be sensitive to variations of several factors such as temperature, salinity, nutrients and toxicants, reflecting the physiological status of bivalves and their adaptation to the environment. The present work aims to determine the antioxidant enzyme response, markers of oxidative damage and reactive oxygen species production in hemocytes of *Pinna nobilis* under anthropogenic pressure.

Pinna nobilis (20 individuals) were collected from several locations along Mallorca waters during May-June 2011 attending to different degree of human impact. The first station was located in a marine protected area off Cabrera Archipelago National Park (Western Mediterranean), located 9 km southeast of Mallorca island and was considered as a clean non-polluted area. The second set of stations because of the low *P. nobilis* densities, the difficulties to find large specimens and in order to minimize the impact in their populations were collected in two sites (Magalluf and Port d'Andratx) with similar characteristics and individuals were considered as human impacted. Hemolymph was withdrawn from the posterior abductor muscle of each individual into a 1-mL hypodermic syringe. The cell fraction was obtained by centrifugation at 900 x g at 4ºC for 10 min. After centrifugation hemocytes were resuspended in the initial volume with PBS and freshly used for ROS production assay or frozen at -70ºC for biochemical assays.

No significant differences were evidenced in *Pinna nobilis* shell length and shell width collected in Cabrera and Magalluf-Andratx stations. The ten individuals from Cabrera station were measured with a mean length of 40.6±3.2 cm and a mean width of 16.9±1.4 cm. For the second station, ten individuals were measured with a mean length of 39.0±3.0 cm and a mean width of 16.3±1.0 cm. Specimens collected in the impacted areas showed increased activities of antioxidant enzymes –catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase-, and myeloperoxidase activity and reduced glutathione levels. No differences in oxidative damage markers –malondialdehyde and carbonyl index- were evidenced between the pristine and polluted areas. Hemocytes from the polluted area presented increased capability to generate ROS and nitrite/nitrate when activated with PMA and zymosan.

The present work provides evidence of the anthropogenic impact on hemocytes of the marine fan mussel *Pinna nobilis* supported by the increased biomarkers of oxidative stress. All antioxidant enzymes from *P. nobilis* sampled in Magalluf presented higher activities indicating a more stressful environment in the impacted area. However, markers of oxidative damage in lipids and proteins did not reported evidence of cellular damage suggesting that the increased antioxidant system was enough to avoid oxidative alterations in biological structures. The increased GSH in hemocytes from the polluted area is probably a response of cells to maintain their redox status in the adequate range. Hemocytes obtained from Magalluf seemed to be primed for the oxidative burst as evidenced by the increased capability to produce ROS when activated. When NO is generated in biological fluids by the action of nitric oxide synthases (NOS), it rapidly combines with oxygen to form the nitrogen oxides nitrite and nitrate. The nitrite and nitrate accumulation was significantly higher in Magalluf reinforcing the idea that pollution induced a preactivation state in hemocytes of P. nobilis.In conclusion, *Pinna* nobilis hemocytes are clearly affected by the anthropogenic impact. Haemocyes responds to pollution with an antioxidant response capable to avoid oxidative damage to lipids and proteins. Hemocytes from the pollute site are also primed for the oxidative burst as evidenced with zymosan and PMA activation.

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Heavy metal contamination of the discharge areas of the main drainage basins of Lisbon in the Tagus estuary, Portugal

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In the city of Lisbon, the existing three discharge areas of drainage basins (Alcântara, Chelas and Beirolas) drain directly onto the Tagus estuary (Portugal) as did an untreated effluent (Terreiro do Paço) that was closed in 2011 and is now connected to the Alcântara discharges. To understand if these discharges could interact with the water metal concentrations and/or be an additional source of heavy metal contaminations to the surrounding environment sampling took place during September of 2011, collecting both sediments and water samples, and dissolved oxygen and pH were measured in situ. In three of the sites (Alcântara, Terreiro do Paço and Chelas) three samples were taken near the discharge outfall (impact stations) and four further away (control stations), while in Beirolas, four treatment and nine control stations were analysed. Samples were spaced 50 m starting from the effluent outfall in the impact stations and from the estuarine margin in control stations. Concentration of lead, chromium, nickel, copper, zinc, cobalt and cadmium were quantified in suspended particulate matter and dissolved in the water column. In bottom sediments, the concentration of heavy metals were also analysed as well as the total organic content. Additionally total chlorophyll (as productivity indicator) and ammonium (as possible contamination source) in the water column were also determined. To investigate differences in the total metal content in sediments, a Metal Pollution Index (MPI) was used. MPI did not revealed differences between treatment and control stations indicating that the effluents may not be a significant source of heavy metals to the environment. However, significant differences were found for sites revealing that Chelas had the lower contamination of heavy metals and Alcântara and Beirolas had the highest contents leading to believe that the sediments could have others sources or previous accumulated contaminations. It was also found that stations closer to the margin had higher contaminations indicating that a possible source could be the drainage from terrestrial grounds. Multivariate analysis revealed that the heavy metal contamination could have different sources in the different sites studied and are related to the form in which the heavy metals are available. The uppermost area (Beirolas) revealed that the main source of heavy metals derived from the accumulation in sediments while the downstream sites were more associated with contamination derived from the water column. In Alcântara, with higher contaminations of heavy metals in the suspended particulate matter and higher values of ammonium in the water column, some influence of the sewage discharges onto the environment seem more visible in the stations closest to the discharges outfall than those further away. On the other hand, in Terreiro do Paço, where the heavy metals dissolved in the water are higher, contamination could probably due to circulatory patterns of heavy metals in the estuary although some previous accumulated contamination could not be ruled out. Corroborating the MPI data, in Chelas sediment contamination was low although some contamination could be associated to availability of heavy metals in suspended particulate matter. In general, it does not seem that the discharges of the major drainage basin of the city of Lisbon could be an important source of heavy metal contamination to the environment. Instead, the discharges into the river seem to interact with the dissolved metal concentrations, leading to the establishment of bounds between metal ions and the introduced particulate organic matter (POM) due to the high chemical affinity between both metals and POM.

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Do mollusc assemblages differ among seawalls and natural rocky shores in the highly urbanized Ría de Ferrol?

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In the last decades, the concentration of human population in coastal areas is having a significant impact on marine habitats and biodiversity. Among other factors, increasing urbanization of the shoreline usually results in fragmentation and destruction of natural habitats and loss of biodiversity. Alternatively, proliferation of new landscape features such as seawalls, breakwaters and pilings may add a novel habitat. Their possible value to sustain marine life has, however, been scarcely assessed in many parts of the world in spite of being a matter of global concern. In some cases, the addition of such structures to the coastline may contribute to increase local biodiversity. This occurs, for instance, when hard substrata are artificially provided to areas mostly dominated by soft substrata; this allows the establishment of, at least, some sessile and mobile epifaunal taxa such as mussels, macroalgae or limpets. Besides, when this is done on natural hard substrata, it is necessary to assess whether these new habitats might support similar assemblages and populations of organisms; otherwise, there is a risk of becoming sinks for propagules supplied by populations established in patches of natural habitat, or directly of resulting in the loss of biodiversity.

The Galician rias (NW Iberian Peninsula) constitute a particular kind of embayment whose peculiar orography and hydrodynamic features allows a high primary production, which has translated in a high economic importance. Not surprisingly, most of the human population of the region concentrates there and subsequent increase of commercial, fishing and industrial activities has demanded the construction of a variety of supporting infrastructures such as shipyards, docks, jetties, etc. This is particularly common at the Ría de Ferrol where, in addition, many patches of the coastline are protected by seawalls, which are usually made up of the natural rock in the area, i.e. granite. Preliminary observations pointed out that those seawalls support a number of taxa including several mobile mollusc species. It is, however, not known whether those assemblages differ from those present on natural rocky shores. Therefore, to test hypotheses about differences in the composition, abundance and frequency of occurrence of mollusc taxa on intertidal natural and artificial habitats, several seawalls and rocky shores were sampled in the Ría de Ferrol.

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Sampling was done in four intertidal stations located in the middle channel of the Ría de Ferrol; the shoreline there has extensive vertical seawalls interspersed with horizontal rocky shores suitable for the intended sampling. Inner and outer areas of the ria were not sampled because they lacked adequate rocky shores and/or seawalls. Sampling was done in four different seasons (from summer 2008 to spring 2009) to test whether patterns would be consistent across a temporal scale. At each station, two seawalls and two rocky shores were sampled. On each location (seawall or rocky shore), three heights representing different assemblages were chosen: highshore (3-3.5 m; upper level of distribution of barnacles -mostly *Chthamalus* spp.-), midshore (2-2.5 m; most of the space covered by barnacles) and lowshore (1.5-2 m; *Mytilus galloprovincialis* belt); heights varied slightly depending on the place. Molluscs were not sampled in the macroalgae-dominated assemblage below 1.5 m because this level was not available for each habitat at all locations. All stations were located in the semi-exposed zone of the Ría de Ferrol and apparently experienced similar wave-wash due to the intense maritime traffic. Two sites, about 5 m long and separated by 5-10 m were selected at each tidal level on each seawall and rocky shore. Five replicate quadrats (20 x 20 cm each) were randomly positioned in each site but always placed at least 10 cm away from crevices or similar features of the substratum. Quadrats within the same site were separated about 50 cm. In each quadrat, any mobile mollusc was identified and specimens of each taxon counted.

In general, there were no consistent differences in composition of mollusc assemblages among natural rocky shores and seawalls; significant differences were generally found at the level of sites within locations or among locations within the same habitat. This contrasts with work done elsewhere which points out that differences are found at high- and midshore levels (Chapman & Bulleri, 2003; Bulleri *et al*., 2005). Similarly, differences in abundance of common species were mostly detected at the scale of sites and scarcely between habitats; when the latter were found those were not consistent among locations or seasons. Differences between the two habitats were, however, detected when frequencies of occurrence of taxa were compared (i.e. number of quadrats in which each species was present in each habitat across all locations). Thus, two different patterns were found: some species were more common on seawalls than on rocky shores at high- and/or midshore (*Patella vulgata*, *Gibbula umbilicalis*) or mid-lowshore (*Nucella lapillus*) while the opposite pattern was found for some snails at high-midshore (*Littorina saxatilis*) and mid-lowshore (*Osilinus lineatus*, *Littorina littorea*); those were also consistent across seasons for the same tidal level.

This suggests that these patterns might be related to differences in availability and/or use of microhabitat(s) as shown in previous work (Chapman, 2003). Further experimental work will be needed to test models explaining these results, which is currently being done.

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Effects of the anthropogenic activities on gills of *Pinna nobilis* using oxidative stress biomarkers

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Aquatic organisms are exposed to anthropogenic contaminants that may strongly affect their performance and survival. The periodical oxygen deficiency in coastal areas can induce the production of an excess of reactive oxygen species (ROS). An increase in the formation of ROS and/or a reduced function of the physiological antioxidant defence systems results in oxidative damage to cellular biomolecules. *Pinna nobilis* is the largest endemic bivalve in the Mediterranean Sea, appearing in coastal areas between 0.5 and 60 m depth. *P. nobilis* is under strict protection and all forms of deliberate capture or killing them are prohibited (EEC, 1992). This study aims to estimate the effects of the anthropogenic activities on gills of *Pinna nobilis* through environmental stress biomarkers (nNOS, HIf-1 alfa), determination of antioxidant enzyme response (Cat, Sod, Gpx, Gr) and biomarkers of oxidative damage (MDA). In bivalve molluscs, the gills are an organ highly exposed to environmental factors due to their large surface and their involvement in gas exchange and feeding. The nitrogen monoxide (NO) belongs to the family of free radicals. The production of NO takes place in a reaction catalyzed at the level of several tissues by different isoforms of a monooxygenase known as NOS (nitric oxide synthase). The enzyme has three known isoforms: nNOS, neuronal tissue constitutive enzyme, eNOS, constitutive of vascular endothelial cells and iNOS, an enzyme inducible by cytokines in the liver and activated macrophages. In the nervous system, NO plays multiple roles such as neurotransmitter, is also involved in sensory processes, in synaptogenesis, and in the synaptic plasticity. NO also participates as a molecular mediator of the non-specific immune response against bacterial infections in invertebrates. It is we established that pollution stimulates the gene expression of this enzyme. Furthermore, NO regulates the activity of many transcription factors such as HIF-1α. The neuroepithelial cells, distributed along the axis of the gills and lamellar filaments in the vertebrates, play an important role in oxygen chemoreception and modulate a variety of responses such as oxygen tension in the arteries in response to hypoxia. The HIF-1α is the key gene in hypoxia signalling, being activated under hypoxic stress. In conditions of normoxia, the HIF-1α is not expressed, degraded and kept inactive; on the contrary, when the oxygen tension is reduced HIF-1α is expressed and enters in the cellular nucleus. The hypoxia is characterized by the production of ROS in the cells. The production of ROS and the oxidative stress associated have been recognized as major players in the mediation of a great number of diseases caused by lack or excess of oxygen. An oxidative stress situation occurs when the balance between antioxidants and oxidants is altered and the redox equilibrium is disturbed, resulting in overproduction of free radicals. The relationship between the production of ROS, the several levels of oxygenation and the pathological state of the organism is very complex. The redox homeostasis is subjected to displacement due to an overabundance of factors such as disease states, deficiency or excess of oxygen, infection, inflammation and oxidative damage. The defence systems known to oppose the formation of oxygen radicals are divided in enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX); and in non-enzymatic antioxidants (such as vitamin E, ascorbic acid and reduced glutathione). The activity of these enzymes is revealed, therefore, as useful biomarkers for assessing the quality status of aquatic environments. For this study, 20 individuals of bivalve *Pinna nobilis* were collected during May-June 2011 in 2 stations with different degree of human impact; the first station located in the Magalluf bay (Mallorca, Esp) along Mallorca Island coasts is considered as a polluted site and the second station in Cabrera Archipelago National Park, (BI, Esp) is considered as a clean non polluted area. The fan mussels were sampled at 8-10 m depth by SCUBA diving, measured and dissected on board in order to remove gills. A portion was frozen in liquid nitrogen for biochemical assays and another portion was stored in paraformaldehyde for histomorphological and immunohistochemical analysis. Histological sections were mounted on glass slides and stained with haematoxylin/eosin to visualize typical morphological features. The morphological staining revealed in individuals of *Pinna nobilis* sampled at Cabrera a regular morphology in the gills with filaments and well organized lamellae, while there were evidenced distinct alterations in the epithelium of gills from the organisms sampled in Magalluf, where it was possible to see thickening and thinning of the lamellar apices close to the filament. The gills of individuals, collected in the waters of Magalluf when analyzed by indirect

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immunofluorescence using antibody anti-nNOS, showed a rather intense immunopositivity in the cells and in the haemocytes of the gill lamellae, compared to those from the reserve of Cabrera. The epithelium of gills from organisms collected to Magalluf showed a greater number of HIF-1α immunopositive haemocytes and cells to respect to what was observed in the gills of the individuals sampled at the control site. All antioxidants CAT, SOD, GPx, GR and GST measured as markers of oxidative stress in gills showed higher activities in Magalluf station respect to the Cabrera. MDA concentration in gills, as a marker of lipid peroxidation, was also increased in the specimens sampled in Magalluf bay. The results suggest that the intense production of vasoactive substances, such as NO found in connective fibbers of gills, may be a local reaction to hypoxia. Due to environmental stress, there is a greater induction of the nNOS, in the nervous elements and in the neuroendocrine-type cells that it can be correlated with the modulation of the chemiosensorial mechanisms for the oxygen and the osmotic control, as it has emerged from the presence of numerous HIF-1α immunopositive cells in the samples from the polluted site. The presences of nNOS and the transcription factor HIF-1α represent adaptive and protective mechanisms. Among the biological assays useful for the assessment of environmental quality, NO is therefore a useful biomarker in providing early information on the presence of environmental changes. We can conclude that human activity induces a biological stress in the fan mussel *Pinna nobilis* which was further highlighted by an increase in the antioxidant enzyme activities and in the lipid peroxidation.

A temporal study of the macroinfauna of the beach most affected by the Prestige oil-spill (O Rostro, NW Spain)

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Exposed beaches are considered poor habitats where wave action and sediment instability limit the development of biological assemblages, with low productivity compared with other coastal environments (McLachlan, 1983). In temperate latitudes, supralittoral levels of beaches are usually occupied by an assemblage constituted by semi-terrestrial amphipods, isopods and insects that are linked to the food supply from algal wrack and the tidal organic material. Intertidal levels are usually occupied by marine amphipods, isopods and polychaetes.

Oil-spills are considered one of the most important environmental disasters on coastal areas and particularly, the "Prestige" oil-spill (autumn 2002 to spring 2003) was particularly harmful due to its magnitude. An important scientific effort was done in intertidal beaches and estuaries affected by the oil-spill, with special emphasis on macroinfaunal assemblages (Junoy *et al*. 2005; de la Huz *et al.* 2005; Puente *et al*. 2008, 2009). Medium and long-term studies are suitable to discern between the rates of natural and/or anthropogenic disturbances, however, these studies are scarce on sandy beaches.

In the present study, a before-after environmental study was conducted in O Rostro beach (42º58´N- 9º15´W, Figure 1), the most affected beach by the "Prestige" oil-spill and pristinely characterized by low macroinfaunal abundances and diversity (Junoy *et al*. 2005; Lastra *et al*. 2006). The morphodynamic of the beach is well known after the monitoring plan and studies developed with occasion of the "Prestige" catastrophe (Bernabeu *et al*. 2006; CEPRECO, 2006; González *et al*. 2009).

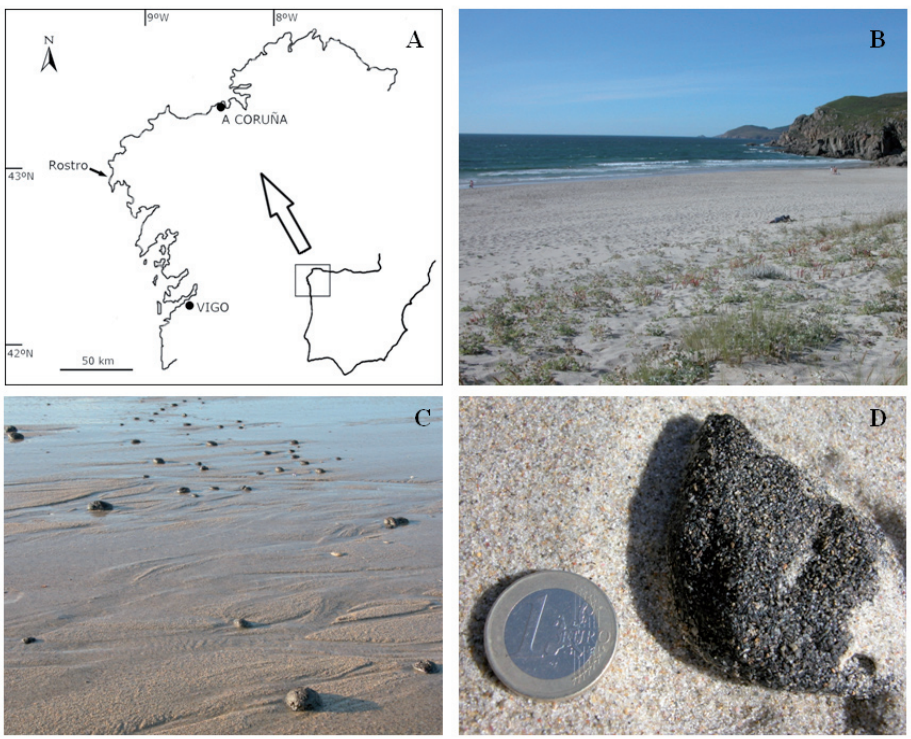

Figure 1. A) Location of the O Rostro beach; B) Photo of the north end of the O Rostro beach at high tide on May 2003; C) Tar balls (15-30 cm diameter) observed at the intertidal on first campaign (May 2003); D) Small pellets still present at O Rostro beach at the last campaign (May 2007).

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This beach was sampled in six sampling campaigns: before oil-spill (September 1995), six months after the oil-spill (May 2003) and after, in yearly campaigns (May 2004, May 2005, May 2006 and May 2007). Big tar balls (15-30 cm diameter) were obvious on the sediment in May 2003 (Fig. 1c); moreover small pellets about 1-2 cm were collected at the end of the sampling period, in May 2007 (Fig. 1d). In each sampling campaign 6 transects were extended from above the drift line (supralittoral level) to below the swash line. Beach division was based on Salvat's zonation scheme (Salvat, 1964, 1967) adding an extra supralittoral level (Pollock & Hummon, 1971): (1) 2 m above the drift line, (2) drift line, (3) retention, tidal level (3-2 m); (4) resurgence, tidal level 2-1 m, and (5) saturation, tidal level 1-0 m. At each level six 0.05 m^2 replicates (1 m apart each other) were taken and sieved through a 1 mm sieve, it is extensively used in former studies in the Galician beaches because of their sediment grain size. The residue was preserved in 7% formalin; the macroinfauna was later sorted from the sediments, identified, and counted. At each level, one yearly sediment sample was collected for grain size analysis and organic matter content.

Multivariate analyses of sample data were performed using PERMANOVA (Anderson 2001) and SIMPER (Clarke, 1993). Furthermore a level and a yearly analysis were studied by cluster and CAP (Anderson & Robinson 2003, Anderson & Willis 2003). PERMANOVA was used to study the differences between the yearly and spatial-temporal variation, it was performed in a 2 factors model design. The factors employed in the model were level and year, both were considered as fixed and crossed. Permutation of residuals under a reduced model was the permutation method chosen by 9999 permutations; moreover Monte Carlo test was used. The samples were studied through main test and pair-wise test. Further subsequent one-way SIMPER analyses were done on the fauna matrix to check the fauna variables which note the differences among the samples with significant results from PERMANOVA pair-wise test. Canonical discriminant analyses were performed through CAP analyses to check the differences among the different years for each level.

The dominant sedimentary type was medium sands (0.2-0.5 mm diameter) throughout the study period in both tidal levels (supralittoral and intertidal). Gravel and, silt and clay are scarce (< 1.3%). Organic matter content was characterized by low values (< 1.2%). Beach index (BI) ranged from 2.23 (2004) to 2.61 (2005). Dean´s parameter (Ω) estimated at the sampling times (May) ranging from 3.2 (2007) to 3.8 (2004). However, the beach slope (1/x) varied largely in O Rostro beach, from 21.85 (2004) to 48 (2006). These observations are in concordance with the data of CEPRECO (2006) and González *et al*. (2009). These authors noted that the modal state of the beach corresponds with the Rhytmic Bar and Beach; however the beach exhibits a great temporal distribution of morphodynamic states, from Transversal Bar and Rip to Longhsore Bar and Trough.

The overall macroinfaunal abundances varied largely throughout the study period, ranging from 74 ind (2005) to 423 ind (2007). These differences were mainly due to the abundance variations of crustaceans, the dominant taxonomic group (Table 1, Figure 2). Species richness remained with slight variations throughout the study period, with the exception of 2003 campaign, six months after the spill that showed the lowest richness (5 taxa). Supralittoral levels were characterized by low macroinfaunal abundances throughout the study period, ranging from 25 ind (2005) to 166 ind (2007). The most abundant taxa were Oligochaeta (114 ind), the isopod *Tylos europaeus* (158 ind) and the talitrid amphipods *Talitrus saltator* (174 ind) and *Talorchestia brito* (77 ind). The remaining species were scarce (< 20 ind). Intertidal levels varied largely throughout the study period, ranging from only one specimen six months after the spill (2003) to a maximum of 291 before it (1995). The most abundant species were the isopods *Eurydice naylori* (257 ind) and *E. pulchra* (247 ind), and the amphipod *Pontocrates arenarius* (256 ind), followed by the mysid *Gastrosaccus roscoffensis* (89 ind).

Table 1. Mean abundances (± SE) of macroinfaunal species from O Rostro beach throughout the study period. * Data kindly provided by Dr. Mariano Lastra, Universidad de Vigo.

Species	1995*		2003		2004		2005		2006		2007	
	Supra-	Inter-	Supra-	Inter-	Supra-	Inter-	Supra-	Inter-	Supra-	Inter-	Supra-	Inter-
Spio martinensis	0	1 ± 1.4	0	0	0	0	0	0	0	0	0	0
Oligochaeta	2 ± 2.8	0	0.5 ± 0.7	0	32 ± 38.1	0	2.5 ± 3.5	0	16.5 ± 23.3	0	2.5 ± 3.5	0
Pontocrates arenarius	0	0	0	0.5 ± 0.7	0	26.5 ± 33.2	0	42 ± 28.2	0	26.5 ± 33.2	0	42 ± 28.2
Talitrus saltator	3.5 ± 0.7	0	0	0	18 ± 19.7	0	58.5 ± 82.7	0	6.5 ± 9.1	0	58.5 ± 82.7	0
Talorchestia brito	11 ± 0	0	0	0	0	0	13.5 ± 6.3	0	4 ± 5.6	0	13.5 ± 6.3	0
Talorchestia deshayesii	7 ± 0	0	0	0	0.5 ± 0.7	0	0.5 ± 0.7	0	0	0	0.5 ± 0.7	0
Eurydice affinis	0	0.5 ± 0.7	0	0	0	0	0	0	0	0	0	0
Eurydice naylori	0	0	0	0	0	1 ± 1.4	0	37 ± 31.1	0	1 ± 1.4	0	37 ± 31.1
Eurydice pulchra	0	119.5 ± 169	0	0	0	0	0	0	0	0	0	0
Tylos europaeus	8.5 ± 2.1	0	47 ± 66.4	0	1 ± 1.4	0	3.5 ± 4.9	0	14.5 ± 19.0	0	3.5 ± 4.9	0
Gastrosaccus roscoffensis	0	14 ± 19.8	0	0	0	0.5 ± 0.7	0	8.5 ± 0.7	0	0.5 ± 0.7	0	8.5 ± 0.7
Cafius xantholoma	0	0	0	0	0	0	0	0	0.5 ± 0.7	0	0	0
Hypocaccus dimidiatus	0	0	1 ±1.4	0	0	0	0	0	1.5 ± 2.1	0	0	0
Coccinelidae	0	0	0	0	0	0	0	0	0.5 ± 0.7	0	0	0
Tabanidae	4 ± 5.6	0	0.5 ± 0.7	0	0	0	2 ± 2.8	0	0	0	2 ± 2.8	0
Diptera sp	2 ± 2.8	0	0	0	0	0	2.5 ± 2.1	0	0	0	2.5 ± 2.1	0
Aracnea	0.5 ± 0.7	0	0	0	0	0	0	0	0	0	0	0
Carinomidae	0	0	0	0	0	1 ± 1.4	0	0	0	1 ± 1.4	0	0

In 2003, post oil-spill conditions, macroinfaunal assemblages were only represented by only one intertidal specimen of the amphipod *Pontocrates arenarius* and four supralittoral taxa: Oligochaeta, the tabanidae (Diptera) larvae, the coleopteran *Hypocaccus dimidiatus maritimus* and the isopod *Tylos europaeus*. *T. europaeus* dominated overwhelmingly (94 ind. 94.94% overall abundance). To lesser extent, some differences can be discerned between before (1995) and after oil-spill (2003 onwards). In 1995, some species were well-represented (the amphipod *Talorchestia deshayesii*, Diptera sp. and the polychaete *Scolelepis squamata*) and posteriorly their densities abruptly decreased or even disappear after the oil-spill episode. In contrast, several species were important components of macroinfauna assemblages during recent campaigns (2006-2007) (*Talitrus saltator* and *Pontocrates arenarius*). Discrepancies with the *Eurydice* species collected, *E. pulchra* in 1995 and *E. naylori* after the spill, are related with the description in 1997 of the latter species (Jones & Pierpoint, 1997).

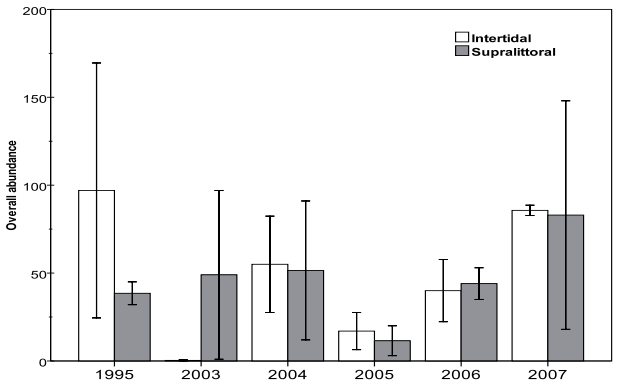

Figure 2. Overall macroinfaunal abundances (± SE) in intertidal and supralittoral stations throughout the study period (oil-spill: winter 2002).

Pair-wise test from PERMANOVA showed that the years 1995, 2004, 2006 and 2007 didn't have significant differences between them in reference to richness and abundances, in contrast the years 2003 and 2005 which had noticeable differences with the rest of years. Cluster analysis presented an aggregation of the two years with less abundance, 2003 (six month after the spill) and 2005, moreover to highlight a different behaviour between intertidal and supralittoral levels (Fig.3)

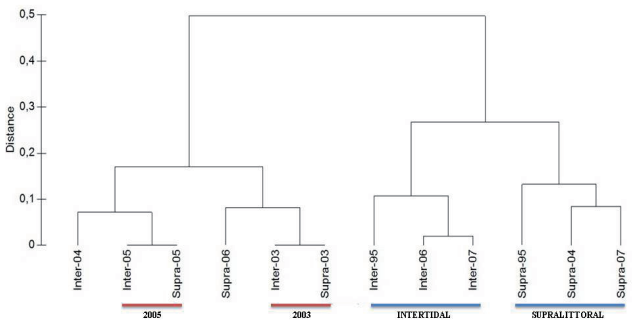

Figure 3. Cluster analysis of levels (Supra: supralittoral; Inter: intertidal) and years (95: 1995; 03... 07: 2003... 2007).

A specific abundance study through CAP, subsequently to the oil spill, revealed a difference between years in the first canonical axe: i) 2003 ii) 2005 and iii) 2004, 2006 and 2007. The second canonical axe shows the difference between the two levels, supralittoral and mediolittoral, except for the first sampling campaign, 2003 (Fig. 4)

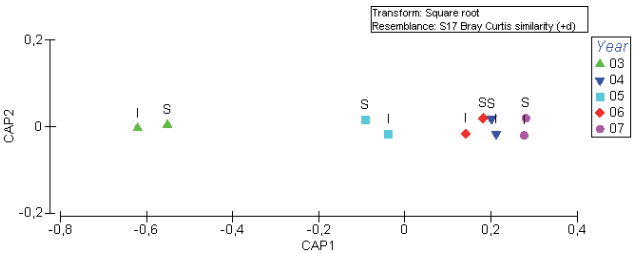

Fig.ure4. CAP analysis. S: supralittoral levels; I: intertidal levels; year 2003 (left) and 2005 are separated from 2004, 2006 and 2007.

The main problem for the assessment of the ecological effects of the "Prestige" oil spill was the lack of the baseline studies in many of the ecosystems affected. Fortunately, the former data (1995) from Galician beaches make possible to compare data from before and six months after the oil-spill (2003). In this context, a long-term spatial analysis with many beaches sampled has proved to be useful to detect the general impact in an 11 short term before-after analyses (De la Huz *et al*. 2005; Junoy *et al*. 2005). A reduction of macroinfaunal abundances and species richness was encountered six months after the spill. These shifts were particularly accentuated in scarce species, represented by low abundances at the studied beaches, which were eliminated. These authors showed changes in macroinfaunal composition in the intertidal and supralittoral zones. In supralittoral, macroinfaunal variations consisted of the disappearance of insects, and in some cases, a sharp increase of oligochaetes. This increase was related with the oil deposit at upper beach levels. Talitrid amphipods and the isopod *Tylos europaeus* showed a clear reduction in their assemblages. In the intertidal, they observed a marked decrease of polychaetes (*Scolelepis*) and isopods abundances (*Eurydice*) and the increase of abundances of the amphipod *Pontocrates arenarius*. The "Prestige" oil-spill did not markedly affect macroinfaunal diversity and richness (De la Huz *et al*. 2005; Serrano *et al*. 2006; Puente . 2009), like occurred in other oil-spills (Kingston . 1995; Feder & Blanchard 1998; Fukuyama . 1998).

However, an expected macrofaunal recovery was observed but within natural variability. In contrast, a significant effect was observed in other spills (Pielou 1975; Gómez-Gesteira . 2003). Other accident oil-spills enhanced the abundance of opportunistic species, mainly polychaetes, and a sharp decrease of sensitive species, mainly amphipods (Gómez-Gesteira & Dauvin 2000, 2005; Peterson 2001). This smaller effect of the oil-spill could be explained by the physico-chemical characteristics of the oil, season of the spill (winter), rough seas, sea temperature and quick removal of oil from most of the beaches (de la Huz . 2005; Junoy . 2005). The present study extends the beach macroinfaunal comparison to five years after the oil-spill, and the results are in

contrast with the previous studies on Galician beaches. At short-time study, O Rostro beach suffer a reduction in macroinfaunal abundance and species number (1995 *vs* 2003, six months after the spill). Significant differences were found according to abundance and richness along the 2003-2007 periods. Ecological studies on interannual variations are scarce in Spanish coasts, and are crucial to determine the temporal variations of the macroinfaunal assemblages. Ganning . (1984) showed the importance to conduct studies in order to define natural variability in the ecosystems; this is particularly noticeable in intertidal exposed sandy beaches where constant ecological variations occur throughout the year (McLachlan & Brown 2006).

Green & Montagna (1996) showed that natural disturbances have possibly hindered the identification of the generalised effects of oil-related activities on macrofauna assemblages. Thus, the responses of opportunists to natural and anthropogenic disturbances are not easily distinguishable (Clark 1982; Spies 1987). One possible explanation for the present data is that the oil-spill changed macrofaunal assemblages in the short-term (weeks or even months) but a mobile sandflat is recovered very quickly as expected. Thus, a naturally highly mobile and variable environment makes rather difficult the detection of anthropogenic stress. Moreover, it has been observed that the temporal response of faunal impoverishment and the peak of opportunistic species are still driven mainly by the presence or absence of natural disturbances.

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Impactos del buceo recreativo sobre las poblaciones de la ascidia solitaria *Halocynthia papillosa* (Linnaeus, 1767) en el Mediterráneo español.

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Halocynthia papillosa (Linnaeus, 1767) ha sido catalogada como especie sensible e indicadora del impacto del buceo en los ecosistemas marinos mediterráneos. Efectos como la resuspensión de sedimentos o el contacto físico directo por parte de los buceadores, pueden afectar sensiblemente a las poblaciones de esta especie, sensible a este tipo de perturbaciones. El objetivo de este estudio fue evaluar el impacto del buceo en áreas protegidas y no protegidas, sobre las poblaciones de la ascidia solitaria *H. papillosa*, especie bioindicadora abundante en el coralígeno de las costas de Alicante y Murcia, en el Mediterráneo español. Se planteó un diseño de muestreo balanceado, con cinco factores de variación con dos niveles cada uno, tres fijos (Tiempo: antes y después del verano, época principal de buceo; Protección: áreas protegidas y no protegidas; e Impacto: con buceo y sin buceo) y dos aleatorios (Localidad y Sitio). Las variables medidas y analizadas fueron la abundancia media, las tallas media y máxima de los individuos y el nivel de exposición. Se realizaron ANOVAs multifactoriales y las técnicas de análisis multivariantes MDS y PERMANOVA para detectar y evaluar las posibles diferencias entre los niveles de los factores establecidos. La abundancia tendió a ser mayor en los lugares donde no se bucea. Después de la presión de la campaña estival, aumentó la abundancia en los sitios donde no hubo actividad de buceo, mientras que en los que sí se buceó, disminuyó respecto a antes de comenzar la temporada alta. Respecto a la talla media, la talla máxima y el nivel de exposición, las diferencias significativas fueron encontradas entre las Localidades y los Sitios muestreados en algunas de las variables, mostrando una elevada heterogeneidad espacial. El análisis PERMANOVA mostró diferencias significativas entre las Localidades y los Sitios para el conjunto de las variables seleccionadas. Según nuestros resultados, esta especie muestra una mayor presencia en zonas con menor presión por parte de la actividad de buceo deportivo. Sin embargo, la elevada heterogeneidad espacial observada y la falta de diferencias significativas en algunos de los resultados, recomiendan la utilización conjunta de otros indicadores para poder realizar una adecuada evaluación del impacto del buceo recreativo.

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Development of histological and molecular tools to characterize endocrine disruption effects on thicklip grey mullet (*Chelon labrosus*) populations from the Basque Coast.

Cristina Bizarro [a], Miren P. Cajaraville [a], Maren Ortiz-Zarragoitia [a]

Introduction

The Marine Strategy Framework Directive (2008/56/CE) is the first EU binding law designed to make a significant contribution to the preservation, protection and restoration of marine ecosystems, including pollution reduction and minimization. The Directive aims at achieving good environmental status in the EU marine waters by 2020 at the latest. According to the Directive, EU Member States should undertake a series of steps to progressively achieve this good environmental status which should ensure the maintenance of ecologically healthy, clean and productive seas as well as reduce adverse human impacts on marine ecosystems. Each Member State has to work to implement measures both at the national and regional scale. The MSFD also recognizes the precautionary principle and requests the application of an ecosystem-based approach to the management of human activities in order to minimize their impacts on the marine ecosystems. Traditionally, water quality monitoring actions have focused on physical and chemical measurements. It is widely recognised that the use of other indicators, in addition and complimentary to traditional chemical and physical water quality monitoring techniques, can greatly enhance the assessment and management of aquatic ecosystems. Consequently, biological monitoring, or biomonitoring, is an important tool in assessing the condition of aquatic ecosystems (Cajaraville ., 2000; Viarengo ., 2007). The use of biomarkers within environmental biomonitoring programs has gathered importance in environmental risk assessment and has been recommended by many international organizations and governmental agencies. In recent years numerous biomarkers have been developed and applied to assess the biological effects and responses of organisms after exposure to pollutants (Cajaraville ., 2000; Galloway ., 2004). Biomarkers are considered measurements carried out on body fluids, cells, or tissues that indicate in biochemical or cellular terms the presence of contaminants (exposure biomarkers) or the magnitude of the host response (effect biomarkers or biomarkers of stress) (McCarthy and Shugart, 1990; Livingstone ., 2000). Because biomarkers can be used as early warning signals to predict changes at higher levels of biological organization, i.e., population, community or ecosystems, they could be defined as short-term indicators of long-term biological effects (Cajaraville ., 2000; Viarengo ., 2007).

The chemical compounds known as endocrine disruptors (ED) and environmental factors linked to climatic change (such as temperature elevation or hypoxia) may alter the functions of the endocrine system and consequently cause adverse health effects to an organism, its progeny or (sub)populations (EEA, 1997; Devlin & Nagahama, 2002). EDs alter reproduction and development by changing hormonal homeostasis, altering genes related to reproduction or interacting with hormonal signals (Crews ., 2000). Such changes can be harmful not only for the health of the concerned individual, but also modify their reproductive capacity significantly reducing the viability of affected populations, even resulting in their disappearance (WHO/IPCS, 2002). The main source of EDs is of human origin and their presence in the aquatic environment is well documented (Langston ., 2005). EDs tend to accumulate in sediments but also in exposed organisms, reaching high bioaccumulation levels (van der Oost ., 2003). Different factors such as stages of development and species sensitivity, nutritional health, age, genetics and season may play different roles in the effects of these chemicals. This makes it difficult to distinguish between direct and indirect effects and primary versus secondary effects of exposure to EDs (Devlin and Nagahama, 2002; WHO/IPCS, 2002). The EU Water Framework Directive (WFD, 2000/60) provides a list of 33 priority chemicals that pose a significant risk to the aquatic environment, some of which are classified as EDs: they include nonylphenol, some PAHs, bisphenol-A, and tributyltin. Several cases of endocrine disruption have been described in aquatic organisms, especially in species that inhabit continental waters with high pollutant burdens. Among EDs, xenoestrogens constitute a particular class of endocrine disruptors with the ability to mimic natural estrogens and have received special attention (WHO/IPCS, 2002; Goksøyr ., 2003). Xenoestrogens in fish, both in laboratory and in the field, cause abnormal gonad development (Jobling ., 1996, 1998; Gimeno ., 1996), alterations in sex steroid titres (Folmar ., 1996), retarded sexual maturity, alteration of the ovaric cycles, lower eclosion rate of eggs, apparition of intersex individuals, sex change inductions and total reproduction blockage (Matthiessen, 2003; Piferrer, F., 2001). Xenoestrogens may elicit effects through a number of divergent pathways including direct binding and activation of the estrogen receptor (er), binding to other nuclear receptors which interact with an estrogen response element (ere), and through other receptor and/or signal transduction pathways such as

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retinoid-x-receptor, rxr (Gillesby and Zacharewski, 1998; Arukwe and Goksøyr, 2003). Both er and rxr belong to a structurally related family of proteins called the nuclear receptor superfamily whose main function is as a DNA-binding transcription factor that regulates gene expression. These are concretely ligand-activated transcription factors that after being activated by binding to a hormone, form homodimers or heterodimers. These then bind to response elements in regulatory regions of target genes in the DNA (Melamed ., 1996; Jisa and Jungbauer, 2003) and affect transcription of specific genes implicated in fundamental biological processes (Keller ., 1995).

In fish, the best described process of feminization is the production of vitellogenin (VTG) in males and immature organisms (Arukwe and Goksøyr, 2003). VTG is a large phospholipoglycoprotein, precursor for the formation of the yolk, synthesized in the liver of adult oviparous females under the regulation of estrogenic hormones. VTG synthesis in males and juveniles is therefore used as a specific biomarker of xenoestrogen exposure in environmental monitoring programs (OECD 2001; WHO/IPCS, 2002). Organizations such as OECD and ICES include VTG as a marker of exposure to xenoestrogenic compounds in fish stocks (ICES, 2005). Expression patterns of different p450 genes, such as cyp19 aromatases, are sensitive to xenoestrogens, and could affect the normal development and metabolism of exposed organisms (Williams ., 1998; Trant ., 2001; Ortiz-Zarragoitia ., 2006). cyp19 aromatase genes are responsible for the synthesis of steroid hormones, converting androgens like testosterone into estrogens like estradiol (E2) (Williams ., 1998). Among aromatases two different cyp19 genes have been found in many teleost fishes. These genes encode two structurally and functionally different aromatase isoforms: cyp19a1 and cyp19a2. Cyp19a1 is preferentially expressed in the ovary, while cyp19a2 is expressed mostly in the brain. Cyp19 aromatases play an important role in sex differentiation and determination in fish (Trant ., 2001; Devlin and Nagahama, 2002). The OECD has proposed the measurement of steroidogenic activity pathway as a potential marker of endocrine disruption (OECD, 2009).

Recent studies conducted by our group in the Biosphere Reserve of Urdaibai have described the presence of thicklip grey mullet *Chelon labrosus* with intersex gonads (testis ova). Males and immature individuals, captured downstream of the wastewater treatment plant of Gernika, showed elevated levels of plasma VTG, that correlated with high levels of nonylphenol metabolites in bile (Puy-Azurmendi ., in press). No further works have reported the presence and effects of EDs on the South Bay of Biscay. Thus, the aim of the present work was to develop and apply a battery of biological tools based on the biomarker approach to monitor the presence of ED effects on mullets from different estuaries of the Basque Coast (South East Bay of Biscay). Selected biomarkers were plasma levels of VTG, transcription levels of genes associated with gametogenesis and reproduction such as vtg, er, rxr and cyp19a1 and a2 aromatases. For the detection of intersex fish a detailed histopathological study of the gonads was carried out in mullets. Mugilids are used worldwide as sentinel organism of coastal pollution. Biomarkers of exposure and effect have been applied in mullets, together with contaminant bioaccumulation studies and endocrine disruption works (Orbea ., 2002; Ferreira ., 2005; Fossi ., 2005; Puy-Azurmendi ., in press). However, little is known about their reproduction and gametogenic cycle in the Basque Coast. Therefore a two year study was completed in order to characterize the gametogenic cyle in mullets from the Basque Coast and to select the most appropriate seasonal period for the study of endocrine disruption effects.

Materials and methods

Mullets were collected from seven estuaries of the Basque coast, showing different pollution loads. Mullets from Arriluze and Plentzia were collected in May 2010, from Gernika in both May 2010 and 2012, from Santurtzi, Ondarroa and Deba in May 2012. The mullet population from Pasaia was selected for a more detailed study, in order to characterize the gametogenic cycle of mullets in the Basque Coast. This population showed individuals present all along the year and easy to capture facilities. This population was sampled in May 2010 and monthly from September 2010 to September 2011 and bimonthly from September 2011 to July 2012. At each sampling point and time 12-30 mullets were collected by fishing rod and immediately processed. Mullets were anesthetized in a saturated solution of MS-222, following the accepted legislation rules for animal experimentation. Length (cm) and weight (gr) of each organism was recorded. From each fish, samples of blood, liver, brain, gonad and bile were collected as follows. Blood samples (approximately 1 mL per fish) were extracted in heparinized syringes and immediately centrifuged for plasma separation. Plasma was transferred to a cryotube, frozen in liquid nitrogen and stored at -80ºC. Liver and gonads were dissected out and weighted in order to calculate the gonadosomatic (GSI) and hepatosomatic (LSI) indexes. A piece of each was placed on neutral buffered formalin for histopathological studies and another piece cut on small fragments and placed on RNAlater for gene expression studies. Brain was sliced on small size fragments and embedded on RNAlater for gene expression assays. Finally, a sample of bile was collected from each fish and directly frozen on liquid nitrogen for the detection of ED metabolites. Histopathological study was focused on the detection of gamete alterations, such as intersex gonads. Gene expression assays measured the transcription levels of vtg, er, rxr and cyp19a1 and cyp19a2 by TaqMan assay. VTG levels in fish plasma were quantified by a specific ELISA. Bile metabolites, such as estrogenic hormones (natural and synthetic), alkylphenols, bisphenol A and phthalates, were isolated by solid phase extraction and calculated by gas chromatography-mass spectrometry.

Results and discussion

Mullets collected in Pasaia from September 2010 to September 2011 showed that gametogenesis started at the end of summer and proceeded during autumn. Mature gonads were observed at the end of autumn and spawning occurred during winter months. Post-spawning gonads were identified during spring-early summer months. No significant differences were detected in gamete development between male and female mullets, but males got gonad maturity slightly earlier than females. Accordingly, GSI values

resembled the same trend described for gametogenesis in both male and female mullets (Figure 1). LSI values also followed the same trend showed for GSI in females, but not in males (Figure 1).

Transcription levels of *cyp19a1*, *er* and *rxr* in the gonads

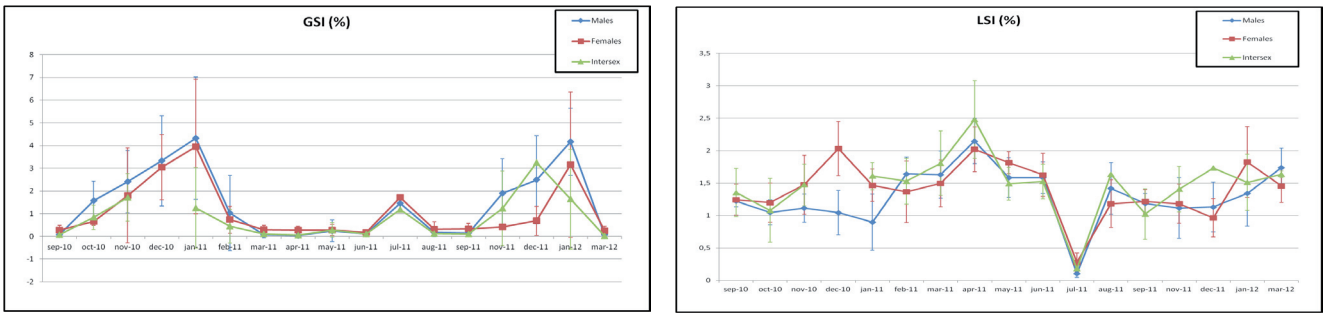

Figure 1. Left: gonadosomatic index (GSI). Right: Hepatosomatic index (LSI). Both organosomatic indexes show values for male, female and intersex mullets collected in Pasaia harbor from September 2010 to March 2012 completing two reproduction cycles.

of female mullets followed the same trend described for gametogenesis, being lower in spring-summer months (post-spawning gonads) and increasing during autumn (gametogenesis) to drop again in winter at mature stage. Accordingly, vitellogenin plasma protein levels and transcription levels in the liver also increased in female fish during autumn months peaking in winter. No such trends were observed in the case of male mullets. *rxr* expression levels in male gonads decreased as gonad got maturity. Transcription of *er* in the liver of female mullets showed a similar trend as the one described in gonad, with high transcription levels at vitellogenesis (late autumn-early winter) and low at spawning and post-spawning stages (spring-summer). In male mullets, *er* transcription in the liver was overall low except in March 2011. Transcription of *er* and *rxr* in the brain of studied mullets did not show significant differences between genders. Transcription of *cyp19a2* in the brain of female mullets decreased during gametogeneis (autumn) and the lowest values were calculated at mature-spawning time (winter months). Male mullets showed the opposite trend from brain *cyp19a2* expression levels. Transcription was high during mature gonad and low at gametogenic gonad.

During the whole sampling period, with the exception of December 2010, intersex mullets were detected (up to 26% of total analyzed mulletd in November 2010). These organisms showed histologically male gonad tissue containing interspersed free oocytes. These oocytes were always at previtellogenic-stage, while the surrounding testis tissue changed according to the gametogenic stages observed for non-affected males at each sampling month. Intersex mullets showed *cyp19a1* expression levels in the gonads between values calculated for male and female mullets, but a similar trend to that described for female mullets could be suggested. Highest *cyp19a1* expression levels in intersex fish were measured during autumn-winter months (September-January) and lower values at spring months (March-May). Intersex mullets showed a similar trend to that observed in female mullet gonads for *er* transcription, with up-regulation between September and November, followed by a drop in expression levels in January. High expression levels were measured again in May. No significant changes were detected for *rxr* expression levels in the gonads of intersex mullets. Intersex mullets also showed measurable vitellogenin levels in their plasma. No significant differences on transcript abundance of *er* and *rxr* in the liver and brain were observed in intersex mullets when comparing to male mullets. However, *cyp19a2* expression levels in brain were elevated in intersex fish all along the study.

Regarding population specific responses, preliminary data from Arriluze, Gernika, Plentzia and Pasaia in May 2010 showed that mullets inhabiting the estuaries of the Basque Country are exposed to EDs, confirming the first results obtained in 2007 by Puy-Azurmendi . (in press). Intersex gonads were observed in mullets from Gernika (2 out of 14 mullets) and Pasaia (1 out of 10). As described above, these mullets showed previtellogenic oocytes surrounded by testicular tissue. Almost all analyzed male mullets contained measurable levels of VTG in plasma. Males from Gernika and Plentzia showed very high plasma VTG levels in comparison to male fish from Arriluze and Pasaia. VTG plasma levels measured in male mullets from Plentzia were at the same level of VTG plasma levels obtained for female fish. *cyp19a2* expression levels in the brain were elevated in male mullets from Pasaia and Plentzia in comparison to values recorded in the brain of male fish from Arriluze and Gernika. Furthermore, gonad *er* transcription levels were high in male fish from Pasaia. Bile metabolite analysis confirmed the uptake of EDs by mullets from selected populations. Mullets from Arriluze and Gernika contained high levels of alkylphenols and bisphenol A, in Plentzia they were enriched on alkylphenols and synthetic estrogenic hormones, and in Pasaia the most detected metabolites were bisphenol A and synthetic estrogenic hormones.

Conclusions

Overall, all these data indicate the presence of endocrine disruption effects in mullet populations from the Basque Country. The battery of biomarkers applied in the present study has been demonstrated as a valuable tool for the assessment of endocrine disruption in estuarine fish. Based on the recommendations for the implementation of a biomonitoring program proposed by the European Water Directive Framework and the Marine Strategy Framework Directive and in order to characterize the presence and effects of EDs in the Basque coast, we suggest the development

of a survey using mullets as sentinels. These biomonitoring can be divided in a two tier approach. In a first step, rapid and cost-effective procedures such as gonad histopathology, measurement of organosomatic indexes and detection of plasma VTG levels, are suggested. In a second step more mechanistic tools can be applied such as quantification of transcription levels of genes associated with reproduction and development in fish tissues, accompanied by bile metabolite analysis, with the aim of establishing the causes of detected alterations.

Acknowledgements

Work funded by Basque Water Agency (URA 10/02, URA 11/03, URA 12/04); Basque Government throught grant to consolidated research groups (GIC07/26-IT-393-07) and UPV/EHU (UFI 11/37). C.B. is recipient of a pre-doctoral fellowship from the UPV/EHU.

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Comunidades bentónico-demersales en caladeros de los volcanes de fango del golfo de Cádiz: Caracterización y actividad pesquera

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El Golfo de Cádiz representa una zona de convergencia de placas litósfericas, donde se han localizado unos cincuenta volcanes de fango, que representan estructuras submarinas formadas por las emisiones de fluidos ricos en hidrocarburos procedentes de la desgasificación de los sedimentos del subsuelo marino (Díaz del Río ., 2006). Estas estructuras, junto a las depresiones submarinas, las dorsales diapíricas, los canales y sedimentos contorníticos y los montículos carbonatados aumentan la variabilidad geomorfológica y ambiental del fondo marino del Golfo de Cádiz y, por tanto, pueden tener cierta implicación en el incremento de la biodiversidad en la zona (Judd y Hovland, 2007, Mata ., 2009). En general, se tiene poco conocimiento sobre las variables implicadas en los patrones de distribución de hábitats y comunidades bentónico-demersales asociadas a los caladeros situados sobre los volcanes de fango del golfo de Cádiz, pero la profundidad, el grado de actividad de los volcanes, el esfuerzo pesquero y los tipos de sustrato (fango, chimeneas y enlosados, restos de corales, etc.) son factores importantes a tener en cuenta, al igual que se ha observado en otras zonas del mundo.

La actividad humana sobre el medio marino está provocando cambios, con efectos negativos, en hábitats y comunidades asociadas, representando una de las principales amenazas para la biodiversidad marina. En este sentido, hay que centrar la atención en los ecosistemas marinos vulnerables (VMEs) (FAO, 2009) y en los daños provocados por la actividad pesquera de arrastre de fondo que es particularmente importante en zonas de arrecifes de corales de aguas frías o en praderas de fanerógamas marinas, entre otros muchos tipos de hábitats. Debido a la situación geográfica del Golfo de Cádiz, donde confluyen especies del Mar Mediterráneo, del Océano Atlántico Norte, de la costa africana, de especies anfiatlánticas o de especies endémicas (ejemplos para moluscos en Gofas ., 2011; Rueda ., 2011), el estudio tanto de las comunidades como de sus hábitats, es importante a la hora de caracterizarlas y poder evaluar el posible impacto de la acción pesquera sobre éstas. En el caso del golfo de Cádiz, existe una importante actividad pesquera de arrastre de cigala (*Nephrops norvegicus*), merluza (*Merluccius merluccius*) y de gamba blanca (*Parapenaeus longirostris*), entre otras especies (Sobrino ., 1994; Ramos ., 1996), y sería interesante y novedoso realizar un seguimiento de esta actividad pesquera mediante datos de posicionamiento VMS ("*vessel monitoring system*") de los barcos que faenan en la zona para así poder evaluar su influencia en los patrones de distribución de las diferentes especies, comerciales o formadoras de hábitats.

Este estudio es parte del proyecto LIFE + INDEMARES/ CHICA, cuyo objetivo es estudiar y caracterizar los hábitats y sus comunidades bentónico-demersales en diferentes áreas del Golfo de Cádiz, incluyendo los volcanes de fango y sus fondos adyacentes. Estos volcanes de fango se encuentran a diferentes profundidades, incluyendo algunos someros (300-400 m) (Albolote y Gazul) y otros situados a profundidades medias (500-600 m) (zona del Triángulo y Chica). Tanto la zona del volcán Gazul (más cercano a la costa) como la del Triángulo (volcanes Anastasya, Tarsis y Pipoca) representan importantes caladeros de especies comerciales (cigala, gamba blanca, merluza, etc.), y por tanto están sometidas a una fuerte presión pesquera por parte de las flotas de arrastre (Ramos ., 1996). En este sentido es de alta importancia localizar y caracterizar las comunidades bentónico-demersales para una mejor conservación y protección de aquellas zonas más sensibles a los impactos antropogénicos dentro del Golfo de Cádiz y contribuyendo a una mejor gestión sostenible de sus recursos, en línea con la Directiva Marco de la Estrategia Marina.

El área de estudio se encuentra en aguas españolas del Golfo de Cádiz, entre los 350 y los 700 m, en el talud superior y medio, próximo a la dorsal diapírica del Guadalquivir, (Somoza ., 2003) donde se localizan diferentes volcanes de fango (Albolote, Gazul, Anastasya, Tarsis, Pipoca, Chica) que, junto con los fondos contorníticos y las características hidrográficas del área, promueven una alta variabilidad ambiental (Fernández-Salas ., 2012).

En la campaña oceanográfica INDEMARES CHICA-0211, realizada a bordo del B/O Cornide de Saavedra (Instituto Español de Oceanografía), se recolectaron muestras faunísticas mediante el arte de arrastre de tipo "Baca 40/60", el cual es ampliamente utilizado en las campañas españolas del Grupo de Trabajo Internacional *Botton Trawl Survey* (IBTS WG) del Consejo Internacional para la Exploración del Mar (ICES/CIEM). Se realizaron un total de 12 lances con una duración de 60 minutos por lance. La velocidad de arrastre fue de 3 nudos con una longitud media recorrida de 5.260 m, siendo su abertura horizontal entre 17 y 18 m. Los datos de abundancia y de biomasa de cada lance fueron estandarizados a un área de 1 Km^2 , para así realizar comparaciones entre las distintas zonas muestreadas. En todos los lances, las especies capturadas fueron separadas e identificadas hasta el nivel taxonómico más bajo, anotándose su abundancia y biomasa. La caracterización de las comunidades bentónico-demersales se llevó

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a cabo de acuerdo con: i) Riqueza específica (S), ii) Abundancia (N), iii) Índice de dominancia (D), iv) Índice de equitatividad (J) y v) Índice de Shannon-Wiener (H´). El estudio de similitud entre las comunidades faunísticas se realizó usando datos cuantitativos (raíz cuarta de las matrices de abundancia y biomasa) y el índice de Bray-Curtis, así como técnicas de ordenación y clasificación. Las comparaciones entre grupos de muestras se realizaron con el ANOSIM estableciéndose factores como zona y la profundidad de los lances. Estos análisis se realizaron mediante el software PRIMER v6.

Las interacciones entre las actividades pesqueras y las comunidades bentónico-demersales se han podido realizar usando los datos de VMS ("*vessel monitoring system*"), facilitados por la Secretaría General del Mar. El Sistema de Vigilancia de Buques (VMS) se utiliza en la pesca comercial y toma datos de identidad del buque, fecha (día, mes y año), hora (hora y minutos UTC en la que se haya determinado la posición del buque), latitud, longitud, rumbo, velocidad actual y si está realizando actividad pesquera o no. Este sistema registró, en el período entre 2000 a 2011, un total de 154 embarcaciones en el área de estudio de las cuales un 83% del total realizaron pescas con el arte de arrastre de fondo, la cual ha sido la técnica de pesca elegida para este estudio. Para identificar los registros de VMS que se correspondían a arrastres en la zona se tuvo en cuenta la velocidad del barco (hasta 3 nudos y medio) y su posición geográfica aprovechándose, de este modo, un 70% de los 251.841 registros analizados.

En las muestras recolectadas con el arte de arrastre de tipo baca se han capturado un total de 148 especies, siendo los peces el grupo más diverso (54 spp.), seguido de crustáceos (44 spp.), moluscos y equinodermos (15 spp.), cnidarios (11 spp.) y poríferos (9 spp.). En relación con la abundancia, el 41,63% de las especies son crustáceos, seguido de peces (35,86%), equinodermos (20,33%), moluscos (1,32%) y, finalmente, los cnidarios y poríferos (menor del 1%).

La abundancia y riqueza específica fué alta en la zona de los volcanes Gazul y Chica y bajos en Anastasya (Fig. 1). La biomasa mostró una tendencia similar con valores altos en zonas con escasa actividad de pesca de arrastre, como son los alrededores de los volcanes Gazul, Chica y Tarsis/Pipoca, mientras que las de baja biomasa se localizan en zonas adyacentes al volcán Anastasya o la zona externa al campo de volcanes, donde se da a su vez una alta actividad de pesca de arrastre (Fig. 2). La diversidad de Shannon-Wiener (H') y la equirrepartición mostraron patrones muy similares siendo altos en todas las zonas estudiadas, excepto en la zona de Chica.

Las especies comerciales dominantes son la bacaladilla (*Micromesistius poutassou*) con alta abundancia en Gazul, la merluza (*M. merluccius*) con alta abundancia en la zona externa al campo de volcanes, Anastasya, Gazul y Chica, la cigala (*N. norvegicus*) con alta abundancia en Gazul, Anastasya y Chica, y la gamba (*P. longirostris*) con alta abundancia en Gazul, Anastasya, dentro del triangulo y su zona externa. Otros crustáceos de importancia pesquera (*Aristaeomorpha foliacea, Aristeus antennatus*) mostraron sus mayores abundancias en la zona dentro del triángulo, Tarsis/Pipoca y Chica.

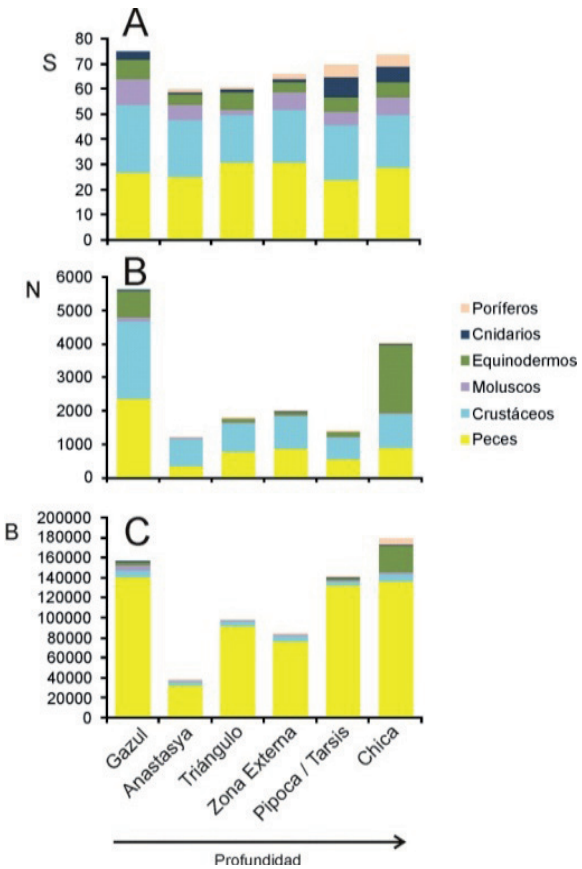

Figura 1. A) Número de especies; B) abundancia (individuos); C) Biomasa (gramos) total en relación a diferentes grupos faunísticos y zonas

Los análisis multivariantes (Cluster, MDS) mostraron una ordenación de las muestras en relación a la profundidad y también a las diferentes zonas, con 3 agrupaciones de muestras que presentaban diferencias significativas en relación a la profundidad y las diferentes zonas de estudio (ANOSIM, $p<0,001$). Estas agrupaciones se corresponden con muestras de Gazul (zona de menor profundidad), lances de Anastasya, Triángulo y Zona Exterior (estrato entre 500-600 m.) y lances de la zona de Chica y Pipoca/Tarsis (estrato > 600 m.), presentando estos últimos una mayor variabilidad (Figura 2).

En las muestras estudiadas se han hallado un total de 148 especies bentónico-demersales, un número similar al hallado a profundidades y esfuerzos de muestreo similares en el Mar de Alborán (Abad ., 2007) o la zona del Cachucho (Le Danois Bank) (Sánchez ., 2008), usando el mismo método de muestreo. Uno de los factores influyentes en la composición faunística es la ubicación geográfica del Golfo de Cádiz, lo cual se traduce en la presencia de especies con una distribución cosmopolita (*Chaulodius sloani*, *Argyropelecus hemigymnus*, *Funiculina quadrangularis*, *Parapenaeus longirostris, Aristaeomorpha foliacea, Aristeus antennatus*), otras cuya distribución principal se sitúa en el Atlántico Norte y en el Mar Mediterráneo (*Micromesistius*

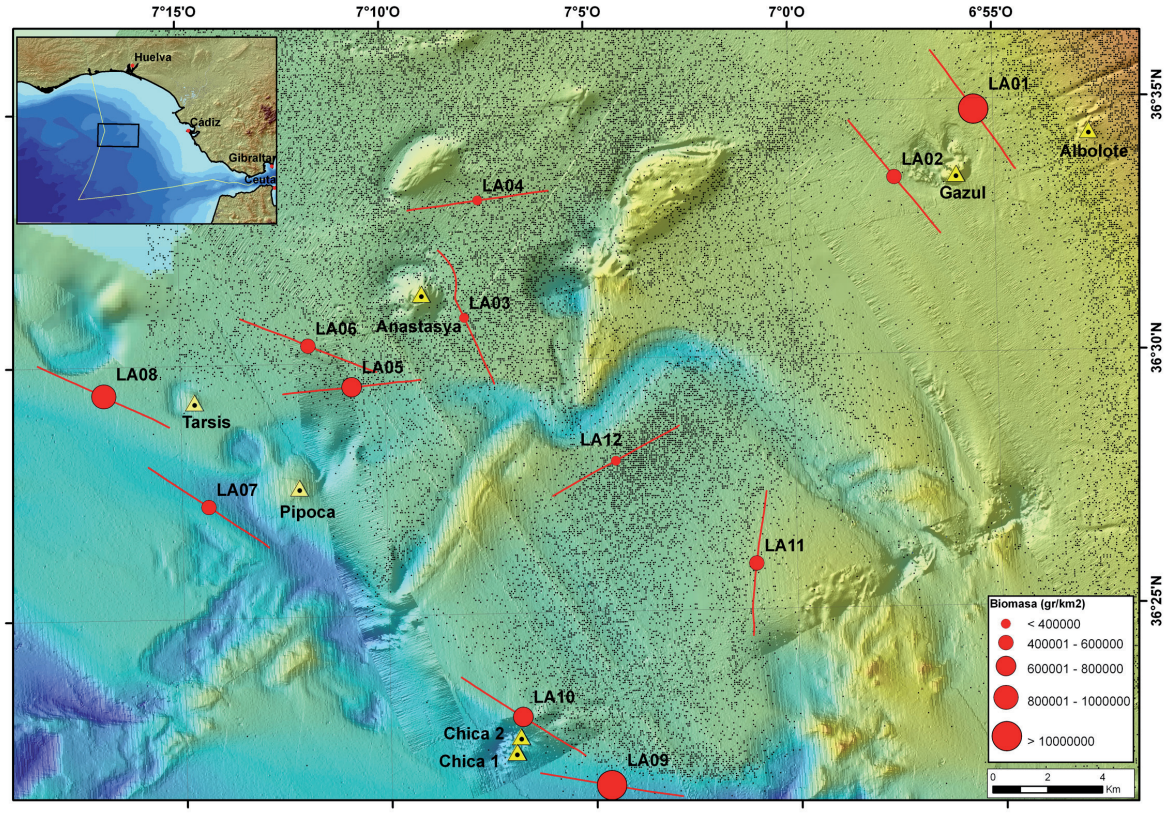

Figura 2. Localización de muestreos lineales realizados con arte de arrastre de tipo "Baca 40/60", biomasa capturada (círculos rojos) y localizaciones VMS de la flota de arrastre entre 2000 y 2011 (puntos pequeños grisáceos).

poutassou, *Merluccius merluccius*, *Galeus melastomus*, *Phycis blennoides*, *Nephrops norvegicus*), especies endémicas del Golfo de Cádiz y del Mar de Alborán como *Galeus atlanticus* o el bivalvo *Limopsis angusta*, especies propias del Mar Mediterráneo (*Plesionika gigliolii*) o especies tropicales anfiatlánticas como el asteroideo *Hacelia superba* (Rueda ., 2011). Algunas de estas especies son importantes desde el punto de vista comercial, por lo que el estudio, tanto de estas comunidades como de su hábitat, es importante a la hora de evaluar el posible impacto de la acción pesquera y así poder establecer medidas de gestión.

La biomasa y riqueza específica fué alta en la zona del volcán Gazul (300-400 m profundidad), la cual está cercana al borde de la plataforma, presentando especies dominantes de peces bentopelágicos y organismos filtradores que son indicadores de masas de aguas con una alta productividad (ej. *M. poutassou, Leptometra* spp.) (Colloca ., 2004; Abad ., 2007). Ello podría ser el resultado de una menor influencia de la corriente cálida y salina del agua mediterránea profunda en esta zona del talud superior, a diferencia de otras zonas algo más profundas (ej. El Laberinto) (Fernández-Salas ., 2012).

En la Zona Externa y la zona del Triángulo, la composición faunística y las especies dominantes (*Chimaera monstrosa*, *Galeus melastomus*, *Etmopterus spinax, Nezumia aequalis*) son muy parecidas entre sí y características de este rango de profundidad (zona de transición de 500 a 600 m) (Abad ., 2007; Sánchez ., 2008). Este estrato se favorece por presentar características de transición entre los distintos estratos (< 500 m. y > 600 m.) (Carney, 2005), y la menor variabilidad espacial de la fauna podría deberse a una mayor similitud sedimentológica con fondos de tipo fangoso, la influencia del agua Mediterránea profunda o a una mayor actividad de la flota arrastrera en esta zona, en la cual abundan especies comerciales como *M. merluccius, Lophius budegassa, N. norvergicus* o la gamba *P. longirostris*. De hecho, la presión pesquera representa un factor de gran importancia en la distribución espacial de las comunidades bentónico-demersales (Koslow , 2001), siendo alta en la zona del Triángulo y Anastasya. Ello seguramente tiene repercusiones negativas en los hábitats presentes en algunas de estas zonas (ej. Anastasya), como son las comunidades de pennatuláceos y megafauna excavadora, o la comunidad de especies quimiosintéticas ligadas a emisiones de gases y que son endémicas del Golfo de Cádiz.

En la zona de los volcanes Pipoca/Tarsis y Chica (> 600 m. de profundidad), aparecen especies propias de la zona de transición, pero también poblaciones de elasmobranquios de gran tamaño (ej. *Centrophorus granulosus* o *Dalatias licha*), que son típicos de estratos más profundos y que muestran una baja abundancia, baja tasa de natalidad y una edad reproductiva tardía, lo cual les hace ser particularmente vulnerables a la sobreexplotación pesquera. Estas poblaciones seguramente se ven favorecidas en este estrato de profundidad por la presencia de canales contorníticos excavados

por la acción de la salida de agua profunda del Mediterráneo (Fernández-Salas ., 2012), que posiblemente presentan una mayor disponibilidad de alimento para especies de gran tamaño y por una menor actividad pesquera de arrastre y, por tanto, de baja destrucción de hábitats y de especies de lento crecimiento.

Agradecimientos

Este estudio está cofinanciado por la Unión Europea y se encuadra en el subproyecto "Chimeneas de Cádiz", dentro del proyecto LIFE + INDEMARES "*Inventario y designación de la Red Natura 2000 en áreas marinas del Estado español*" coordinado por la Fundación Biodiversidad. Queremos agradecer la ayuda prestada por todos los componentes del equipo científico que participó en la campaña INDEMARES/CHICA 0211 así como a la tripulación y al capitán del B/O Cornide de Saavedra, en el cual se ha empleado equipamiento del programa FEDER FICTS-2010-01. También agradecer a la Secretaría General del Mar por facilitar datos de la actividad pesquera (cajas azules) en la zona de estudio.

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Anfípodos asociados al fouling de las instalaciones de acuicultura en el Mediterráneo Occidental

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Los avances en la construcción e instalación de jaulas flotantes para la acuicultura intensiva, han permitido la expansión de este sector en zonas costeras, llegando a ser el principal método de producción en el Mediterráneo. La instalación de estas estructuras, en zonas relativamente profundas y expuestas, durante un largo periodo de tiempo, ha supuesto la creación de una gran cantidad de hábitat artificial disponible en pleno mar abierto.

Diferentes especies son capaces de colonizar estas estructuras de forma que, redes, cabos y boyas son rápidamente cubiertos por comunidades de fouling, dominadas principalmente por bivalvos, hidrozoos y algas. Los anfípodos, tanto gammáridos como caprélidos, tienen la capacidad de colonizar estas instalaciones, alcanzando grandes densidades de población, superiores en muchas ocasiones, a las encontradas en otras comunidades naturales cercanas.

Los objetivos de este estudio fueron: en primer lugar, identificar la composición de especies del poblamiento de anfípodos asociados a las comunidades de fouling en jaulas de acuicultura; en segundo lugar, determinar la consistencia espacial de este poblamiento a una escala de 100´s de kilómetros, y por último, profundizar en el conocimiento de las relaciones ecológicas y tróficas que estos establecen con el medio.

El poblamiento de anfípodos fue muestreado cualitativamente mediante raspados, prestando especial atención a los principales hábitats presentes en la instalación: comunidades de mejillón, algas e hidrozoos. Las muestras fueron tomadas de siete instalaciones dedicadas al engorde de dorada (*Sparus aurata*) y lubina (*Dicentrarchus labrax*), localizadas a lo largo de la costa mediterránea española, desde Andalucía hasta Cataluña.

Los resultados obtenidos reflejan una composición de especies similar en todas las localidades. Las especies más abundantes fueron: *Elasmopus rapax*, *Jassa marmorata*, *Ericthonius brasiliensis*, *Caprella equilibra* y varias especies del género *Stenothoe*. Por otro lado, otras especies sólo aparecieron puntualmente en ciertas instalaciones, como *Caprella grandimana* en Salobreña o *Caprella dilatata* en Alicante.

Aunque la composición de especies asociada al fouling fue similar entre las diferentes instalaciones, dentro de cada una de ellas se apreciaron diferencias en función del hábitat muestreado. Así, en el caso de Alicante, la especie más importante en la comunidad formada por mejillón fue *E. rapax*, mientras que *Stenothoe tergestina* lo fue en la dominada por algas. Por otro lado, los caprélidos *C. equilibra* y *C. dilatata* se vieron beneficiados por la presencia de hidrozoos.

Las especies presentes en las jaulas de acuicultura se caracterizan por ser especies cosmopolitas y resistentes a contaminantes, que también suelen encontrarse en zonas como puertos y plataformas petrolíferas. Sin embargo, las densidades de población observadas para algunas de estas especies en las jaulas de acuicultura son muy superiores a aquellas que se desarrollan en otras comunidades de fouling.

En general, la composición del poblamiento de anfípodos asociados al fouling de las instalaciones de acuicultura fue consistente a lo largo de la costa mediterránea española. Dentro de cada instalación, las especies dominantes cambiaron dependiendo del hábitat estudiado, sugiriendo una relación de éstos con sus requerimientos ambientales.

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Evaluación del efecto del buceo deportivo sobre el briozoo *Myriapora truncata* (Pallas, 1766) mediante análisis de imagen

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El buceo deportivo es una actividad recreativa en expansión constante en el Mediterráneo, pero los efectos de esta actividad en sus ecosistemas marinos han sido relativamente poco estudiados. El briozoo *Myriapora truncata* (Pallas, 1766) es una especie con un frágil esqueleto calcáreo, fácilmente identificable y muy abundante en el Mediterráneo español, características que la convierten en una potencial especie indicadora de este tipo de impacto. En este trabajo se evaluó el impacto del buceo en áreas protegidas y en áreas son protección, sobre las poblaciones de *Myriapora truncata*. Se planteó un diseño de muestreo balanceado, con cinco factores de variación con dos niveles cada uno, tres fijos (Tiempo: antes y después del verano, época principal de buceo; Protección: áreas protegidas y no protegidas; e Impacto: con buceo y sin buceo) y dos aleatorios (Localidad y Sitio). Dado que existe una elevada heterogeneidad espacial y una amplia distribución en el recubrimiento de briozoos, un estudio detallado de la distribución de estos organismos y la detección estadística de diferencias entre los tratamientos resulta complicada si el nivel de réplicas es muy bajo, o la muestra tiene una superficie muy pequeña. Por lo tanto, se procedió a aplicar una metodología de muestreo mediante fotografía submarina para aumentar la superficie estudiada y permitiendo así el aumento en el número de réplicas. Se fotografiaron todas las colonias de briozoos presentes en cada réplica, y posteriormente se gestionaron mediante el programa de tratamiento de imagen Image-J. Las variables medidas y analizadas fueron el número de colonias, el área media de las colonias, el área máxima, el área total, el perímetro medio y total de las colonias, así como su nivel de agregación y de exposición. Se realizaron ANOVAs multifactoriales y las técnicas de análisis multivariantes MDS y PERMANOVA para detectar y evaluar los posibles efectos de los factores establecidos. Se observó que en las zonas donde se bucea, el número de colonias de briozoos fue menor que en aquellas donde no se practica esta actividad, aunque dicha tendencia no fue significativa. El área media de las colonias sólo mostró diferencias significativas entre las áreas protegidas y no protegidas, siendo mayor en estas últimas, mientras que el área máxima y la total tendieron a ser mayores en las zonas donde no hay impacto del buceo. Se observó una tendencia similar para el perímetro total, mientras que no estuvo muy clara para el perímetro medio, que tuvo un comportamiento variable. Respecto al nivel de exposición se observó que, después del verano, las colonias se encontraban en sitios menos expuestos en los lugares donde se bucea con intensidad. El nivel de agregación no mostró diferencias en ningún caso. PERMANOVA mostró, para el efecto conjunto de las variables, que existen diferencias significativas entre los lugares con diferente intensidad de buceo. Estos resultados muestran que el buceo con equipo autónomo afecta en alguna medida a las poblaciones de esta especie, por lo que futuros estudios podrían considerarla como una posible especie indicadora del impacto de esta actividad deportiva.

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Application of passive samplers and biomonitors to assess the contamination due to organic compounds in the Oiartzun estuary waters (South-eastern Bay of Biscay)

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In the last decades more than 250.000 man-made new chemical compounds have been found in the marine environment. Special interest in organic compounds is growing due to their ecological effects in marine and riverine biota. Most of these compounds are classified as Persistent Organic Pollutants (POPs) by OSPAR convention, UNEP and Stockholm Convention. Environmental quality and its management programs requirements are established by the Water Framework Directive (WFD 2000/60/EC), and allowed concentrations of these organic compounds have been fixed for the following years. Measuring organic compounds in water is not a simple task. Their concentrations in water are very variable and usually at trace levels. For this reason, spot water sampling is not appropriate, as huge amount of water should be sampled and analysed, carrying expensive and long processes. In last years, passive sampling techniques have appeared as an alternative to solve these problems and to give response to the European legislation. These techniques allow measuring labile dissolved concentrations of pollutants in water, mimicking biota uptake. Passive samplers are simple, low cost, can be deployed for long time periods, large volume of water is sampled, time-weighted average concentrations are provided and they are less sensitive to disturbances and more reproducible than biomonitors.

The area of study is the Oiartzun estuary, in the South-eastern Bay of Biscay. This estuary is an altered area surrounded by human and industrial settlements (agriculture, farms, shipyards, metallurgy, paper mill, textile...). Due to these activities this estuary has suffered high contamination for many decades, causing the deterioration of its waters and consequently the impoverishment of the biological communities. This study shows the application of the Silicone Rubber passive sampler in the Oiartzun estuary for measuring Poly Chlorinated Biphenyl Compounds (PCB) and Poly Aromatic Hydrocarbons (PAH) in comparison with the concentration of these compounds in the tissues of transplanted mussels.

The Silicone rubber sampler consists in small sheets (5.5x9.0cm) of special silicone (AlteSil ™ Altec Products Ltd (UK), 0.5 x 600 x 600mm, translucent, 60 Shore A) deployed in a stainless steel frame. The silicone rubber sheets are pre-treated in the laboratory to eliminate oligomer traces with a Soxhlet process during 96 hours, using acetylacetate as solvent. After eliminating the solvent and soaking the sheets with methanol [1], silicone rubber sheets were spiked under constant shaking in methanol with a selection of Performance Reference Compounds (PRC) (from AccuStandard, Inc.) in order to get an *in situ* calibration [2]. Two sampling sites with different contamination level were selected in the Oiartzun estuary to deploy the samplers. One was located in a semi-enclosed basin characterised as a highly polluted area (Herrera Kaia) and another one was located in a cleaner area in a mooring for small boats (Pasaia San Pedro). At each sampling point two silicone rubber samplers (used as replicates) and a mussel net (with 50 individuals) were deployed at 2 m depth during one month. Each sampler consisted in 6 silicone sheets. Mussels (*M. galloprovincialis*) were collected in a clean area and transplanted to the sampling sites after being depurated for 24 hours in the laboratory. Afterwards, the condition index (CI) was calculated in a subsample (10 individuals).. Blanks at each station consisted on controls not deployed but used to assess any potential contamination of the samplers during preparation, to determine PRC initial concentrations before exposure, and to calculate the limits of detection (LOD) and quantitation (LOQ). Mussels control sample was used as well to analyse the organic compounds at initial time (t=0). After exposure, each sampler was extracted, concentrated, cleaned-up and analysed by GC-MS. Mussels were measured for CI calculations and prepared for their analysis by GC-MS.

The results of the organic compounds uptake by silicone rubber and by biota will be compared.

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