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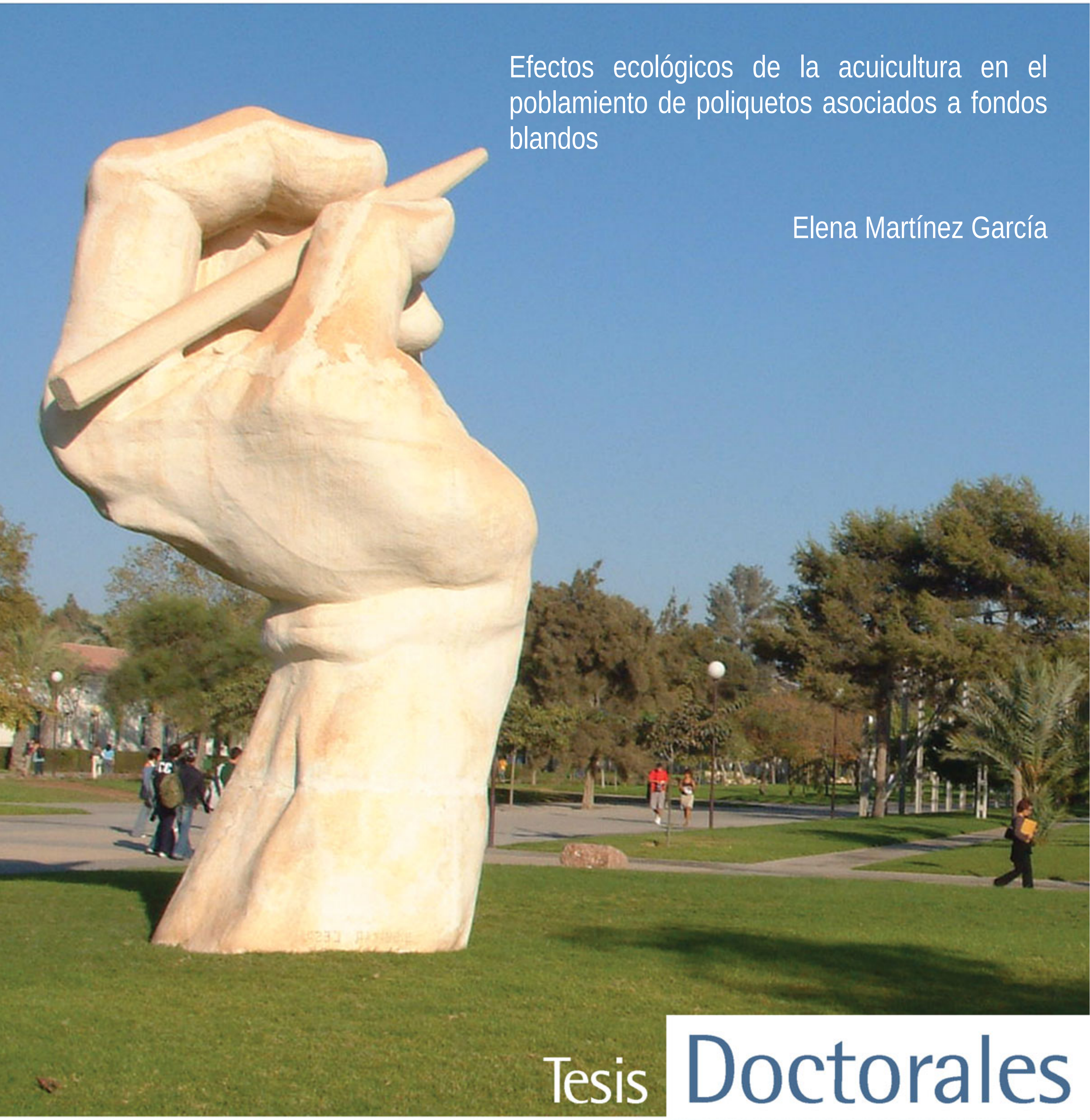

# Efectos ecológicos de la acuicultura en el poblamiento de poliquetos asociados a fondos blandos

Elena Martínez García

Tesis Doctorales

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# ECOLOGICAL EFFECTS OF AQUACULTURE ON POLYCHAETE ASSEMBLAGES ASSOCIATED TO SOFT SEDIMENTS

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# EFECTOS ECOLÓGICOS DE LA ACUICULTURA EN EL POBLAMIENTO DE POLIQUETOS ASOCIADOS A FONDOS BLANDOS


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**Tesis presentada para aspirar al grado de**
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CERTIFICAN:

Que la memoria de Tesis doctoral titulada "Ecological Effects of Aquaculture on Polychaete Assemblages associated to Soft Sediments" presentada por ELENA MARTÍNEZ GARCÍA, ha sido realizada bajo su dirección en el Departamento de Ciencias del Mar y Biología Aplicada. Y para que conste a los efectos oportunos, firman en Alicante, a 7 de Octubre del año dos mil dieciséis.

Fdo: Pablo Sánchez Jerez

Fdo: José Luis Sánchez Lizaso,

A mi tía Paqui

que tanto amor nos dio.

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# General Introduction

# Chapter 1

Photo: Leslie Harris and Elena Martínez

# 1. General Introduction

## 1.1. Ecology of soft sediments

The majority of the Earth's surface is covered by oceans (70.8%), most of which are underlain by soft sediments such as muds, sands and gravels (Snelgrove 1999). Soft sediments are defined as a weakly-structured habitat, and are characterised by the mobility and the instability of their particles (Peterson and Peterson 1979). Subtidal sediments are influenced by many of the same physical and chemical forces as intertidal areas, particularly currents, which erode and relocate sediments, determining their depth and particle-size composition, and physicochemical processes, which control the rate of diffusion of oxygen and nutrients into the sediments (Morrisey *et al.* 1998). Hereafter all subsequent references to soft sediments will relate only to subtidal environments. Waves are important in distributing and affecting sediments down to depths of 100 m, but the effect decreases exponentially with depth and so the dominant subtidal influences on sediment transport are currents (Cater 2002). There are, of course, many exceptions to this pattern, such as areas around the entrances to estuaries and bays, where tidal currents are concentrated, and near wave-exposed shores (Malvarez *et al.* 2001). Sediments range from coarse gravels, in areas subjected to major wave and current action, to muds, typical of low-energy areas, and fine silts and clays in deep-sea sediments (Gray and Elliot 2009). Moreover, sands and muds may contain gravel derived from older underlying deposits and from the shells of molluscs living within the sediment or brought into the area by currents. This environment is usually free from macrovegetation, but in some areas macrophytes (e.g. seagrass or macroalgae) may have an important role.

These sediments, as well as other ecosystems in coastal areas, are susceptible to impacts from human activities at local or global scale (Nogales *et al.* 2011). The overexploitation of natural resources, some commercial / industrial activities such as aquaculture or desalination, the introduction of exotic species and, sewage and other discharges (which can contain high levels of organic and inorganic compounds), are some of the factors that may alter the natural ecological processes in soft sediments. Some activities (e.g. aquaculture) are recommended to be performed over soft sediments because of their higher resilience and resistance to perturbation, but these impacts may have a negative effect on the environment and interfere in the proper function of the ecosystems if their carrying capacity is surpassed (Rapport *et al.*

1985). In ecology, resilience refers to the capacity of an ecosystem to recover from environmental stresses, i.e. to absorb both natural and human pressures (Odum 1994). Carpenter *et al.* (2001) defined three characteristics of resilience: 1) the ability of the system to resist a disturbance so that it is not overwhelmed, but instead retains its functions, 2) the capability of the system for self-organisation, and 3) the ability to learn from and incorporate disturbances as mechanisms for adaptive capacity. Moreover, resilience and resistance are influenced by the role of key species that maintain feedback interactions between biological and either chemical or physical processes (Townsend and Trush 2010).

Regarding the utility of this ecosystem, soft sediments are well known for providing ecosystem goods and services (Erlich and Mooney 1983, Gray *et al.* 1999). Ecosystem "goods" are the tangible resources that can be extracted and utilised by humans, such as food or raw materials. Ecosystem "services" are the abilities of ecological system to provide favourable conditions for humans by processing material or providing intrinsic benefits (Townsend and Thrush 2010). Ecosystem services are for example: I) **trophic links**; for which microphytobenthic algae living in the sediment make an important input to primary production along with pelagic phytoplankton (Troell *et al.* 2005). Filter-feeders and deposit-feeders use this phytoplankton and integrate it into the trophic chain, thus transferring energy to other benthic and epibenthic organisms, and subsequently to fish (Cloern 1982, Officer *et al.* 1982, Loo and Rosenberg 1989); II) **nutrient recycling** and sediment oxygenation processes are closely associated with services of detoxification and waste disposal, given that nutrients are regenerated and contaminants degraded in sediments. These processes are directly regulated by microbial organisms, and by bioturbating organisms, which strongly influence oxygenation and the physical movements of contaminants (Henriksen *et al.* 1983, Pelegri and Blackburn 1995, Gray *et al.* 1999, Weslawski *et al.* 2004); III) **habitat structure**; marine habitats provide living space for species and so are a prerequisite for the provision of all other goods and services (Townsend and Trush 2010); IV) **provisioning of food**, with a great influence in society due to its commercial and cultural importance, particularly for fish and shellfish, but also for algae in parts of Asia. The aquaculture sector is also involved in this category as it has a requirement for fishmeal and/or a natural supply of food by phytoplankton (Weslawski *et al.* 2004) and V) **recreational activities** such as water sports and fishing (Troell *et al.* 2005). Additionally, soft sediments goods and services are related to adjacent ecosystems, particularly the 'true water column' and the coastal zone. The maintenance of these goods and services will depend on the resistance capacity and the resilience of soft sediments, which are related to the intrinsic features of each type of sediment such as grain size and associated fauna.

Concerning these biological processes, the benthic fauna of soft sediments plays a key role in the maintenance of these goods and services. Benthic fauna communities are frequently characterised by their species richness, abundance and biomass (Rosenberg 2001), which depend on the sediment type and the sedimentary process (Bellan 1985). There are different ways to classify the benthic fauna: whether the organisms are mobile, sedentary or sessile; their position in relation to the sediment, hyperbenthic animals (which can move around the water column for short periods of time), epibenthic (which live over the sediment) and infauna (which live in the sediment). The dominant taxa in soft sediments are (in order of abundance): polychaetes, crustaceans, echinoderms and molluscs. These organisms are subject to a variety of physical and biological disturbances which vary in frequency and intensity, as well as in temporal and spatial extents. The structure of these communities (i.e. their species composition and relative species abundance) and their stability may vary markedly in response to anthropogenic disturbances (Turner *et al.* 1995).

### 1.2. Aquaculture development

Aquaculture is one of the human activities that may affect marine ecosystems. The FAO (1990-2016) provide a broad definition as the farming of aquatic organisms: fish, molluscs, crustaceans, aquatic plants, crocodiles, alligators, turtles, and amphibians. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. Farming also implies individual or corporate ownership of the stock being cultivated. For statistical purposes, aquatic organisms which are harvested by an individual or corporate body which has owned them throughout their rearing period contribute to aquaculture, while aquatic organisms which are exploitable by the public as a common property resource, with or without appropriate licences, are the harvest of capture fisheries. Mariculture is an additional subset of aquaculture in which the cultivation of the end product takes place in seawater, such as fjords, inshore and open waters and inland seas in which the salinity generally exceeds 20‰. Earlier stages in the life cycle of these aquatic organisms may be spent in brackish water or freshwater.

The earliest records of aquaculture date back to around 2000-1000 B.C. in China. The aquaculturist S.Y. Lin was cited by Hickling (1962) as the first person to use the common carp (*Cyprinus carpio*, Linnaeus 1758) for husbandry reasons. However, there is no written record of this, as it was an unbroken tradition in the population. In Europe it is hard to determine, but there is evidence that during the Roman Empire there were small constructions for fish culturing. The larger development of European aquaculture commenced in the late 19$^{th}$ century, achieving its maximum expansion with increases in biological knowledge and new technologies (FAO-HAKI 1999). Marine finfish in European aquaculture has become most intensive since 1988 (Read and Fernandes 2003). Modern fish farming in the Mediterranean started in 1980s with gilthead sea bream (*Sparus aurata,* Linnaeus, 1758) and European sea bass (*Dicentrarchus labrax*, Linnaeus, 1758).

At present, in the European Union, the main aquaculture products are fish and molluscs; aquaculture of crustaceans, algae and other invertebrates has generally been on a much smaller scale. In Spain, the species with highest productions are (in decreasing order): mussels (*Mytilus galloprovincialis,* Lamarck 1819), sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*) and rainbow trout (*Oncorhynchus mykiss,* Walbaum 1792). With the exception of the rainbow trout, the other three species are produced in marine waters (APROMAR 2016). Mussels are cultivated in rafts and long-lines, which are floating structures. For rafts the culture ropes hang from floating platforms, while for long-lines culture ropes hang at intervals along a rope back-bone that is supported by plastic floats (Figure 1.1). Sea bream and sea bass are cultivated in floating cages, which consist on very large rigid floating plastic rings that support underwater nets (Figure1.2). Floating cages are currently used in three different approaches: coastal farming (less than 500 m from the coast and less than 10 m from the sea bottom depth), off-coast (up to 3 km from the coast and between 10-50 m depth) and offshore (several km from shore and deeper than 50 m) (Holmer 2010).

Figure 1.1. Facility of mussel aquaculture in Galicia (Photo: Pablo Sánchez)

The FAO considers that aquaculture contributes to the effective use of natural resources, to food security and economic development, with a limited and controllable impact on the marine environment (FAO 2014). However, there are still several concerns about the environmental impact of aquaculture, which we will discuss in the following section.

Figure 1.2. Aquaculture facility of sea bream and sea bass in Guardamar (Photo: Pablo Sánchez)

### 1.3. Environmental impacts of aquaculture

Along with aquaculture development, fish farm facilities have been studied to understand their influences on various aspects of the marine environment:

**1.3.1.** Wastes from foods; there are four components of waste originated from food: I) Uneaten food arising from artificial feeding, generally due to bad husbandry, fish sickness or unsuitable environmental conditions (Figure 1.3). II) Undigested food, produced mainly by bivalves, which do not have sufficient control of intake and repletion. Thus, they ingest more than they can process and release intact micro algae in the form of faeces called pseudo-faeces. III) Indigestible compounds, due to complex molecules that are split into small molecules that cannot cross the intestinal border during digestion. Those that cannot be absorbed, due to their size or shape, are rejected as particulate matter (faeces). IV) Excreta produced by physiological phenomena, by which molecules that come into the body and become dissolved in plasma are released after being processed and degraded. These are soluble compounds that are discharged into the water through particular organs, such as gills (Dosdat 2009). These wastes produce a nutrient enrichment that may either affect the seabed (widely described in section 1.4) or the water column. Nutrient enrichment of the water column could cause eutrophication, resulting in excessive algal growth and subsequent effects on the wider environment such as reduced water clarity, physical smothering of biota, or extreme reductions in dissolved oxygen due to microbial decay of the algal biomass (Cloern 2001, Forrest *et al.* 2007). Significant depletion of dissolved oxygen in the water column due to finfish aquaculture overseas has usually only occurred when cages are heavily stocked or where they are located in shallow sites with weak flushing (La Rosa *et al.* 2002).

**1.3.2.** Chemical discharges; medicines, disinfectants and anti-fouling products are introduced into the marine system through aquaculture (Costello *et al.* 2001). Some of them may accumulate and persist in the marine environment, resulting in harmful consequences for the biota (Hansen and Lunestad 1992) or in bioaccumulation of these substances at higher trophic levels (Kiviranta *et al.* 2000).

**1.3.3.** Genetic issues; the major genetic risks of aquaculture include the loss of genetic diversity within populations, loss of genetic diversity among populations and loss of fitness (Waples *et al.* 2012, Segovia-Viadero *et al.* 2016); but, also introduced species that may become invasive in the new environment (Minchin *et al.* 2009).

**1.3.4.** Fouling; surfaces immersed in the marine environment become colonised by marine organisms, the most common are bivalves (particularly mussels), algae, hydroids and ascidians (Sarà *et al.* 2007, Fitridge *et al.* 2012), and also epifaunal organisms such as amphipods (Fernandez-Gonzalez and Sanchez-Jerez 2014). Fouling reduces the efficiency of material and equipment, so the use of antifouling products is quite common. Fouling can compete for resources with cultured organisms and can include predators and diseases (Willemsen 2005).

**1.3.5.** Escapees; an escape event is defined as the process by which one or several fish lose their confinement and reach the unenclosed marine environment (Dempster *et al.* 2013). The definition is also extended to fertilised eggs due to spawning in fish cages (Jørstad *et al.* 2008). Escaped fish have different interactions at different trophic levels: when escapees are able to survive in the wild they can exploit natural resources, affecting predator-prey interactions and interspecific competition (Toledo-Guedes *et al.* 2014); genetic interactions as explained above; pathogenic, as escaped fish spread parasites and diseases (Arechavala-Lopez *et al.* 2013); and fisheries, as escapees are available for professional fisheries, resulting in an extra income for them (Izquierdo-Gomez and Sanchez-Jerez 2016).

**1.3.6.** Wild animals; marine fish are attracted by aquaculture cages and nets, and as a consequence fish farms may affect the presence, abundance, residence times and diets of fish in a given area (Sanchez-Jerez *et al.* 2011). Marine birds use aquaculture facilities as resting areas and a source of food, attacking the cultivated species. In order to avoid these attacks, farmers use nets and/or other devices which may cause bird mortality (Quick *et al.* 2004). Marine mammals such as dolphins and seals are also attracted by fish farm facilities, and can be trapped in the nets (Díaz-Lopez and Berna-Shirai 2007) or can bioaccumulate chemicals such as antibiotics or antioxidants.

### 1.4. Organic matter and sediment biogeochemistry

Of the environmental impacts discussed above, the wastes originating from uneaten food and faeces have received a lot of attention, because the accumulation of organic matter on the sea bottom is often an environmental concern regarding the expansion of aquaculture in coastal areas. This organic enrichment has a direct influence on the sediment, resulting in chemical, physical and biological changes (Pearson and Rosenberg 1978). Sediment metabolism during fish farming can be up to 10 times higher than usual in coastal areas (Holmer and Kristensen 1992). The organic matter that is not consumed by benthic macrofauna is degraded to inorganic compounds through bacterial processes using different paths with different acceptors as oxidants (Canfield *et al.* 1993). When there is oxygen availability, aerobic processes such as heterotrophic respiration and aerobic chemosynthesis are most common in the upper layer of the sediment (Holmer *et al.* 2005a). Aerobic bacteria are capable of mediating the entire diagenetic sequence leading to complete particulate organic carbon oxidation to $CO_2$ and $HO_2$ (Canfield 1994). However, marine sediments are reduced environments covered only by a thin oxic surface layer, from millimetres to centimetres depending on whether there are productive shallow waters or oceanic sediments in oligotrophic conditions (Reimers *et al.* 1986, Glud *et al.* 1994, Kristensen 2000). When this oxygen is consumed, mutualistic consortia of bacteria accomplish anaerobic decomposition, because no single type of anaerobic bacterium seems capable of complete mineralisation (Fenchel *et al.* 1998, Kristensen 2000). The energetic output of respiration with different electron acceptors generally decreases according to the sequence oxygen ($O_2$), nitrate ($NO_3^-$) manganese (MnIV), iron oxide (FeIII), sulphate ($SO_4^{2-}$) and $CO_2$. Sulphate reduction easily becomes the predominant respiration route (Christensen *et al.* 2000). Moreover, organic enrichment from finfish farm wastes stimulates sulphate-reducing bacteria more than other sources of organic matter (Holmer *et al.* 2005b). The final product of sulphate reduction is hydrogen sulphide ($H_2S$), which is highly toxic for most benthic fauna (Wang and Chapman 1999, Vaquer-Sunyer and Duarte 2010). $H_2S$ accumulation is counteracted by oxidation or precipitation-forming non-toxic sulphur with $O_2$, $NO_3$, Fe oxides and Mn oxides, which also use $O_2$ by spontaneous or microbially-mediated redox reactions (Thamdrup 2000, Schippers and Jørgensen 2002, Canfield *et al.* 2005). In methanogenesis, which occurs when there is no more sulphate to reduce, electrons are transferred from organic matter to $CO_2$, through a $H_2$ intermediary (Canfield *et al.* 2005).

The other process affected by organic enrichment is nitrogen cycling (Casado-Coy *et al.* 2016). This effect has usually been studied where agriculture is affecting the marine environment to a greater extent than aquaculture (Paerl and Piehler 2008). Low levels of organic enrichment provide substrate for heterotrophic denitrifiers, which oxidise organic carbon with $NO_3^-$ in the nitrification process (Laursen and Seitzinger 2002), as explained above. Nevertheless, at high levels of organic enrichment, when the competition for electrons acceptors rises and the environment become sulphidic, nitrogen mineralization pathways can shift form net nitrogen removal through denitrification or anammox to produce ammonium ($NH_4^+$) via dissimilatory nitrate reduction (Holmer and Kristensen 1992, Christensen *et al.* 2000).

Figure 1.3. Process of fish feeding in aquaculture through pellets (Photo: Pablo Sánchez)

### 1.5. Environmental management of aquaculture

The successful development of aquaculture in the coastal zone requires the establishment of a regulatory framework for sustainable aquaculture, considering important aspects such as site selection, environmental impact assessment and monitoring (Sanchez-Jerez *et al.* 2016). At present, the Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC) set the guidelines for all Member States to define the Environmental Quality Objectives and develop national legislation for water ecosystems (Figure 1.3). In Spain, the law of environmental evaluation (Law 21/2013, 9 of December) requires that aquaculture facilities with production of more than 500 tons per year must carry out an environmental impact assessment. However, beyond this threshold regional governments are responsible for setting the legal system and the criteria for the monitoring of environmental impacts. This leads to an inconsistent legal framework, and depending on the regional laws, environmental monitoring programmes can incorporate different measures and proceedings.

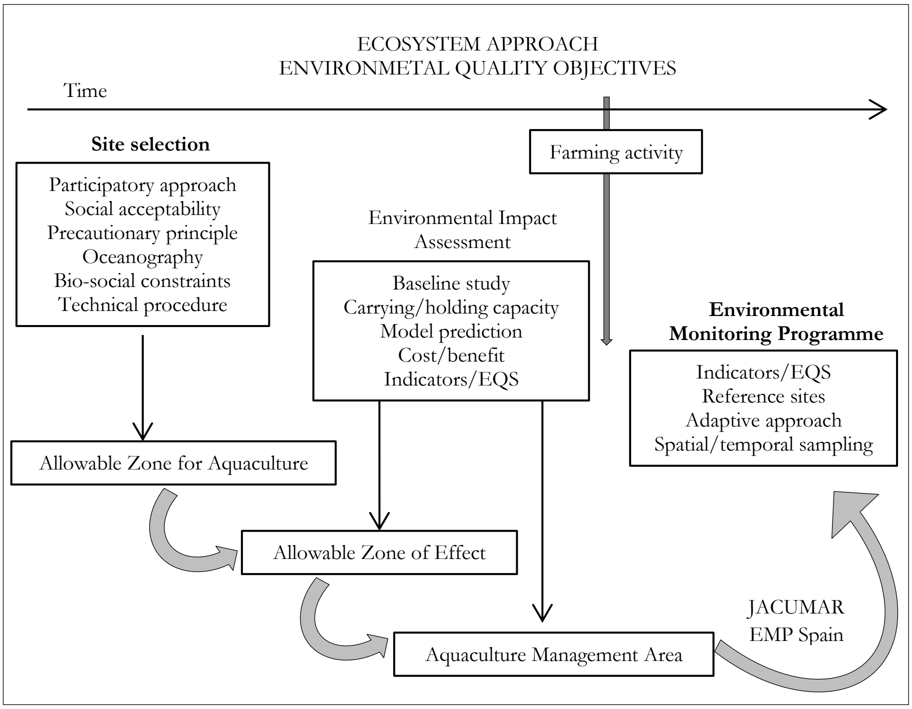


Figure 1.3. Technical procedures for the environmental management of aquaculture (modified from Sanchez-Jerez *et al.* 2016).

In order to deal with this issue, in 1999 the National Advisory Board of Marine Fish Farms (JACUMAR) developed some projects and protocols to standardise the criteria and methodologies for evaluating the possible effects of marine aquaculture. One decade later, an environmental monitoring plan (EMPs) for Spain was published (Aguado-Giménez *et al.* 2012a), which provides different environmental quality standards (EQS) for benthic and pelagic habitats (Figure 1.3). This standardisation was a significant advance in terms of environmental monitoring plans application, given that many different protocols and indicators had been developed since the WFD was published. As discussed above, different regional laws have caused high variation in EMP procedures, as a range of environmental variables can be used to assess the EQS. However, it was (and still is) necessary to find a proportionate balance between the information required and the effort made.

Some of the new variables to evaluate the environmental impact are biological indicators. A bioindicator is a species or group of species that readily reflects the abiotic or biotic state of an environment; it represents the impact of environmental changes on a habitat, community or ecosystem; and/or is indicative of the diversity of a subset of taxa or the whole diversity within an area (Gerhardt 2009). There are positive indicators, also referred to as pollution-tolerant or opportunistic species, whose abundance typically increases in response to environmental changes, and whose dominance often gives a low diversity of other macrofauna in the samples; and negative indicator species, also referred to as sensitive or intolerant species, whose abundance typically decreases (Rygg 1985, Warwick 1988). One of the first lists of pollution-tolerant species was produced by Pearson and Rosenberg (1978); this document was a review of all information about species used as bioindicators up to that date. They also described the stages of macrofauna succession in relation to organic enrichment. Since then, different authors have described indicator species and their habitat preferences or trophic strategies, and many macrofauna species have been catalogued as tolerant or sensitive (Davis 1987, Méndez *et al.* 1998, Cañete *et al.* 2000, Belan 2003, Harkantra and Rodrigues 2004, Giangrande *et al.* 2005, Pagliosa 2005, Tomassetti and Porello 2005, Lee *et al.* 2006, Dean 2008). The use of bioindicators has also evolved along time. On one hand, it is possible to look for these species throughout macrofauna assemblages and to give information related to their presence or absence. Another approach is a method to summarise this information using a number, called index, early biodiversity indices were based on the relative abundances of each species, such as the Shannon diversity index (Shannon and Weaver 1963) and Pielou's evenness index (Pielou 1966), while later indices were based on the number of different species, such as Simpson's index (Simpson 1949) or specific richness. These indices are widely used (Rosenberg *et al.* 2004). In the last decade, some biotic indices have appeared adding information about macrofauna sensitivity (tolerance or not to a pollutant) and summarising this as a number (Aguado-Giménez *et al.* 2015). This value is associated with a scale that ranges from very good or very healthy to severely disturbed, a fact that makes indices more understandable for managers (Chainho *et al.* 2007). The most common indices in Europe and Mediterranean areas are the AZTI Marine Biotic Index (AMBI-Borja *et al.* 2000), the Benthic Quality Index (BQI-Rosenberg *et al.* 2004), the Bentix index (Simboura and Zenetos 2002), BOPA (Dauvin and Ruellet 2007), M-AMBI (Muxica *et al.* 2007), BO2A (Dauvin and Ruellet 2009) and the Mediterranean Occidental index (MEDOCC- Pinedo *et al.* 2015). However, these indices are under constant revision, due to the differences among regions, between organisms, and for all types of anthropogenic disturbances; so, no biotic index is able to be applied worldwide (Dauvin *et al.* 2007, Keeley *et al.* 2012a). Another handicap of some of these indices is the high taxonomic resolution needed, which requires specialist taxonomic expertise as well as time-consuming procedures (de-la-Ossa-Carretero *et al.* 2012a). Reducing the entire soft bottom macrobenthic community to the study of polychaete assemblages could represent a pragmatic solution for the above concerns (Aguado-Giménez *et al.* 2015). Some authors have suggested taxonomic sufficiency, which consists on identifying organisms only to higher levels of the taxonomic scale, such as family level, arguing that there is not a significant loss of information at the scale required for environmental impact studies (Ferraro and Cole 1990, Bacci *et al.* 2009), particularly in relation to organic enrichment disturbance. Moreover, these higher identification levels, analysed by multivariate methods, give accurate information about the influence of fish farming, being more appropriate and statistically valid than univariate benthic indices (Aguado-Giménez *et al.* 2007, Callier *et al.* 2008, Quintino *et al.* 2012).

### 1.6. Polychaete assemblages

In the context of environmental impact monitoring, annelids, and more specifically polychaetes, have been widely used mainly in soft sediment bottoms (Crema *et al.* 1991, Elias 1992, Grall and Glémarec 1997, Olsgard and Somerfield 2000, Solis-Weiss *et al.* 2004, Giangrande *et al.* 2005, Domínguez-Castanedo 2007, Del-Pilar-Ruso *et al.* 2015) and specifically in fish farm monitoring (Tomassetti and Porrello 2005, Lee *et al.* 2006, Dean 2008, Aguado-Giménez *et al.* 2015). However, most studies of polychaete sensitivity have described the level of tolerance to perturbations at species level. Thus, there is still little information about general tendencies at the family level. The class Polychaeta contains 11 688 accepted species (up to 2016) distributed in a complex taxonomic classification with 87 accepted families (WORMS-www.marinespecies.org). An additional thousand species have been named and considered invalid (Rouse and Pleijel 2001). It should be noted that the great efforts of taxonomists in updating the Polychaeta database and, nowadays with technological development, have made it much easier for ecologists to make use of all this information.

There are many reasons why polychaetes are highly suitable for environmental impact monitoring. They are more or less ubiquitous and are usually the most abundant taxon in benthic communities, not only in numerical abundance but also in the number of different species (Beesley *et al.* 2000, Dean 2008). They occur in a wide range of habitats, from inland salt lakes and fresh waters to the ocean floor. These organisms show a large variety of feeding types and strategies and can be divided into omnivores, herbivores, carnivores, filter feeders, surface deposit feeders and burrowers (Gambi and Giangrande 1986, Pagliosa 2005). The duration of the entire life cycle of many species of polychaetes is often in the order of days or weeks, and reproductive rates are also very high, which allows a rapid response to any perturbation (Dean 2008).

Understanding the relationship between polychaetes and the sediment is very important for two reasons. Grain size is considered to be a super-parameter for benthic organisms, and particularly for polychaetes (Jansson 1967, Fresi *et al.* 1983, Gambi and Giangrande 1986). On the other hand, polychaetes play a major role in the breakdown, subduction and incorporation of organic matter into sediments and their aeration (Kristensen and Kostka 2005, Heilskov *et al.* 2006).

### 1.7. Justification and general aims

After the development of European aquaculture in marine ecosystems with the implementation of legislative and management frameworks, environmental impact monitoring has undergone substantial improvements during the last decade. However, it is still necessary to homogenise protocols and most importantly, to make them economically feasible for aquaculture operators and easier to interpret for managers. Polychaete assemblages seem to be an accurate tool for these purposes, but still require a deep understanding of the underlying ecological process by which aquaculture may have environmental impacts on polychaete assemblages. Specially, there is a general lack of information about how the sediment structure affects biogeochemistry under different organic enrichment conditions. Therefore, the general aim of this Doctoral Thesis was to improve our understanding of the interaction between different organic enrichment circumstances arising from aquaculture and polychaete community structure, in different sediment types, and ultimately to provide recommendations for monitoring programs that will help to achieve sustainable aquaculture.

In order to achieve the aforementioned general goals, a series of objectives were accomplished:

Chapter 2

· To assess the consistency of the proposed variables and design of the environmental monitoring plan proposed by the Spanish Ministry of Agriculture to correctly detect the environmental impacts of fish farming.

· To identify possible improvements for future environmental monitoring plans.

Chapter 3

· To detect which families of polychaetes are the best indicators of fish-farming impacts on soft benthic sediments along the Western Mediterranean Sea and nearby Atlantic Ocean.

· To assess which geochemical variables best explain the changes in polychaete assemblages at a scale of hundreds of kilometres.

Chapter 4

· To understand the effects of grain size on biogeochemical processes affected by aquaculture wastes by comparing two types of sediments: sand and mud.

· To comprehend the effects of bioturbation and bioirrigation related to polychaetes on these biogeochemical processes.

Chapter 5

· To observe the effects of grain size on polychaete recolonization under organic enrichment conditions using an experimental approach.

· To evaluate the detection of environmental impacts from fish farming using different indices and multivariate analyses using experimental units, and to define the most important species that reflect changes on assemblage structure.

· To test the effectiveness of experimental units as instruments to evaluate environmental quality through recolonization.

# From paper to practice: Implementation of the Environmental Monitoring Plan for Fish Farming proposed by Jacumar

# Chapter 2

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Photo: Pablo Arechavala López

# 2. From paper to practice: Implementation of the Environmental Monitoring Plan for fish farming proposed by JACUMAR

## 2.1. Summary

The National Advisory Board of Mariculture (JACUMAR) developed an initiative to unify methodologies between the regions of Spain, in which they proposed the implementation of site-specific "Environmental Monitoring Plans" (EMP). In this study, we tested the feasibility of the EMP in a fish farm on the Mediterranean Sea. We found that the methods and tools proposed in the EMP are highly useful for environmental monitoring of aquaculture. However, spatial heterogeneity figured prominently in a univariate analyses with environmental variables, and a multivariate analyses of polychaete assemblages. This variability may be due to habitat patchiness, and thus may be solved by an improved experimental design, e.g. by adding replication in order to increase statistical power. Multivariate analyses of polychaete assemblages provided accurate information about the quality of the sediment. This information could also be improved using ecological information about key polychaete families in order to avoid possible misleading results. Thus, the JACUMAR EMP has proved useful in providing precise information about the ecological status of marine benthic habitats, meeting the requirements of current European Directives. However, we suggest that some modifications may be required in order to account for possible misleading thresholds for environmental quality standards, spatial heterogeneity and increasing power analyses.

## 2.2. Introduction

The identification of indicators for the monitoring of aquaculture activities has been on the agenda of the General Fisheries Commission for the Mediterranean of FAO in recent decades (Massa and Bourdenet 2016). However, it is only since the European Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC) were proposed and Member States were required to achieve a “Good Environmental Status” in their marine waters, that the need for effective monitoring protocols has increased. It is acknowledged that monitoring protocols for aquaculture, either as a mandatory or voluntary process, are highly inconsistent between countries and regions, ranging from very exhaustive studies to little or no requirements (Read and Fernandes 2003, Telfer *et al.* 2009). Thus, the broad variation in monitoring programs and indicators used has resulted in a range of different approaches and conclusions about the spatial extent and severity of these effects (Kalantzi and Karakassis 2006).

In Spain, specific guidelines were developed in order to maintain the ecosystem goods and services provided the aquaculture (FOESA 2011). This document suggested focusing on aspects such as a reduction of conversion factors, the compliance codes of good practices in aquaculture, and the implementation of environmental monitoring plans. Regarding the last point and because the high legislative heterogeneity at regional level, the Spanish Ministry of Agriculture, Food and Environment, through the National Advisory Board of Mariculture (JACUMAR), developed an initiative to unify methodologies, proposing the implementation of site-specific “Environmental Monitoring Plans” (EMP) (Aguado-Giménez *et al.* 2012a).

This EMP approach focuses on the interactions between aquaculture and benthic ecosystems, particularly increases in organic enrichment (from a combination of uneaten food and fish faeces) and the sensitiveness of benthic assemblages, to detect environmental impacts (Papageorgiou *et al.* 2010, Martinez-Garcia *et al.* 2013, Mangion *et al.* 2014). This incremental increase in organic matter (OM) over the benthos may affect sediment biogeochemistry (Karakassis *et al.* 2005), causing oxygen depletion, augmentation of nutrient efflux and decreased benthic fauna diversity (Pearson and Rosenberg 1978, Hargrave *et al.* 2008, Martinez-Garcia *et al.* 2015). When oxygen concentration is reduced, aerobic metabolism is replaced by anaerobic metabolism, sulphate reduction and methanogenesis, producing sulphide and methane which are harmful for macrobenthic fauna (Holmer *et al.* 2005a, Hargrave *et al.* 2008). For that reason, a combination of biogeochemical variables and macrofauna are normally selected as bioindicators of aquaculture environmental impacts. Regarding macrofauna, this effect has been evaluated by different methods: by multivariate analyses of the macrofauna assemblage and/or by using benthic biotic indices, which simplify the complex multivariate benthic assemblage up to a single value to describe the ecological status (Karakassis and Hatziyanni 2000, Hoey *et al.* 2010, Aguado-Giménez *et al.* 2015).

Among all macrobenthic faunal groups, the polychaete assemblage is commonly used for the analyses of disturbances produced by organic enrichment, due to their widespread distribution in the benthos, trophic flexibility and quick response to disturbances (Dean 2008). Differences in trophic strategies among species and families can lead to different responses to organic pollution. Some polychaetes are regarded as pollution tolerant, because they can survive in advanced stages of disturbance, while other species are regarded as pollution sensitive, because they are not able to persist under stress conditions (Pearson and Rosenberg 1978, Giangrande *et al.* 2005). Some authors have tested the use of polychaete assemblages to study disturbances originating from fish farm facilities (Tomassetti and Porrello 2005, Martinez-Garcia *et al.* 2013, Aguado-Giménez *et al.* 2015), and concluded that this assemblage gives accurate information about the quality of the environment.

The general aim of this study was to assess the feasibility of the EMP defined by JACUMAR for monitoring of the aquaculture effects in a Spanish fish farm. The proposed EMP includes a specific design with hierarchical sampling along a gradient of distance from the fish cages, taking into account different physicochemical variables (sulphides, OM, pH, redox potential (Eh) and $\delta^{15}N$) and the polychaete assemblage as bioindicators, followed by an adjustable monitoring program. Thus, following the JACUMAR EMP guidelines (see Material and Methods section), we evaluated the environmental impact of a Mediterranean fish farm, dedicated to seabream and seabass farming, with particular focus on the identification of possible improvements for future EMPs, and to examine the spatial consistency of the proposed variables necessary to correctly detect environmental impacts.

## 2.3. Material and Methods

### 2.3.1. JACUMAR Environmental Management Plan

JACUMAR EMP (Aguado-Giménez *et al.* 2012a) is based on Environmental Quality Objectives/Environmental Quality Standards (EQO/EQS) and 'Allowable Zones of Effects' (AZE) approaches (Fernandes *et al.* 2001). In this EMP, the AZE is considered to be the area within the limits of the administrative lease. Two EQOs are defined for the cage-specific fish farming environment interactions: I) environmental changes within the AZE should not exceed the limits proposed for a set of environmental variables (in contrast with reference areas), and II) tolerable adverse effects should not reach beyond the AZE. To comply with these EQOs, an experimental design was defined including 4 zones: zone A, inside the perimeter of the lease-hold area physically underneath the facilities; zone B, the area surrounding the administrative concession, no more than 50 m outside the limits and; two zones C, reference or control areas with no influences from cage cultures, located at least 500 m away from the aquaculture activity. Specific EQS are defined for each zone and indicator variable (Table 2.1). For soft-sediment bottoms, the monitoring consists of one sample per year during the maximum productivity period. At each zone, three random nested sites (S) must be selected for integrating the natural spatial variability. Univariate analyses, using ANOVA, corresponds to physicochemical variables of the sediment (total free sulphides, sediment finest fraction, OM, pH, Eh and $\delta^{15}N$) following the model of sources of variability:

$$Xij = \mu + Zi + T_j + Z_i x T_j + S_k(Z_i x T_j) + \text{Residual}$$

where Z refers to the zone (fixed and orthogonal with 4 levels: A, B, C1 and C2), T refers to the sampling campaigns (random and orthogonal with levels up to the number of campaigns done) and S refers to the sites (random and nested in Z, with 3 levels: Site 1, Site 2, Site 3). The environmental effects of aquaculture are evaluated by comparison of zones A and B versus the two control locations (C1 and C2), to which they must not have statistical differences. The EQS´s are shown in Table 2.1. In special cases, when the aquaculture facilities are located over an area with high natural concentration of OM (e.g. the mouths of rivers or watercourses), then, a hypothesis contrasting to the control values without significant differences will be required for pH and Eh.

Polychaete identification to family level using a taxonomical-sufficiency approach (Del-

Pilar-Ruso *et al.* 2010, Martinez-Garcia *et al.* 2013, Aguado-Giménez *et al.* 2015) is proposed in the EMP. The multivariate treatment corresponds to polychaete assemblages following the model above; a multivariate analyses of the variance through permutations (PERMANOVA-Anderson *et al.* 2008) was undertaken. A multidimensional analyses (MDS-Clarke 1993) of the polychaete families' abundance is also proposed to observe the spatial ordination of the zones between sampling times, and a similarity test (SIMPER-Clarke and Gorley 2006) in the same manner. Polychaete assemblages in zone A should not have less than 75% number of families and 75% similarity than zones C. In zone B, the number of families should not be less than 50% of zones C and the dissimilarity should not be less than 50%.

If these EQS were not achieved, it may require some modification of the EMP, as part of an adjustable EMP with potential application of administrative and management measures. Initially a new campaign will be required over the next six months, with the analyses of Total Free Sulphide (TFS) and polychaete assemblage. If the effect of the organic enrichment continues to exceed the permitted values of the EQS, the requirements will be stronger, such as reducing production or moving the facilities to another location.

Table 2.1. Summary of the Environmental Quality Standards (EQSs) proposed by the JACUMAR EMP. Zone A: inside the perimeter of the lease-hold area physically below the facilities; Zone B: area surrounding the administrative concession, no more than 50m outside the limits.

| | Environmental Quality Standars (EQSs) | |
|---|---|---|
| | Zone A | Zone B |
| Total Free Sulphide (TFS) | < 3000 µM<br>< 3 samples over 5000 µM | < 3000 µM<br>< 50% increase than zones C |
| Finest fraction | < 50% increase than zones C | < 25% increase than zones C |
| Organic matter (OM) | < 50% increase than zones C | = zones C |
| pH | Between 7 and 9<br>Special case: = zones C | Between 7.5 and 8.5<br>Special case: = zones C |
| Redox potential (Eh) | > -200 mV<br>Special case: between -50 and -100 mV than zone C | Between -50 and -100 mV than z. C<br>Special case: = zones C |
| $\delta^{15}N$ | < 6‰ or<br>< 4 units than zones C | = zones C |
| Polychaete assemblage | < 75% nº of families than zones C<br>< 75% dissimilarity than zones C | < 50% nº of families than zones C<br>< 50% dissimilarity than zones C |

### 2.3.2. Study area and experimental design

The study was carried out in 2009 in a sea bream and sea bass floating-cage farm at 25-30 m depth in Guardamar bay, Southeast of Spain. The Segura River flows into Guardamar bay, so this would be classified as 'a naturally-high organic concentration at a regional scale', as described in the JACUMAR protocol. Samples were collected in late summer during the period of warmest water and maximum productivity. Four zones were sampled in the vicinity of the fish farm according analyses to EMP: zone A (just below the cages); zone B (50 m from the cages, at the edge of the farm facilities defined by the delimitation buoys); and zones C1 and C2 (reference area placed 1 km away from the fish farm). Three sites in each zone were randomly selected, and each had three random replicates collected for sediment analyses and three separate replicates for polychaete assemblage structure. Samples were collected using a Van Veen grab (0.04 $m^2$). Immediately after collection, the samples for the macrobenthos analyses were sieved with seawater through a 1 mm mesh net and the residues were preserved in 10% buffered formalin. At the laboratory, polychaetes were removed and preserved in 70% alcohol, and were later identified to family level. TFS content was measured with an ion-selective electrode (silver/sulphide combination electrode 9616 BNWP) following the method described by Wildish *et al.* (1999). Fractions of silt and clay (finest fraction: < 0.0625 mm) were determined by the wet sieving method described by Buchanan (1984). OM was measured by loss on ignition (400 ºC, 4 h). The pH and Eh were measured with CRISON electrodes. $\delta^{15}N$ isotopic composition was measured using an EA-IRMS (Thermo Finnigan) analyser in continuous flow configuration, combined with a stable ratio mass spectrometer Deltaplus. The $\delta^{15}N$ isotopic composition is expressed as:

$\delta^{15}N(‰) = \left[(R_{sample} / R_{standard}) - 1\right] \cdot 10^3$ where R = $^{15}N$ / $^{14}N$, atmospheric $N_2$ being the standard and 0.1‰ the analytical precision (Peterson and Fry 1987).

### 2.3.3. Statistical analyses

The data were analysed according to a 2-factor model as suggested in the JACUMAR EMP (Aguado-Giménez *et al.* 2012a), using the following model of sources of variability: Xij= μ + Zi + Sj(Zi) + Residual, where Z refers to the zone (fixed and orthogonal with 4 levels: A, B, C1 and C2) and S refers to the sites (random and nested in Z, with 3 levels: Site 1, Site 2, Site 3). Analyses of variance (ANOVA) were used to analyse the environmental variables using the above model. Heterogeneity of variance was tested with Cochran's test and data were transformed when necessary (Underwood 1997). Where variance remained heterogeneous, untransformed data were analysed and the α-value was set at 0.01, as ANOVA is robust for heterogeneity of variances, particularly for large, balanced experiments (Underwood 1997). Where significant differences were found, data were subsequently investigated using an SNK test (Student-Newman-Keuls) to determine which samples were involved in the differences. We used the software R (R Development Core Team 2011) with the GAD package (Sandrini-Neto and Camargo 2014) for ANOVA analyses. To investigate the effects on the polychaete assemblage, the model was analysed using permutational multivariate analysis of variance (PERMANOVA); pairwise tests and Monte Carlo tests were used to detect differences between levels of the factor Z (Anderson and Robinson 2003). Differences in polychaete assemblage structure were explored using non-parametric multidimensional scaling (MDS-Clarke 1993) and similarity percentages (SIMPER) between zones (Clarke 1993). To assist with the interpretation of analyses, the variability at each spatial scale was expressed as a component of variation (sum of all pseudo-variance components) (Anderson *et al.* 2005). Multivariate statistical analyses were performed using PRIMER-E software (PRIMER software- Clarke and Gorley 2006) with the add-on package PERMANOVA+ (Anderson *et al.* 2008).

### 2.4. Results

TFS was the most sensitive environmental variable to the fish farming activity, showing significant differences for Zone factor (Table 2.2), with zone A having significant higher values than the other zones (SNK: A>B>C1>C2, Figure 2.1). The rest of indicators did not reveal significant differences caused by farming activity. There was high spatial variability across sites for all indicators except pH, TFS and $\delta^{15}N$, for which Eh was the most spatially inconsistent (Table 2.2). The components of variation showed that TFS and Eh had the highest variation among zones, at the scale of hundreds of meters, while the proportion of finest fraction, pH, OM and $\delta^{15}N$ reflected a higher proportion of variation among replicates at a scale of meters (Table 2.2). In spite of the significant differences, TFS concentration did not surpass the concentration limits proposed by JACUMAR, with values between 493-1269 μM at zone A and 378-787 μM at zone B (Figure 2.1). At zone A, there was no sample above 5000 μM.

Table 2.2. Results of ANOVA with 2 factors. Df: degrees of freedom, MS: mean square, F: F-distribution. Levels of significance: *p<0.05, **p<0.01 and ***p<0.001. -a indicates that there was no homogeneity of variance, the levels of significance were *p< 0.01, **p<0.001. CV: components of variation.

| Source of | | TFS | | | | % Finest fraction | | | | Organic matter | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| variation | df | MS | F | P | CV | MS | F | P | CV | MS | F | P | CV |
| Zone | 3 | 3.06 | 30.78 | **0.000** *** | 81% | 0.02 | 1.66 | 0.252 | 10% | 0.11 | 1.36 | 0.322 | 6% |
| Site (zone) | 8 | 0.10 | 1.43 | 0.235 | 2% | 0.01 | 2.65 | 0.031 · | 31% | 0.08 | 2.29 | 0.055 · | 28% |
| Residual | 24 | 0.07 | | | 17% | 0.00 | | | 59% | 0.03 | | | 66% |
| Cochran's C test | | C=0.37631, P>0.05 | | | | | | - a | | C=0.35787, P>0.05 | | | |
| Transformation | | log (x+1) | | | | arc sin (x) | | | | log (x+1) | | | |
| Source of | | pH | | | | Eh | | | | $\delta^{15}N$ | | | |
| variation | df | MS | F | P | CV | MS | F | P | CV | MS | F | P | CV |
| Zone | 3 | 0.21 | 0.95 | 0.461 | 0% | 26206 | 5.79 | 0.021 · | 52% | 0.02 | 0.69 | 0.585 | 0% |
| Site (zone) | 8 | 0.22 | 0.44 | 0.885 | 0% | 4529 | 4.19 | **0.003** * | 25% | 0.03 | 0.66 | 0.723 | 0% |
| Residual | 24 | 0.50 | | | 100% | 1082 | | | 23% | 0.05 | | | 100% |
| Cochran's C test | | | | -a | | | | -a | | C=0.30955, P>0.05 | | | |
| Transformation | | | | none | | | | none | | | | none | |

However, at zone B, the increase of TFS concentration was 267% and 179% higher than C1 and C2 respectively. The percentage of finest fraction did not exceed the EQS because zones A and B were not 50% and 25% higher than zones C1-C2 respectively (Figure 2.1). OM did not exceed the limit established by the EQS proposed by JACUMAR, as its concentration in zone A was not 50% higher than its concentration in zones C1 and C2. The pH values in zone A were inside the allowed range, but the values in zone B were outside the limits (Figure 2.1). This represented the special case considered in the EMP for areas with a naturally-high OM content (due to the outflow of the Segura River), and the hypothesis contrasting relating to differences between zones C1-C2 and zone B in pH, which had no significant differences (Table 2.2). Eh values in all zones fell outside the allowed range proposed by JACUMAR. Likewise, as it was an area with a naturally-high content of OM, the EMP proposed a hypothesis contrasting, for which zone A was no more electronegative than C1-C2 (Figure 2.1), and zone B did not differ significantly from zones C1-C2 (Table 2.2). $\delta^{15}N$ values were very similar across zones and did not exceed the EQS in any sample (Figure 2.1).

PERMANOVA analyses of polychaete families showed significant differences among zones in spite of the spatial variability among sites (Table 2.3). When a pair-wise test was applied, only zone A was significantly different to zones B, C1 and C2. Zone B was not significantly different to zones C1 and C2 (Table 2.3).

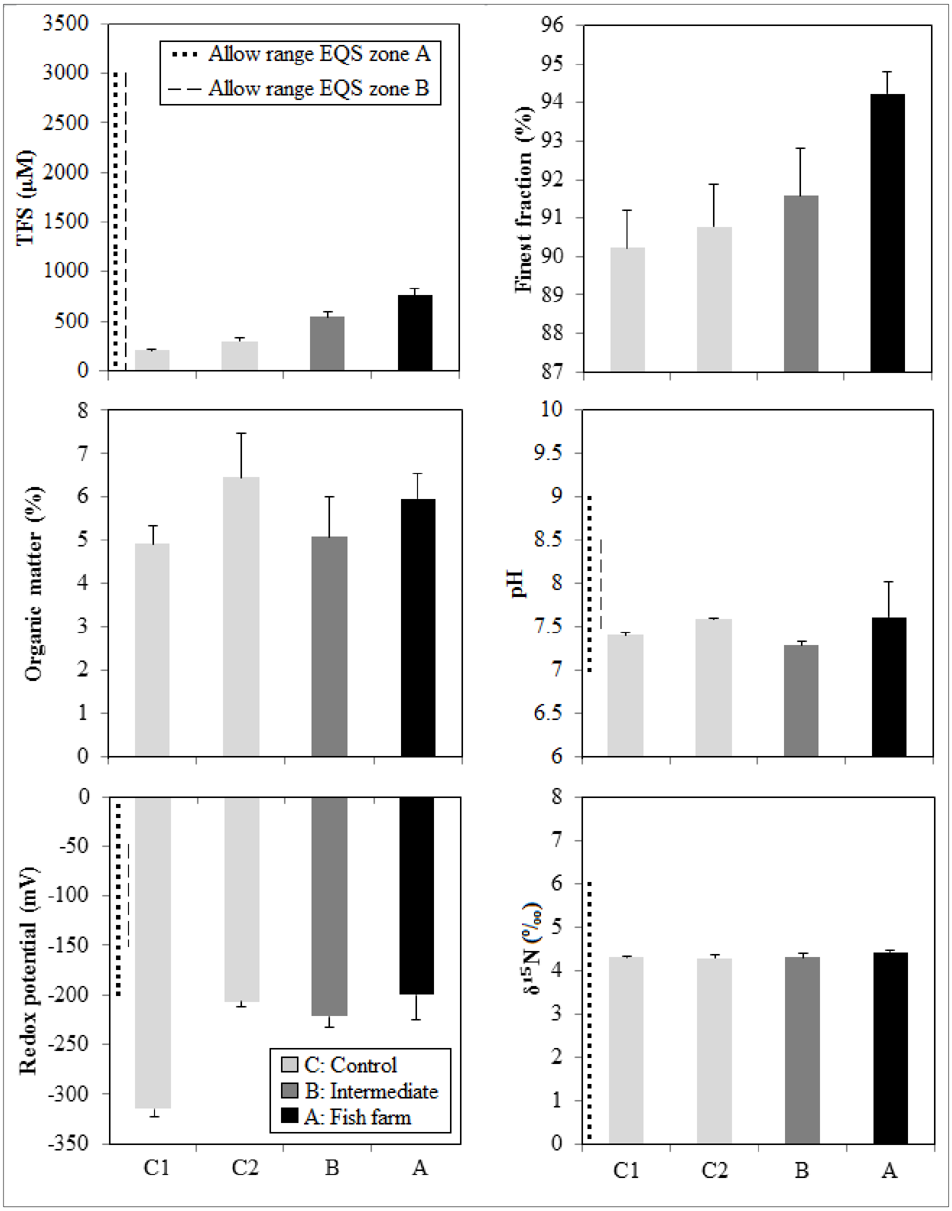


Figure 2.1. Mean values (± standard error) of Total Free Sulphides (TFS), finest fraction (%), organic matter (%), pH, redox potential (mV) and δ15N.

No significant differences were observed between C1 and C2. The composition of variance showed that the variability was produced at a scale of meters between replicates, and at zone scale of hundreds of meters (Table 2.3).

Table 2.3. Results of PERMANOVA and pair-wise test of polychaete assemblage. Df: degrees of freedom, MS: mean square. MC: Monte Carlo test. Levels of significance: *p<0.05, **p<0.01 and ***p<0.001. CV: components of variation. A: fish farm, B: intermediate, C1: control 1, C2: control 2

| Source of variation | df | MS | P | | CV |
|---|---|---|---|---|---|
| | Polychaete assemblage | | | | |
| Zone | 3 | 9793.50 | **0.0004** | *** | 37% |
| Site (zone) | 16 | 1968.40 | **0.029** | * | 16% |
| Residual | 24 | 1467.90 | | | 47% |
| Total | 35 | | | | |
| Pairwise test | | P | P -MC | | |
| A - B | | 0.0956 | **0.0029** | ** | |
| A - C1 | | 0.1034 | **0.0007** | *** | |
| A - C2 | | 0.0977 | **0.0003** | *** | |
| B - C1 | | 0.1019 | 0.06 | | |
| B - C2 | | 0.1957 | 0.1809 | | |
| C1 - C2 | | 0.1 | 0.0538 | | |

The MDS analyses of the polychaete assemblage structure was broadly consistent with the PERMANOVA results, as zones C1, C2 and B were tightly clustered, and zone A appeared to be separated in the plot. Moreover, zone A showed higher scatter between its samples (Figure 2.2). The SIMPER test indicated that the dissimilarities between zone A and C1-C2 were 83.87% and 83.82%, respectively. Zone B was less dissimilar than control zones, with values of 48.95% (C2) and 53.973% (C1), in the latter case slightly exceeding the EQS (Table 2.4). The number of families in zone A was 64.63% and 70.10% lower than zones C1-C2 exceeding the EQS; conversely, the number of families in zone B was 17.07% higher compared to zone C1 and 1.03% lower than zone C2 (Table 2.4).

Table 2.4. Summary of SIMPER dissimilarities of polychaete assemblages at the four zones, A: fish farm, B: intermediate, C1: control 1, C2: control 2. Aver. Dissim: average dissimilarity. % nº families: percentage of the polychaete families in A and B regarding to C1 and C2. EQS: Environmental quality standard proposed by JACUMAR EMP.

| | Polychaete assemblage | | |
|---|---|---|---|
| Zone: | Aver. Dissim. | % nº families | EQS |
| A - C 1 | **83.87** | **< 64.63** | < 75 |
| A - C 2 | **83.82** | **< 70.10** | < 75 |
| B - C 1 | **53.97** | > 17.07 | <50 |
| B - C 2 | 48.95 | < 1.03 | <50 |
| A - B | 86.53 | | |
| C1 - C 2 | 44.4 | | |

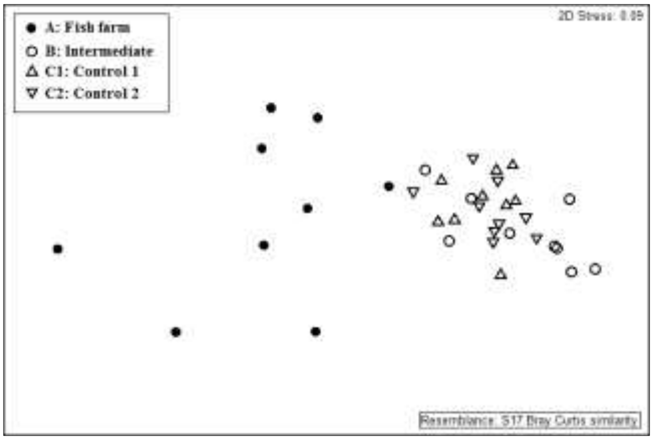


Figure 2.2. Non-metric multidimensional scaling ordination (MDS) analyses on the Bray-Curtis Similarity of non-transformed abundance data at each family.

### 2.5. Discussion

The application of EMPs proposed by JACUMAR led to the detection of an environmental impact due to fish farming, mainly in TFS and polychaete assemblages; therefore we conclude that there was a localised environmental impact just underneath the cages, inside the Allowed Zone of Effect. Among all physicochemical variables, only TFS had significant differences between impacted and control zones, and only TFS in zone B exceeded the EQSs proposed by the JACUMAR approach. Polychaete assemblages at family level were the best indicator of fish farming impacts, as they were sensitive enough to detect an environmental impact in spite of the above-mentioned minor physicochemical effects. However, high spatial variability was observed at different scales for several indicators, which could affect statistical power and increase the probability of a type II statistical error (Underwood 1997). Natural variability between control zones could also negatively affect an environmental impact assessment. From these results we can conclude that although this EMP proposal includes a complementary set of methods and tools that seem to be appropriate for fish farming environmental monitoring, some improvements could be made in order to deal with spatial variability, increased sampling robustness and reducing type II errors, and thus reducing uncertainty when interpreting the results.

The spatial variability for geochemical and biological variables is a normal consequence of benthic assemblage patchiness and irregular disturbance of the seabed by fish farming. This variability can affect the sediment mostly at a scale of meters (Quintino *et al.* 2006, Fernandez-Gonzalez *et al.* 2013), as happened in this study for some geochemical parameters (pH, finest fraction %, OM and $\delta^{15}N$), and for polychaete assemblages. This significant variability at replicate level confirms the well-known importance of selecting a representative sample size in terms of the volume of sampled sediment and the number of replicates (Andrew and Mapstone 1987). Other variables were affected at a scale of hundreds of meters (TFS, Eh and polychaete assemblage), reflecting the interaction of the fish farm cages with the benthic habitat. In some uncertain situations, it may be appropriate to use a higher level of significance (e.g. $\alpha = 0.1$) in order to avoid overlooking slight environmental impacts, thus using a precautionary approach to reduce type II errors, e.g. when the EQS of polychaete assemblages are exceeded but not for physicochemical variables. In this sense, the replication at higher spatial cases (e.g. nested sites within zones), as performed in this EMP (Underwood 1997, Terlizzi *et al.* 2005), will increase statistical power and avoid pseudoreplication (Hurlbert 1984), and would be necessary in order to detect organic enrichment from fish farm facilities over a naturally-patchy environment.

Regarding polychaete assemblages, dissimilarity tests concluded that zone A and even B exceeded the EQSs proposed by JACUMAR. In this aspect of sensitivity, polychaete assemblage at family level appears to be a very good tool to identify changes in the benthic ecosystem, allowing the detection of slight differences between zones even with high spatial heterogeneity. Polychaete assemblage had higher spatial variability below the fish farm than in other zones. This increased variability has been considered to be a general feature of assemblages in stressed environments, and it could be due to different scenarios: changes in total cover or total number of taxa, changes in the variance-to-mean ratio for particular species, or changes in taxonomic composition (Warwick and Clarke 1993, Chapman *et al.* 1995, Terlizzi *et al.* 2005). However, in this case, the dissimilarity results shown in SIMPER, in which zone B is over the limits, are quite misleading regarding the decrease in the number of families, PERMANOVA did not show significant differences between zone B and zones C1-C2, and MDS clustered all zones B, C1 and C2 together. In borderline cases such as this, managers may find it difficult to decide whether to apply administrative measures against the farmer. If the SIMPER result was outside the EQS but PERMANOVA and MDS were inside the EQS, should the manager apply any measure? A precautionary approach could be implemented and mitigation measures could be agreed in consensus with the farmer, following a good practice code (FEAP 2006). However, observing the natural variability and the dissimilitude result between C1 and C2, a revision of the EQS would be appropriate. We suggest that the limit in zone B (50% of families and 50% of dissimilitude comparing with C zone) may be increased to 65% without jeopardizing the achievement of EQO´s. In the studied farm with this new EQS, the results would have more concordance and only zone A would be outside the EQS.

The JACUMAR EMP proposed the analyses of polychaete assemblages from a multivariate perspective. Classification only to family level requires less taxonomic resolution than some biological biotic indices, and consequently facilitates their possible use in EMPs in aquaculture activities (Karakassis and Hatziyanni 2000, Lampadariou *et al.* 2005, Aguado-Giménez *et al.* 2015) as has been used in EMPs of other disturbance types such as desalination plants or sewage discharges (Del-Pilar-Ruso *et al.* 2009). This approach could also be enhanced with ecological information from univariate analyses of polychaete families. Polychaetes have been well documented as bioindicators, specifically species such as *Capitella capitata* or some Spionidae species (Pearson and Rosenberg 1978, Giangrande *et al.* 2005, Tomassetti *et al.* 2005, Mangion *et al.* 2014). Moreover, studying the whole assemblage at family level and following general patterns of family abundance, as described by Martinez-Garcia *et al.* (2013), provides accurate information on the quality of the benthic environment. It will be useful to observe what types of family are appearing or disappearing in each zone, in order to determine whether they are tolerant or sensitive to organic enrichment. In this manner, information provided by polychaete assemblages will not be restricted to the number of families and the dissimilarity value, which is a simplified number that summarises an overall ecological process, as happens with the biotic indices (Aguado-Giménez *et al.* 2015). Therefore, univariate analyses of selected polychaete families should be also carried out in order to provide more information on the ecological processes occurring due to fish farming. Considering that classification of polychaetes at family level is required to perform multivariate analyses, this approach would not require additional taxonomic effort.

Sulphide measurements, such as TFS, are able to show variations in biogeochemical processes of the sediment due to organic enrichment (Wildish *et al.* 1999), even when the input is not high enough to be detected by OM measurements (Martinez-Garcia *et al.* 2015). The method to quantify TFS concentration in the sediment is relatively cheap and easy to conduct (Keeley and Taylor 2015). The potential toxicity to biota has been well described, as well as the relationship between sulphide and the lack of oxygen in the sediment (Holmer *et al.* 2005a, Hargrave *et al.* 2008). Consequently, TFS provides accurate information on the environmental quality of the sediment, making it easier to interpret what is happening in the benthic habitat. Thus, some authors have proposed TFS as an indicator to be included in EMPs (Katavić *et al.* 2005, Aguado-Giménez *et al.* 2015). In these EMPs, sulphide concentration may be compared to control areas, and a hypothesis contrasting in which the control values would not have significant differences becomes more important, which in this study was detecting differences produced by the fish farming. Regarding to the concentration limit set by JACUMAR EQS, zone A and B were inside the allowed limits. Again, this could cause a controversial situation for the managers, as Zone A clearly suffered impacts in relation to the contrary hypothesis, and this result was in line with polychaete assemblage results. By contrast, zone B was slightly altered, outside the limits of 50% higher than controls but, if this data was related to the polychaete assemblage, the MDS and PERMANOVA did not show an important impact caused by the sulphide value at zone B. The EQS limits set by JACUMAR were probably very high in that the impact values in other fish farm facilities may be wrongly considered inside the EQS. We suggest, that coupled with the hypothesis contrasting, an increased percentage threshold with respect to zone C may be added. For areas with naturally-high organic enrichment such as Guardamar bay, the limit for zone A could be significantly different in the hypothesis contrasting, with double the concentration in zone C. Sulphide concentration limit at zone B could also be revised, taking into account that there was a difference of 50% between C1 and C2. Another suggestion for the limit may be to double the concentration of controls. With these new limits, the studied fish farm would have zone A clearly exceeding the EQS, and zone B would be close to the limit but inside the EQS compared to C2 and over the EQS compared to C1.

On the other hand, after many years using Eh to add information on the degree of organic enrichment, some authors do not advocate its use in finfish monitoring; this is due to the problems associated with potential "poisoning" of the probes and the higher variability in the Eh measures and difficulty to reach a stable reading, as we observed in our results (Wildish *et al.* 2005, MER 2008). The $\delta^{15}N$ have been used as a good tracker of fish farm waste (Holmer *et al.* 2007, Ruiz *et al.* 2010), and some authors propose its inclusion in fish farming EMPs because it may be interpreted as a measure of nutrient accumulation (Carballeira *et al.* 2012). However, for our $\delta^{15}N$ results there were no sign of fish farm waste even below the cages; taking into account that an interaction was detected with other variables such as TFS and assemblage polychaetes, the use of $\delta^{15}N$ with a level of acceptance as an EQS for an EMP might be quite misleading. This variable should not be based on concentration alone, but to be indicative of the proportion of $^{15}N$ from the total N. $\delta^{15}N$ is a tracker rather than a direct indicator of impact, so it will be necessary to establish all of the potential sources of $\delta^{15}N$ in order to understand the contribution of the fish farm waste to this total amount of nitrogen in the sediment (Sarà *et al.* 2004). Moreover, the content of marine ingredients in the fish feed has been highly reduced, incorporating a greater percent of terrestrial ingredients in recent years (Yrestøy *et al.* 2015), so the $\delta^{15}N$ signal from fish farm waste may now be reduced. For these reasons, if there is a special need to include $\delta^{15}N$ in the EMP, it would be necessary to revise the protocol and analyse the isotopic signal due to fish farm regularly in relation to the latest types of feed. The $\delta^{15}N$ would give interesting information if measured regularly for the whole source of N in the area, as well as jointly with $\delta^{13}C$. However, regardless of this additional information it remains an expensive variable for standard EMPs.

One important aspect of the EMP is the consideration of special cases with naturally-high contents of OM, and those that are not well oxygenated at a regional scale, as happens in Guardamar bay, for which the OM had elevated values and the finest fraction had a physical homogeneity throughout the study area with high values. As we observed in pH, even mean values in zones B and C were outside the limits of the EQS (slightly acidic) reflecting transitory conditions of the environment towards unacceptable values (Hargrave *et al.* 2008). In these situations, the comparison with control locations would be compulsory for any kind of EMP. The fine sediment can also affect the accumulation of OM, sulphides and ammonium, so the metabolic capacity of the sediment is reduced (Kalantzi and Karakassis 2006, Papageorgiou *et al.* 2010, Martinez-Garcia *et al.* 2015). This fact supports the approach of the EMP, in which decisions are taken based on a comparison of control and impacted zones (Aguado-Giménez *et al.* 2012b), avoiding misinterpretations from the definition of an EQS for impacted locations exclusively.

A more exhaustive EMP with increased periodicity may be required if surveys reveal significant negative impacts. Thus, an adjustable EMP is proposed by JACUMAR for Spain, similar to other countries such as Croatia and Norway (Ervick *et al.* 1997, Katavić *et al.* 2005). However, in some situations, as we have seen with this study, contradictory results could affect the implementation of an adjustable EMP. According to our results, and following an adjustable EMP, it would be necessary to adapt the EMP of the studied fish farm by increasing the sampling periodicity of polychaete assemblages and TFS to a survey every six months, until the values would again be within the EQSs. If the environmental impact persisted over time, mitigation measures would be also required, such as reducing productivity or relocation of facilities to deeper waters.

Consensus about monitoring protocols is needed to ensure that data meet defined standards of quality, with a known level of confidence, in order to be credible, so that data stand up to external review and allow comparisons of data among places, regions and/or agencies. Thus, the proposed EMP of JACUMAR seems to be a very innovative approach and a reliable tool to monitor fish farming along a scale of 1000's of km along the entire Spanish coast, and this kind of proposal could be considered for implementation in other European and Mediterranean countries with fish farming under similar environmental conditions. Moreover, this EMP is pioneering in adding a hypothesis contrasting as an EQS for fish farm monitoring, and for proposing comparisons with control locations as natural background values. However, in some cases higher statistical power with regard to univariate and multivariate analyses is needed in order to accurately determine the effect of fish farming.

Serious or irreversible environmental damage could occur due to a lack of statistical power, because high spatial heterogeneity and/or a lack of appropriate replication at several scales could postpone effective measures to mitigate environmental degradation from fish farming. Therefore, before full implementation of JACUMAR EMP in Spain, it will be necessary to evaluate the need of increasing spatial replication at several scales and by contrasting the information obtained from different pilot cases. Moreover, iterative EMP will be important in order to trace the progression of the fish farm activity in relation to benthic environmental quality (Aguado-Giménez *et al.* 2012a), and long temporal data series from fish farming EMPs carried out with the same methodology will very informative, giving more accurate information about their environmental status, as required by European Directives (Hoey *et al.* 2010). Therefore, we encourage future steps for harmonisation of EMP at national, European or even Mediterranean scale, as this EMP proposed by JACUMAR for Spanish coastlines.

# A Meta-analysis approach to the effect of fish farming on soft bottom Polychaeta assemblages in temperate regions

# Chapter 3

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# Effect of Sediment Grain Size and Bioturbation on Decomposition of Organic Matter from Aquaculture


# Chapter 4

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# Assesment of a New Tool to evaluate the Benthic Aquaculture Impact: Colonization Experimental Units

# Chapter 5

# 5. Assessment of a New Tool to Evaluate the Benthic Impacts of Aquaculture: Colonisation of Experimental Units by Polychaeta

## 5.1. Summary

A range of different protocols and indices have been developed in recent years for the definition of Environmental Quality Standards in aquaculture. However, it can be difficult to compare these protocols or indices between different regions or different habitats due to spatial heterogeneity at different scales. We carried out a field study to estimate the effectiveness of experimental units (sediment-filled trays) as an environmental management tool. The experimental units were filled with two different sediments, sand and mud, and placed underneath two fish farm facilities for one month. Using polychaetes to assess the ecological status, AMBI and multidimensional analyses showed clear results, and had further value when combined with sulphide concentrations measures. Sandy experimental units had a greater sensitivity for the detection of organic matter enrichment. The species with the most pronounced responses in the facilities studied were: *Capitella capitata*, *Spiochaetopterus costarum*, *Capitella minima*, *Pseudopolydora pulchra* and *Aricidea (Strelzovia) claudiae* in the fish farm, and *Gallardoneris iberica*, *Micronephtys stammeri*, *Sternaspis scutata* and *Ampharete lindstroemi* in the reference area without organic enrichment. Experimental units provide a complete and reliable source of information about the environmental quality status of fish farms, and with further refinement they could be used as a management tool for aquaculture monitoring.

### 5.2. Introduction

The effects of aquaculture activities on benthic environments are well documented (Holmer *et al.* 2005a, Martinez-Garcia *et al.* 2013, Tomassetti *et al.* 2016), and the greatest effects are typically due to organic enrichment, derived from an accumulation of uneaten food and fish excretions (Borja *et al.* 2009, Mirto *et al.* 2010, Martinez-Garcia *et al.* 2015). Over the last two decades aquaculture management groups have developed various measures to assess or control this, including general protocols, the European Water Framework Directive (WFD, 2000/60/EC), and environmental indices to define Environmental Quality Standards (Pearson and Rosenberg 1978, Odum 1985, Borja *et al.* 2009, Karakassis *et al.* 2013, Aguado-Giménez *et al.* 2015). Macrobenthic species are often used as biological indicators to monitor the environmental impact caused by organic enrichment (Pearson and Rosenberg 1978, Carvalho *et al.* 2006). Among all macrobenthic groups, polychaetes are remarkably well represented in every benthic habitat (Beesley *et al.* 2000) and their identification represents a useful tool for detecting changes in the benthos caused by fish farming activities (Lampadariou *et al.* 2005, Dean 2008, Martinez-Garcia *et al.* 2013). Diverse groups of polychaetes are present at different organic enrichment levels, from pristine to heavily disturbed areas (Giangrandre *et al.* 2005); their composition and relative abundances can be transformed to a numerical index (Borja *et al.* 2000) or analysed by multivariate parameters to assess the quality of the benthic environmental (Aguado-Giménez *et al.* 2007). One of the main problems in the use of these indicators is the natural variability in the distribution of habitats, as geochemical changes associated with the presence of fish-farm effluents are rarely consistent (Holmer *et al.* 2008, Mirto *et al.* 2010), due to latitudinal changes, different depths, different types of underlying sediment (Kalantzy and Karakassis 2006, Fernandez-Gonzalez *et al.* 2013), patchy substrata (Longdill *et al.* 2007) and different sampling scales (Tataranni and Lardicci 2009). These are also be a problem for the development of reference conditions, primarily because they are chosen randomly and could vary from the fish farm conditions (Forchino *et al.* 2011). Results from different sediments such as sand, mud, meadow, pebbles or shell-hash are difficult to compare. Moreover, the bottom is sometimes covered with hard substrata (including mussel detritus or rubbish from aquaculture), which prevents the usage of drags or cores.

Thus, it is not always easy to conduct management works and to compare levels of pollution between facilities placed in different ecosystems, or in impact versus control assessments, because of the interaction between natural spatial variability and patchy distribution of the habitat. In addition, if the sampling is expensive or should be done quickly due to security reason, the number of replicates could be reduced, which could have a strong effect on the power analyses, potentially leading to the misunderstanding of statistical results (Colquhoun 2014).

A new tool to avoid this problem could be the usage of experimental units (EUs), in which all of the initial physical, chemical and biological conditions are known. The EUs are placed underneath the fish farm facility for a defined time period and then recovered. Once back in the laboratory, geochemical changes in the sediment and recolonisation processes can be studied. Recolonisation experiments in different sediment types have previously been carried out under stress conditions produced by anthropogenic impacts (Guerra-García and García-Gómez 2006, Lu and Wu 2007, Guerra-García and García-Gómez 2009) and concretely under fish farm conditions (Lu and Wu 1998, Fernandez-Gonzalez *et al.* 2016). The later authors described the distribution and succession of benthic species and how the accumulation of toxic metabolites can become a limiting factor in recolonisation. In particular, sulphide concentration is a useful variable to represent stress levels in relation to organic matter degradation caused by fish farm activities (Hargrave *et al.* 2008, Keeley *et al.* 2012a). However, we have found no studies that assessed the use of these recolonisation experiments as a tool for marine environmental impact management, particularly for aquaculture facilities.

The general aim of this paper is to assess the feasibility of EUs (incorporating polychaete species as bioindicators) for the environmental monitoring of fish farming aquaculture, so that they could be applied to a wide range of sampling designs and habitat types. The objectives were: 1) to test the effectiveness of EUs as an instrument to evaluate environmental quality; 2) to compare different sediments in order to observe the effect of grain size in recolonisation under organic enrichment conditions; 3) to evaluate the detection of environmental impacts due to fish farming using different indices based on Pearson and Rosenberg (1978) and the multivariate analyses in EUs, and 4) to define the most important species that reflect changes in assemblage structure.

### 5.3. Material and Methods

#### 5.3.1. Study area

Experiments were carried out at Guardamar del Segura (Figure 5.1) in the southeast of Spain (38°5'45.88"N; 0°36'15.84"W). The study period was between June and August 2010, and was characterised by intensive fish feeding and high water temperature. Guardamar del Segura bay hosts 3 fish farm facilities in water ranging from 23 to 30 m depth; the finfish species were European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*). These facilities are located 4 km off the coast, over a benthos of muddy sediments (Fernandez-Gonzalez *et al.* 2016).

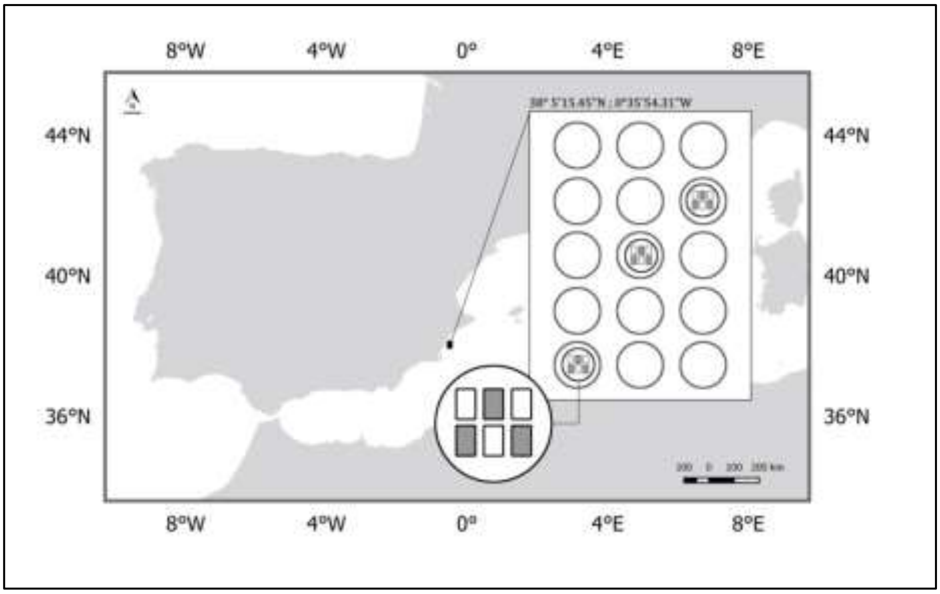


Figure 5.1. Location of the facilities in the western Mediterranean Sea, on the southeastern coast of Spain. The inlay shows the experimental design used in each location, with an example below one of the fish farms. White rectangles represent sandy EUs and grey rectangles represent muddy EUs.

#### 5.3.2. Experimental units and experimental design

The experimental design is represented in Figure 5.1. Two facilities in Guardamar Bay were selected. Three replicated experimental units (EUs) with sandy sediment (plastic trays of 24 x 15 x 6 cm) and three replicated EUs with muddy sediment were set below three random cages (impact) at 25–28 m depth. Two reference areas (control) were also selected 1 km away from the fish farms, with three random sites and three replicated EUs with sandy and muddy sediments respectively in each site, maintaining the same depth and bottom sediment. In total seventy-two EUs were set for the experiment. Sediments to fill the EUs were collected by divers in Guardamar Bay (muddy sediments) and in Alicante Bay (sandy sediments) (38º15'19.4''N, 0º30'24.7''W). Grain size was determined using Bouyucos method (Buchanan 1984). The initial composition of the sandy sediments was 96.48% sand, 0.3% silt and 3.30% clay. In muddy sediments the initial composition was 6.84% sand, 48.8% silt and 44.36% clay. Organic matter content was determined by the loss on ignition method (LOI, 450 ºC for 4 h). Initial organic matter content was 1.18 ± 0.10% in sandy sediments and 5.90 ± 0.5% in muddy sediments. Sediments were defaunated using the methodology described by Guerra-García and García-Gómez (2006). The sediment was frozen for 3 days and then dried first under natural sunlight for one day and later exposed to heat (40 ºC). The process was repeated twice. Divers set the EUs over the bottom of the fish farms and the reference area. After one month, EUs were closed and recovered. A subsample of sediment (0.045 m²) was taken from each EU to determine sediment particle size, organic matter and total free sulphides (TFS) (Wildish *et al.* 1999). The remaining sediment (0.0315 m²) was sieved through a 0.25 mm mesh in order to retain the polychaetes. They were fixed in a 10% formalin solution and later preserved in 70% alcohol. The polychaete specimens were identified to species level where possible; the taxonomic bibliography used is listed in the supplementary material for this article.

### 5.3.3. Statistical analyses

Total abundance, species richness (S), Shannon diversity index (H') (Shannon and Weaver 1963), Evenness (J) and the AZTI Marine Biotec Index (AMBI) (Borja *et al.* 2000) were calculated for each ET. The eighteen most-abundant polychaete species were also statistically analysed. These indices and the species selected were included in a 4-way factorial analysis of variance (ANOVA) to compare sandy sediments and muddy sediments (2 levels, fixed and orthogonal), control and fish farm (2 levels, fixed and orthogonal), locations (2 levels, random and nested) and sites (3 levels, random and nested), as well as the interactions between these factors. Heterogeneity of variance was tested using Cochran's test, and data were transformed when necessary (Underwood 1997). All statistical tests were conducted with a significance level of $\alpha = 0.05$. Where significant differences were found, data were subsequently investigated using the SNK test (Student-Newman-Keuls) to determine which samples were responsible for the differences. Analyses were conducted using the software R

(R Development Core Team 2011), with the GAD package (Sandrini-Neto and Camargo 2014). To explore differences in polychaete recolonisation, non-metric multidimensional scaling (MDS- Clarke 1993) and the percentage similarities procedure (SIMPER- Clarke 1993) were used. The Spearman correlation between polychaete species abundance and abiotic factors, TFS and organic matter, was determined using the RELATE procedure (Clarke 1993). Multivariate statistical analyses were performed using PRIMER-E software (Clarke and Gorley 2006).

## 5.4. Results

After one month, granulometric characteristics and organic matter variables did not show any change in their values (Figure 5.2). However, as expected, there was an increase in TFS under the fish farms in sandy sediments, and to a lesser extent in muddy sediments (Figure 5.2). In sandy sediments under fish farms there were differences in TFS concentration between locations (Table 5.1). Values of total abundance, S, AMBI, H' and J are represented in Figure 5.3. Polychaeta total abundance was always higher in sandy than in muddy EUs (Figure 5.3 and Table 5.1). There was no significant difference in species richness (S) between 'control' and 'impact' treatments for muddy EUs, but sandy EUs had a higher S at impact.

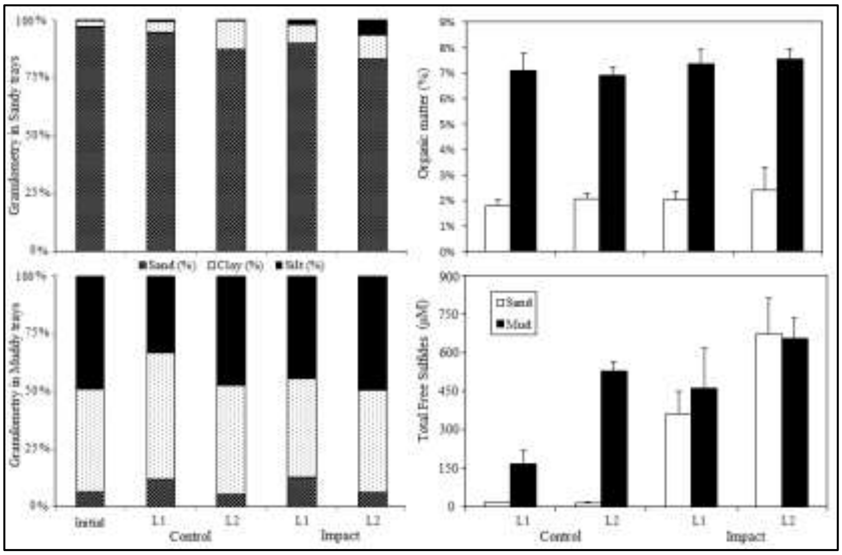


Figure 5.2. Granulometry percentages and mean values (±standard error) of organic matter (%) and TFS: total free sulphide (ppm) in sandy and muddy EUs at two fish farms (L1 and L2). The initial corresponds to sediment used to fill the EUs.

Table 5.1. Results of the analyses of variance (ANOVA) with four factors (Sed: sediment type, OM: impact through organic enrichment, Lo: location, Si: site) on measures of variance in Total abundance, richness (S), Shannon diversity index (H'), AMBI, Evenness (J), total free sulphides (TFS) and eighteen species of polychaetes. Df: degrees of freedom; MS: mean square; F: F-distribution. Levels of significance were * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Dash (-) indicates that there is no transformation. [a] Indicates that there is no homogeneity of variance, the levels of significance being * p 0.01, ** $p < 0.001$.

| | | Total abundance | | Richness | | Shannon | | AMBI | |
|---|---|---|---|---|---|---|---|---|---|
| Source of variation | df | MS | F | MS | F | MS | F | MS | F |
| Sed | 1 | 20.96 | 69.46 * | 666.13 | 383.69 ** | 3.93 | 30.65 * | 0.81 | 3 |
| OM | 1 | 9.72 | 6.28 | 485.68 | 4.8969 | 31.43 | 8.43 | 133.82 | 176.82 * |
| Lo (OM) | 2 | 1.55 | 3.3 · | 99.18 | 5.89 * | 3.73 | 6.69 * | 0.76 | 1.8 |
| Si (Lo (OM)) | 8 | 0.47 | 1.04 | 16.83 | 0.81 | 0.56 | 1.55 | 0.42 | 0.68 |
| Sed x OM | 1 | 1.55 | 5.14 | 435.13 | 250.63 ** | 7.84 | 61.18 * | 3.84 | 14.25 |
| Sed x Lo (OM) | 2 | 0.3 | 0.84 | 1.74 | 0.08 | 0.13 | 0.16 | 0.27 | 0.39 |
| Sed x Si (Lo (OM)) | 8 | 0.36 | 0.8 | 22.06 | 1.06 | 0.82 | 2.27 * | 0.69 | 1.11 |
| Residual | 48 | 0.45 | | 20.87 | | 0.36 | | 0.62 | |
| Cochran's C test | | C= 0.196, P>0.05 | | C=0.218, P>0.05 | | C=0.196, P>0.05 | | | |
| Transformation | | log (x + 1) | | - | | - | | - a | |
| | | Evenness | | TFS | | Ampharete lindstroemi | | Aricidea daudiae | |
| Source of variation | df | MS | F | MS | F | MS | F | MS | F |
| Sed | 1 | 0.1002 | 9.9087 · | 16.77 | 8.37 | 1.02 | 1.43 | 0.9398 | 3.29 |
| OM | 1 | 1.8656 | 12.9062 · | 32.68 | 8.69 | 22.86 | 34.83 * | 2.2549 | 2.74 |
| Lo (OM) | 2 | 0.1446 | 4.6842 * | 3.76 | 19.91 *** | 0.66 | 0.56 | 0.8222 | 12.07 ** |
| Si (Lo (OM)) | 8 | 0.0309 | 2.3742 * | 0.19 | 0.75 | 1.17 | 3.82 ** | 0.0681 | 0.37 |
| Sed x OM | 1 | 0.0151 | 1.4971 | 14.65 | 7.31 | 0.72 | 1.01 | 3.2925 | 11.54 · |
| Sed x Lo (OM) | 2 | 0.0101 | 0.2773 | 2 | 1.99 | 0.71 | 2.53 | 0.2853 | 1.14 |
| Sed x Si (Lo (OM)) | 8 | 0.0365 | 2.8063 * | 1.01 | 4 ** | 0.28 | 0.92 | 0.2511 | 1.36 |
| Residual | 48 | 0.013 | | 0.25 | | 0.31 | | 0.6111 | |
| Cochran's C test | | C=0.20998, P>0.05 | | C= 0.159, P>0.05 | | C=0.19608, P>0.05 | | C=0.190, P>0.05 | |
| Transformation | | - | | log (x + 1) | | log(x + 1) | | $\sqrt[3]{x}$ | |
| | | Capitella capitata | | Capitella minima | | Diplocirrus glaucus | | Exogone naidina | |
| Source of variation | df | MS | F | MS | F | MS | F | MS | F |
| Sed | 1 | 6403 | 26.34 · | 11.22 | 39.23 * | 6.72 | 18.62 · | 19.0139 | 27.94 · |
| OM | 1 | 34804 | 11.07 | 25 | 10.47 · | 9.39 | 8.24 | 23.3472 | 186.78 * |
| Lo (OM) | 2 | 3144 | 3.17 | 2.39 | 2.3 | 1.14 | 2.65 | 0.125 | 0.05 |
| Si (Lo (OM)) | 8 | 991 | 1.41 | 1.04 | 2.38 * | 0.43 | 1.19 | 2.4028 | 1.75 |
| Sed x OM | 1 | 5922 | 24.36 · | 8.57 | 29.96 * | 8 | 22.15 · | 19.0139 | 27.94 · |
| Sed x Lo (OM) | 2 | 243 | 0.65 | 0.29 | 1.17 | 0.36 | 0.55 | 0.6806 | 0.31 |
| Sed x Si (Lo (OM)) | 8 | 372 | 0.53 | 0.24 | 0.56 | 0.65 | 1.81 | 2.1806 | 1.69 |
| Residual | 48 | 705 | | 0.44 | | 0.36 | | 1.375 | |
| Cochran's C test | | | | C=0.224 , P>0.05 | | | | | |
| Transformation | | - a | | log (x + 1) | | - a | | - a | |
| | | Galathowenia oculata | | Gallardoneris iberica | | Levinsenia cf. flava | | Micronephtys stammeri | |
| Source of variation | df | MS | F | MS | F | MS | F | MS | F |
| Sed | 1 | 1.54 | 46.82 * | 0.06 | 0.01 | 2.689 | 51.74 * | 11.21 | 25.64 * |
| OM | 1 | 6.16 | 475.98 ** | 22.58 | 9.99 · | 6.0082 | 479.22 ** | 17.84 | 24.86 * |
| Lo (OM) | 2 | 0.01 | 0.1 | 2.26 | 20.95 *** | 0.0125 | 0.04 | 0.72 | 1.19 |
| Si (Lo (OM)) | 8 | 0.13 | 0.67 | 1.11 | 0.45 | 0.3075 | 1.68 | 0.6 | 2.38 * |
| Sed x OM | 1 | 1.54 | 46.82 * | 5.72 | 12.19 · | 2.689 | 51.74 * | 8.2 | 18.91 * |
| Sed x Lo (OM) | 2 | 0.33 | 0.2 | 0.47 | 1.61 | 0.052 | 0.21 | 1.15 | 1.15 |
| Sed x Si (Lo (OM)) | 8 | 0.16 | 0.83 | 0.29 | 1.21 | 0.2444 | 1.34 | 1.48 | 1.48 |
| Residual | 48 | 0.19 | | 0.24 | | 0.1826 | | 0.25 | |
| Cochran's C test | | C=0.232, P>0.05 | | C=0.141, P>0.05 | | C=0.216, P>0.05 | | C=0.152, P>0.05 | |
| Transformation | | log(x + 1) | | log(x + 1) | | $\sqrt[3]{x}$ | | $\sqrt[3]{x}$ | |
| | | Paralacydoniidae paradoxa | | Prionospio fallax | | Microspio mecznikowianus | | Ophyotrocha sp. | |
| Source of variation | df | MS | F | MS | F | MS | F | MS | F |
| Sed | 1 | 0.48 | 202.47 ** | 6.13 | 6.04 | 5.5556 | 0.5076 | 5.01 | 2.98 |
| OM | 1 | 4.67 | 31.96 * | 8.68 | 15.25 · | 14.222 | 6.9189 | 5.01 | 0.57 |
| Lo (OM) | 2 | 0.15 | 1.55 | 0.57 | 0.31 | 2.0556 | 0.9136 | 8.74 | 1.13 |
| Si (Lo (OM)) | 8 | 0.09 | 0.42 | 1.82 | 1.82 · | 2.25 | 1.209 | 7.71 | 3.03 * |
| Sed x OM | 1 | 0.48 | 202.47 ** | 5.01 | 4.95 | 3.5556 | 0.3249 | 0.68 | 0.41 |
| Sed x Lo (OM) | 2 | 0 | 0.01 | 1.01 | 0.5 | 10.944 | 11.9394 * | 1.68 | 0.47 |
| Sed x Si (Lo (OM)) | 8 | 0.29 | 1.3 | 2.04 | 2.04 · | 0.9167 | 0.4925 | 28.78 | 1.42 |
| Residual | 48 | 0.23 | | 1 | | 1.8611 | | 2.54 | |
| Cochran's C test | | C=0.231, P>0.05 | | C=0.222, P>0.05 | | | | | |
| Transformation | | log(x + 1) | | - | | - a | | - a | |
| | | Pseudopolydora pulchra | | Sphaerosyllis dimenti | | Spiochaetopterus costarum | | Spio decoratus | |
| Source of variation | df | MS | F | MS | F | MS | F | MS | F |
| Sed | 1 | 2.52 | 9.29 · | 62.347 | 121.32 * | 0.01 | 0.15 | 0.96323 | 3.26 |
| OM | 1 | 6.91 | 6.93 | 66.125 | 952.2 * | 6.33 | 4.83 | 2.71626 | 1.25 |
| Lo (OM) | 2 | 1 | 1.4 | 0.069 | 0.02 | 1.31 | 2.12 | 2.17742 | 10.9328 ** |
| Si (Lo (OM)) | 8 | 0.71 | 1.11 | 3.236 | 1.11 | 0.62 | 2.21 * | 0.19916 | 0.83 |
| Sed x OM | 1 | 0.06 | 0.02 | 58.681 | 114.18 * | 3.64 | 65.05 * | 1.48144 | 5.02 |
| Sed x Lo (OM) | 2 | 0.27 | 0.41 | 0.514 | 0.19 | 0.06 | 0.07 | 0.29506 | 1.03 |
| Sed x Si (Lo (OM)) | 8 | 0.66 | 1.03 | 2.681 | 0.92 | 0.77 | 2.77 * | 0.28521 | 1.19 |
| Residual | 48 | 0.64 | | 2.903 | | 0.28 | | 0.239 | |
| Cochran's C test | | C=0.166 , P>0.05 | | | | C=0.193, P>0.05 | | C= 0.233, P>0.05 | |
| Transformation | | log(x + 1) | | - a | | $\sqrt[3]{x}$ | | $\sqrt{x}$ | |

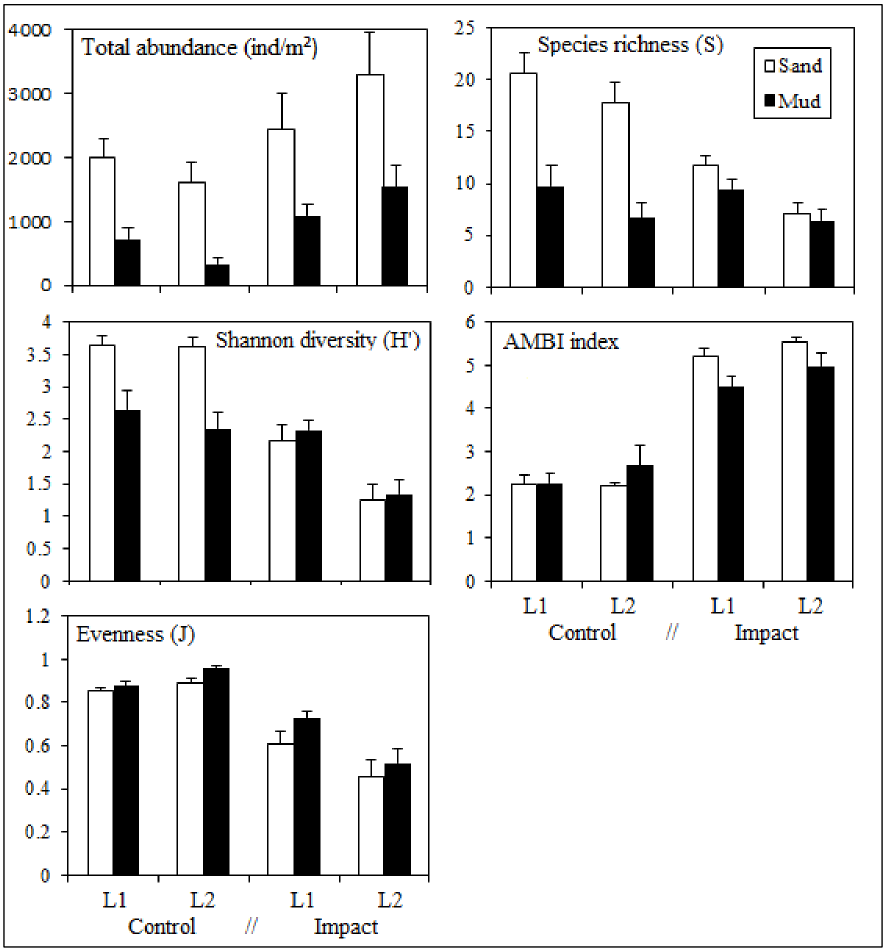


Figure 5.3. Mean values (± standard error) of Total abundance, Species richness (S), Shannon diversity (H'), Evenness (J) and AMBI in the EUs at two fish farms (L1 and L2).

Inside the impacts, there were differences between locations. In controls, sandy EUs had higher S than muddy EUs. H' was significant affected by the fish farm in sandy EUs and in one location of muddy EUs. H' presented no significant differences between sediments in impact EUs; however, there were differences between localities. AMBI software displayed their results with 20% of species not assigned. AMBI had only significant differences between controls and impacts. Controls had values from 2.3 to 2.7, corresponding to slightly disturbed, while impacts had values from 4.5 to 5.5, the limit between moderately disturbed and heavily disturbed, consistently between sediments or localities. J was the less consistent with differences between controls and impacts, also with differences between localities, sites and sediments in many combinations (Figure 5.3, Table 5.1). Table 5.2 summarized all the species found in the EUs: 80 species of polychaetes, belonging to 34 families. In the analyses of the polychaete assemblage structure, the MDS plot had an ordination of the EUs in two main groups according to fish farm presence (Figure 5.4), group control and group impact. Organic matter did not have an influence on the separation of these two groups, but it had an influence in the spatial distribution depending on the original sediment (mud or sand) with a significant but low correlation level in RELATE procedures ($p = 0.01$, Rho = 0.116). TFS seemed to influence the separation of the two groups in MDS, but some points in each group were not consistent with the others (Figure 5.4); RELATE corroborated this correlation ($p = 0.01$, Rho = 0.128). The SIMPER procedure indicated a segregation of those EUs affected by the fish farm with an average dissimilarity of 90.32 compared with the reference EUs. These differences derived mainly from the greater abundance of *Capitella capitata*, *Spiochaetopterus costarum*, *Capitella minima*, *Pseudopolydora pulchra* and *Aricidea (Strelzovia) claudiae* in the fish farm EUs, and to the abundance of *Gallardoneris iberica*, *Micronephtys stammeri*, *Sternaspis scutata* and *Ampharete lindstroemi* in reference EUs (Table 5.3).

Among the eighteen most-abundant species in the EUs (Figure 5.5 and Table 5.2), the species *Ampharete lindstroemi, Diplocirrus glaucus, Exogone (Exogonoe) naidina, Galathowenia oculata, Levinsenia* cf. *flava, Micronephtys stammeri, Paralacydonia paradoxa* and *Sphaerosyllis climenti* had significant differences in the factor ‘control-farm’, with drastic reductions in abundance under fish farms. *Gallardoneris iberica* and *Spio decoratus* had lower abundance under fish farms in sandy samples. The species *Aricidea (Strelzovia) claudiae*, *Capitella minima* and *Spiochaetopterus costarum* had significant differences between farms and controls, but there were also some interactions between sites and localities. *Capitella capitata* showed a tendency to increase its abundance under fish farms. *Microspio mecznikowianus*, *Ophyotrocha sp. Prionospio fallax* and *Pseudopolydora pulchra* did not show any significant difference for any factor (Figure 5.5, Table 5.1).

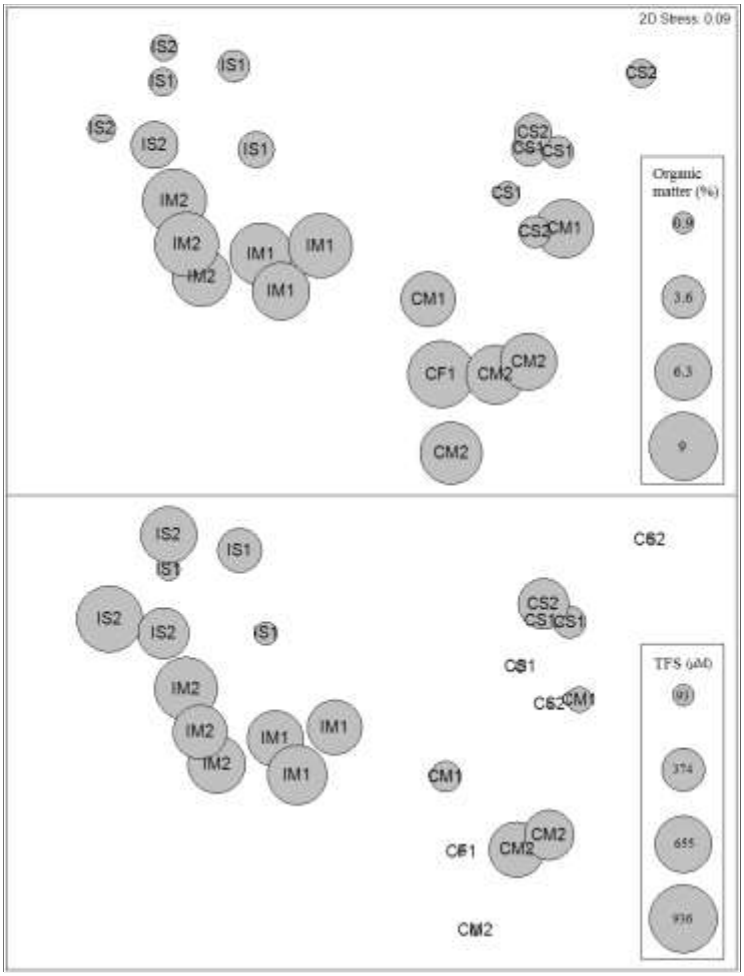


Figure 5.4. Non-metric multidimensional scaling ordination (MDS) with superimposed bubble plots correlating polychaete assemblage and organic matter %, above, and total free sulphide (TFS), below.

Table 5.2. Polychaete species collected in EUs (ind/m²) below fish farms (Impact) and in the reference area (Control), in sandy and muddy sediments, and in two locations (Loc1 and Loc2). * Most-abundant species, eighteen selected.

| | Control | | | | Impact | | | |
|---|---|---|---|---|---|---|---|---|
| | Sand | | Mud | | Sand | | Mud | |
| | Loc1 | Loc2 | Loc1 | Loc2 | Loc1 | Loc2 | Loc1 | Loc2 |
| Ampharetidae Malmgren, 1866 | | | | | | | | |
| **Ampharete lindstroemi* Malmgren, 1867 sensu Hessle, 1917 | 176 | 120 | 159 | 42 | 4 | 0 | 0 | 0 |
| *Amage adspersa* (Grube, 1863) | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| Capitellidae Grube, 1862 | | | | | | | | |
| **Capitella capitata* (Fabricius, 1780) | 99 | 11 | 74 | 4 | 1492 | 2561 | 550 | 1153 |
| **Capitella minima* Langerhans, 1881 | 18 | 25 | 18 | 7 | 713 | 187 | 92 | 42 |
| *Heteromastus filiformis* (Claparède, 1864) | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| *Mediomastus capensis* Day, 1961 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 |
| *Notomastus aberans* Day, 1957 | 7 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| Capitellidae incomp. | 7 | 14 | 0 | 0 | 21 | 4 | 7 | 0 |
| Chaetopteridae Audouin & Milne Edwards, 1833 | | | | | | | | |
| **Spiochaetopterus costarum* (Claparède, 1869) | 42 | 14 | 7 | 0 | 21 | 99 | 78 | 116 |
| Cirratulidae Carus, 1863 | | | | | | | | |
| *Aphelochaeta marioni* (Saint-Joseph, 1894) | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| *Aphelochaeta monilaris* (Hartman, 1960) | 14 | 0 | 4 | 14 | 4 | 0 | 4 | 7 |
| *Caulleriella* sp. | 11 | 21 | 0 | 4 | 0 | 0 | 0 | 0 |
| *Chaetozone setosa* Malmgren, 1867 | 32 | 25 | 11 | 11 | 7 | 0 | 4 | 0 |
| *Chaetozone gibber* Woodham & Chambers, 1994 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Monticellina dorsobranchialis* (Kirkegaard, 1959) | 32 | 11 | 4 | 21 | 0 | 0 | 7 | 0 |
| Cirratulidae incomp. | 4 | 7 | 0 | 0 | 7 | 0 | 0 | 0 |
| Cossuridae Day, 1963 | | | | | | | | |
| *Cossura soyeri* Laubier, 1964 | 14 | 14 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dorvilleidae Chamberlin, 1919 | | | | | | | | |
| **Ophyotrocha* sp. | 14 | 11 | 4 | 0 | 4 | 67 | 0 | 25 |
| Eunicidae Berthold, 1827 | | | | | | | | |
| *Eunice cf. pennata* (Müller, 1776) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| *Eunice vittata* (Delle Chiaje, 1828) | 4 | 0 | 4 | 0 | 0 | 0 | 4 | 0 |
| *Lysidice unicornis* (Grube, 1840) | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Flabelligeridae de Saint-Joseph, 1894 | | | | | | | | |
| **Diplocirrus glaucus* (Malmgren, 1867) | 56 | 32 | 7 | 0 | 0 | 0 | 4 | 0 |
| Glyceridae Grube, 1850 | | | | | | | | |
| *Glycera alba* (O.F. Müller, 1776) | 4 | 4 | 0 | 0 | 11 | 7 | 18 | 11 |
| Goniadidae Kinberg, 1866 | | | | | | | | |
| *Goniada maculata* Örsted, 1843 | 4 | 11 | 7 | 18 | 0 | 4 | 0 | 4 |
| Hesionidae Grube, 1850 | | | | | | | | |
| *Syllidia armata* Quatrefages, 1866 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |
| Lumbrineridae Schmarda, 1861 | | | | | | | | |
| **Gallardoneris iberica* Martins *et al.* 2012 | 279 | 134 | 123 | 74 | 14 | 4 | 71 | 18 |
| *Lumbrineris cingulata* Ehlers, 1897 | 14 | 14 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Lumbrineris nonatoi* Ramos, 1976 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 |

Continuation Table 5.2. Polychaete species collected in EUs (ind/m²) below fish farms (Impact) and in the reference area (Control), in sandy and muddy sediments, and in two locations (Loc1 and Loc2). * Most-abundant species, eighteen selected.

| | Control | | | | Impact | | | |
|---|---|---|---|---|---|---|---|---|
| | Sand | | Mud | | Sand | | Mud | |
| | Loc 1 | Loc 2 | Loc 1 | Loc 2 | Loc 1 | Loc 2 | Loc 1 | Loc 2 |
| Lumbrineridae Schmarda, 1861 | | | | | | | | |
| **Gallardoneris iberica* Martins *et al.* 2012 | 279 | 134 | 123 | 74 | 14 | 4 | 71 | 18 |
| *Lumbrineris cingulata* Ehlers, 1897 | 14 | 14 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Lumbrineris nonatoi* Ramos, 1976 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 |
| Magelonidae Cunningham & Ramage, 1888 | | | | | | | | |
| *Magelona equilamellae* Harmelin, 1964 | 28 | 42 | 4 | 4 | 0 | 0 | 0 | 0 |
| Maldanidae Malmgren, 1867 | | | | | | | | |
| *Clymenura (clymenura) clypeata* (Saint-Joseph, 1894) | 14 | 7 | 7 | 0 | 0 | 0 | 0 | 0 |
| *Euclymene oerstedi* (Claparède, 1863) | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| Sfam. Eudymeninae | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| Nephtyidae Grube, 1850 | | | | | | | | |
| **Micronephthys stammeri* (Augener, 1932) | 339 | 339 | 60 | 18 | 18 | 7 | 11 | 7 |
| *Nephtys cirrosa* Ehlers, 1868 | 11 | 4 | 14 | 4 | 0 | 0 | 7 | 14 |
| Nereididae Blainville, 1818 | | | | | | | | |
| *Neanthes caudata* (Delle Chiaje, 1827) | 0 | 0 | 0 | 0 | 39 | 32 | 11 | 7 |
| Onuphidae Kinberg, 1865 | | | | | | | | |
| *Aponuphis bilineata* (Baird, 1870) | 0 | 0 | 0 | 0 | 7 | 7 | 4 | 0 |
| Opheliidae Malmgren, 1867 | | | | | | | | |
| *Armandia cirrhosa* Filippi, 1861 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| *Ophelina modesta* Støp-Bowitz, 1958 | 14 | 18 | 7 | 0 | 0 | 11 | 0 | 0 |
| Orbiniidae Hartman, 1942 | | | | | | | | |
| *Scoloplos (Scoloplos) armiger* (Müller, 1776) | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oweniidae Rioja, 1917 | | | | | | | | |
| **Galathowenia oculata* (Zachs, 1923) | 74 | 56 | 14 | 14 | 0 | 0 | 0 | 0 |
| *Owenia fusiformis* Delle Chiaje, 1844 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Paralacydoniidae Pettibone, 1963 | | | | | | | | |
| **Paralacydonia paradoxa* Fauvel, 1913 | 46 | 46 | 14 | 35 | 0 | 0 | 0 | 0 |
| Paraonidae Cerruti, 1909 | | | | | | | | |
| *Aricidea (Aricidea) capensis* bansei Laubier & Ramos, 1974 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |
| *Aricidea (Acmira) catherinae* Laubier, 1967 | 35 | 4 | 0 | 4 | 7 | 0 | 14 | 4 |
| * *Aricidea (Strelzovia) claudiae* Laubier, 1967 | 25 | 0 | 4 | 0 | 11 | 4 | 53 | 25 |
| **Levinsenia cf. flava* (Strelzov, 1973) | 71 | 81 | 14 | 4 | 0 | 0 | 0 | 0 |
| *Paradoneis lyra* (Southern, 1914) | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Paraonides* sp. | 0 | 0 | 4 | 0 | 11 | 0 | 0 | 0 |
| Pectinariidae Quatrefages, 1866 | | | | | | | | |
| *Amphictene auricoma* (O.F. Müller, 1776) | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 |
| *Lagis koreni* Malmgren, 1866 | 39 | 14 | 14 | 14 | 18 | 18 | 11 | 11 |
| Phyllodocidae Örsted, 1843 | | | | | | | | |
| *Mystides* sp. | 11 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Phyllodoce cf. mucosa* Örsted, 1843 | 4 | 14 | 4 | 0 | 14 | 18 | 7 | 4 |
| *Paranaitis wahlbergi* (Malmgren, 1865) | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |

Continuation Table 5.2. Polychaete species collected in EUs (ind/m²) below fish farms (Impact) and in the reference area (Control), in sandy and muddy sediments, and in two locations (Loc1 and Loc2). * Most-abundant species, eighteen selected.

| | Control | | | | Impact | | | |
|---|---|---|---|---|---|---|---|---|
| | Sand | | Mud | | Sand | | Mud | |
| | Loc1 | Loc2 | Loc1 | Loc2 | Loc1 | Loc2 | Loc1 | Loc2 |
| Pilargidae de Saint-Joseph, 1899 | | | | | | | | |
| *Pilargis verrucosa* Saint-Joseph, 1899 | 7 | 4 | 4 | 0 | 0 | 0 | 0 | 0 |
| *Sigambra parva* (Day, 1963) | 0 | 0 | 0 | 0 | 21 | 4 | 14 | 0 |
| Polynoidae Kinberg, 1856 | | | | | | | | |
| *Harmothoe cf. impar* (Johnston, 1839) | 0 | 0 | 0 | 0 | 4 | 0 | 4 | 0 |
| Sfam. Harmothoinae | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| Sabellidae Latreille, 1825 | | | | | | | | |
| *Chone collaris* Langerhans, 1881 | 4 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| *Chone cf. duneri̇ficta* Tovar-Hernández *et al.* 2007 | 21 | 35 | 4 | 4 | 4 | 0 | 0 | 0 |
| *Euchone rubrocincta* (Sars, 1862) | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Laonome kroyeri* Malmgren, 1866 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Serpulidae Rafinesque, 1815 | | | | | | | | |
| *Spirobranchus lamarcki* (Quatrefages, 1866) | 7 | 0 | 0 | 4 | 14 | 0 | 11 | 0 |
| *Spirobranchus triqueter* (Linnaeus, 1758) | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| *Spirobranchus polytrema* (Philippi, 1844) | 0 | 0 | 4 | 0 | 18 | 0 | 0 | 0 |
| Sphaerodoridae Malmgren, 1867 | | | | | | | | |
| Sphaerodoridae incom. | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Spionidae Grube, 1850 | | | | | | | | |
| *Aonides paucibranchiata* Southern, 1914 | 21 | 4 | 0 | 7 | 11 | 0 | 11 | 0 |
| *Laonice cirrata* (M. Sars, 1851) | 0 | 4 | 4 | 0 | 0 | 4 | 0 | 0 |
| **Microspio mecznikowianus* (Claparède, 1869) | 11 | 81 | 28 | 0 | 0 | 7 | 0 | 0 |
| *Paraprionospio pinnata* (Ehlers, 1901) | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Prionospio fallax* Söderström, 1920 | 42 | 60 | 21 | 0 | 14 | 0 | 7 | 4 |
| *Prionospio* sp. | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Pseudopolydora pulchra* (Carazzi, 1893) | 56 | 46 | 35 | 0 | 92 | 212 | 53 | 81 |
| **Spio decoratus* Bobretzky, 1870 | 63 | 11 | 18 | 0 | 4 | 0 | 14 | 0 |
| *Spio filicornis* (Müller, 1776) | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Spiophanes bombyx* (Claparède, 1870) | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Spiophanes kroyeri* Grube, 1860 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| Spionidae incomp. | 0 | 18 | 4 | 0 | 0 | 7 | 11 | 0 |
| Sternaspidae Carus, 1863 | | | | | | | | |
| *Sternaspis scutata* Ranzani, 1817 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 |
| Syllidae Grube, 1850 | | | | | | | | |
| **Exogone naidina* Örsted, 1845 | 60 | 78 | 7 | 0 | 0 | 0 | 0 | 0 |
| **Sphaerosyllis climenti* Del-Pilar-Ruso & San Martín, 2012 | 113 | 127 | 7 | 0 | 4 | 0 | 0 | 0 |
| *Syllis gracilis* Grube, 1840 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 |
| *Syllis vittata* Grube, 1840 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 |
| Syllis sp. | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |
| Terebellidae Johnston, 1846 | | | | | | | | |
| *Amphitrite sp.* | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Pista unibranchia* Day, 1963 | 7 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| Terebellidae incomp. | 4 | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| Poecilochaetidae Hannerz, 1956 | | | | | | | | |
| *Poecilochaetus serpens* Allen, 1904 | 14 | 18 | 4 | 0 | 35 | 39 | 0 | 4 |

Table 5.3. Summary of SIMPER analyses results showing the most important polychaete species that influence the two groups made by MDS. Control: control group and Impact: impact group. Contrib.%: percentage of contribution; Cum%: cumulative percentage; Av. Abund: average dissimilarity.

| Av.D = 90.32 % | Control | Impact | | | |
|---|---|---|---|---|---|
| Species | Av. Abund | Av. Abund | Av. Diss | Contrib. % | Cum. % |
| *Capitella capitata* | 0.12 | 3.11 | 10.37 | 11.48 | 11.48 |
| *Gallardoneris iberica* | 3.68 | 0.58 | 9.31 | 10.3 | 21.78 |
| *Spiochaetopterus costarum* | 0.32 | 1.66 | 5.27 | 5.83 | 27.62 |
| *Micronephtys stammeri* | 2.37 | 0.14 | 5.01 | 5.55 | 33.17 |
| *Capitella minima* | 0.08 | 1.42 | 4.16 | 4.61 | 37.78 |
| *Sternaspis scutata* | 2.51 | 0 | 3.33 | 3.68 | 41.46 |
| *Pseudopolydora pulchra* | 0.3 | 1.05 | 3.2 | 3.54 | 45 |
| *Ampharete lindstroemi* | 1.11 | 0.01 | 2.75 | 3.04 | 48.04 |
| *Aricidea (Strelzovia) claudiae* | 0.2 | 0.62 | 2.35 | 2.6 | 50.65 |

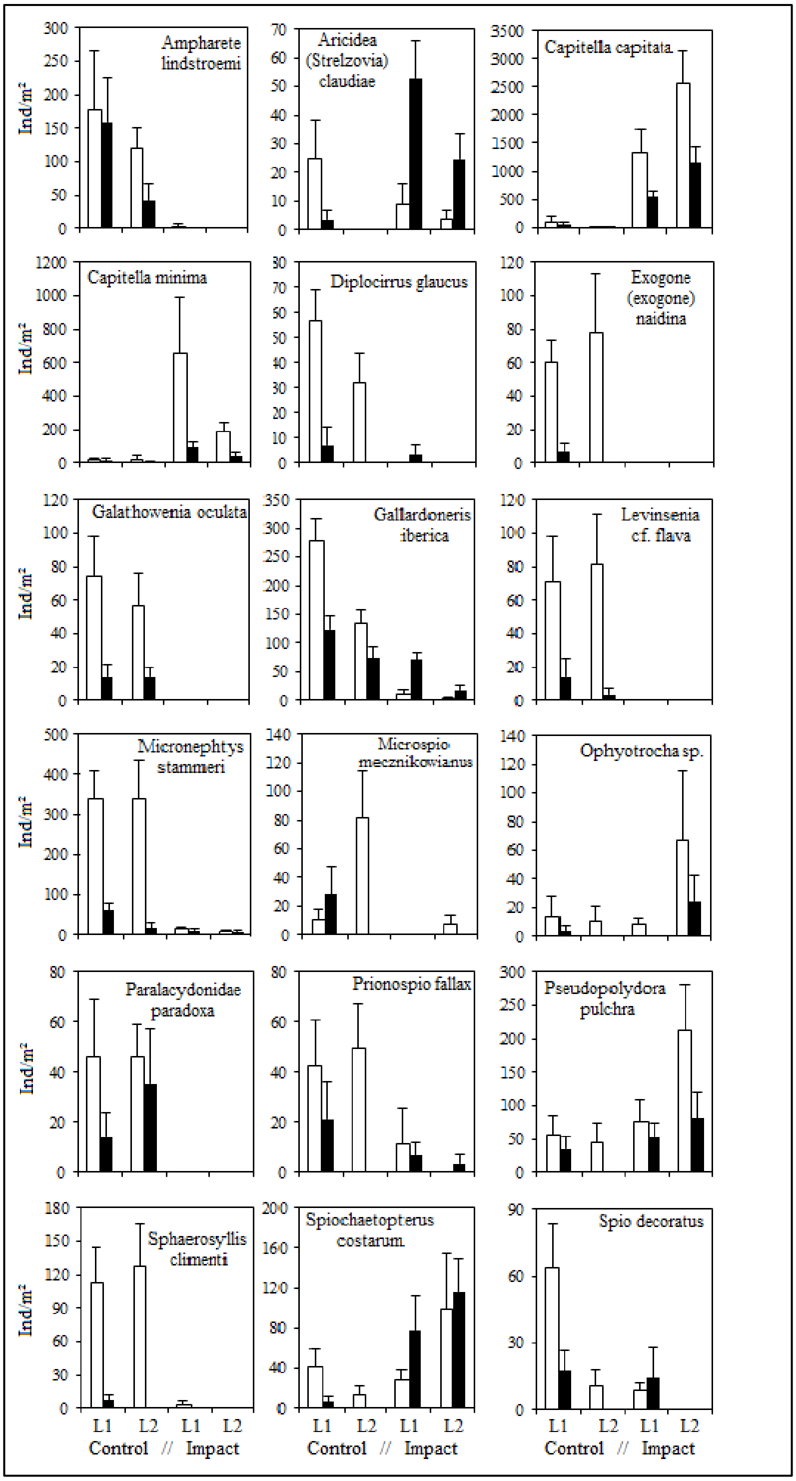


Figure 5.5. Mean values (±standard error) of the eighteen most-abundant polychaetes in the EUs at two fish farms (L1 and L2).

**5.5. Discussion**

The EUs used in this experiment could represent a good tool for the marine environmental impact studies. Accurate information on the status of the environment was provided by both the indices based on Pearson and Rosenberg (1978), and the multivariate analyses of polychaete assemblage in recolonisation processes with sulphide concentration measures. This approach could complement traditional benthos sampling and analyses.

Over the course of one month the EUs did not undergo significant variations in their granulometric characteristics and/or organic matter content as other authors have previously described (Guerra-García and García-Gómez 2006). However, this period of time was enough to detect small changes in TFS concentration due to the fish farms. These changes were more pronounced in sandy EUs due to the low TFS concentration in the reference area, but final TFS level was quite similar between sandy and muddy EUs, at the level set between Oxic A (TFS levels up to 500 μM) and Oxic B (TFS levels between 750 and 1500 μM) (Hargrave *et al.* 2008). Our results are not consistent with some previous laboratory experiments in which muddy sediments accumulated more TFS than sandy sediments (Martinez-Garcia *et al.* 2015), achieving Hypoxic B level (3000-6000 μM) in muddy sediments and Hypoxic A level (1500-3000 μM) in sandy sediments. This could be due to the length of the experiment, which was enough to detect changes in TFS concentration and even differences between fish farm facilities, but not representative of a long period of organic matter enrichment. With these EUs it is possible to measure 'pulse' impacts over a defined period of time. This could be very useful, for instance, to track the short-term effects of changes in the management of a fish farm facility after an administrative sanction due to environmental impacts. On the other hand, EUs could be deployed for a long period of time to determine 'press' impacts over a longer period of fish maturation (Underwood 1992).

Sediment grain size has been used as a variable to interpret other variables such as sulphides or fauna composition (Aguado-Giménez *et al.* 2015). The sediment largely determines the polychaete composition, so responses to fish farms can be different (Giangrande *et al.* 2005, Martinez-Garcia *et al.* 2013). This fact is important when choosing an appropriate reference area, which sometimes can lead to mistaken results (Forchino *et al.* 2011) due to the high sampling variability between small-scales and large-scales and its effects on benthic community spatial patterns (Tataranni and Lardicci 2009, Fernandez-Gonzalez *et* *al.* 2013). In this experiment, sediment grain size was fixed from the start, and the recolonisation process did not depend on it, otherwise factors such as larvae supplied and adults played a major role in the new polychaete assemblage (Guerra-García and García-Gómez 2006). EUs can be filled with sediment similar to the surrounding sediment or standard sediment to test variability between distant localities or different natural sediments. In this experiment, although the surrounding sediment was mud, the sandy and muddy EUs had the same level of pollution under fish farms when H', J and AMBI were calculated with polychaeta recolonisation data. However, when assessing total abundance and S, the sandy EUs had higher values, possibly because at this stage the community was at an earlier stage of the recolonisation process (Guerra-García and García-Gómez 2009). Differences under fish farms are due mainly to the proliferation of *Capitella capitata* and *Capitella minima* in organic enrichment conditions (Pearson and Rosenberg 1978, Tsutsumi *et al.* 1990) in a greater degree in sandy EUs. H' is sensitive to sediments and locations; in sandy EUs the differences between the reference area and the fish farms were noticeable, but in muddy EUs they were not as clear (Aguado-Giménez *et al.* 2007, Borja *et al.* 2009, Tomassetti *et al.* 2016). J graphs seem to be clear but the statistical analyses showed that the differences were due to location or site. The index with the best results was AMBI, which detects the anthropogenic organic enrichment with no interference, regardless of sediment type or location. AMBI has been used widely in assessing the ecological status of benthic communities for the European Water Framework Directive (Muxica *et al.* 2005, Borja *et al.* 2009, Keeley *et al.* 2012a). In the EUs case, it provides precise information when using only polychaetes. Moreover, other reason to use only polychaetes in EUs is that amphipod recolonisation below the fish farm is strongly dependent on the input of amphipods from the high densities of fouling communities (Fernandez-Gonzalez *et al.* 2016), which could interfere with the final results, despite the good results that indices with amphipods taxa have over the sediment as BOPA (Dauvin and Ruellet 2007). Likewise, we do not know how other benthic fauna behaves in relation to recolonisation. Multivariate analyses has similar results to AMBI (Aguado-Giménez *et al.* 2007), so any expert can achieve the same conclusion with the data, especially when there is not enough information on the species and their ecological group, which can be difficult to apply in some indices (Keeley *et al.* 2012b). Multivariate analyses are also used worldwide to detect changes due to fish farms (Aguado-Giménez *et al.* 2007, Martinez-Garcia *et al.* 2013, Tomassetti *et al.* 2016). Consequently, authors should decide which method best suits their environmental studies. In addition, biodiversity indices and multidimensional analyses should be combined with chemical assessment such as TFS to fully determine the degree of disturbance (Tomassetti *et al.* 2016).

In addition, when the eighteen most-abundant species were used to interpret the underlying ecological changes that relate to biological indices, we can observe that each behaves differently from the others when recolonizing sediments. Several species related to conditions stressed by organic enrichment appeared in non-polluted areas, and vice versa (Dean 2008). For instance, we placed *Aricidea (Strelzovia) claudiae* and *Spiochaetopterus costarum*, in group I (species sensitive to organic enrichment and present under unpolluted conditions- Borja *et al.* 2000), but these species were also present in fish farm areas. We also found *Capitella capitata* and *Capitella minima* in these fish farm EUs, which are well documented to be a pollution indicator, and are catalogued by AMBI in group V (first-order opportunistic species - pronounced unbalanced situations - Borja *et al.* 2000). The species with most abundance in the reference area that disappeared under fish farms were catalogued in group I, *Ampharete lindstroemi* and *Diplocirrus glaucus*; group II (species indifferent to enrichment, always present in low densities with non-significant variations with time, from initial state to slight unbalance- Borja *et al.* 2000), such as *Exogone (Exogone) naidina*, *Micronephtys stammeri* and *Paralacydonidae paradoxa*; and group III (species tolerant to excess organic matter enrichment, these species may occur under normal conditions, but their populations are stimulated by organic enrichment, slight unbalance situations- Borja *et al.* 2000), such as *Galathowenia oculata*, *Levinsenia cf. flava* and *Spio decuratus*. Other species were not catalogued yet, such as *Gallardoneris iberica* and the recently described species *Sphaerosyllis climenti*. Therefore, this highlights the importance of studying the changes in the assemblages when monitoring fish farms, and not just focusing on the presence of certain species (Dimitriou *et al.* 2012). However, our results suggest that it is not necessary to study the entire benthic assemblage because polychaete assemblages in recolonisation EUs gave a complete and reliable source of information about environmental quality status (Martinez-Garcia *et al.* 2013). The Polychaeta taxon resolves the problem of monitoring in hard substrata when it seems that most of the fauna are sessile. There are some documented hard-bottom vagile polychaetes that can be used as indicators (see review Giangrande *et al.* 2005).

The time taken to recolonize new sediments could vary depending on the temperature; tropical and subtropical areas with warm waters require less time, and cold waters such as northern Europe require longer periods (Lu and Wu 1998). In northern Africa, Guerra-García and García-Gómez (2009) found that univariate indices such as total abundance, S, H' and J presented similar results for recolonisation and natural sediments after one month, indicating a total recovery of the sediments, whereas the multivariate approach showed a complete recolonisation after 3 months. According to our results, in order to evaluate the impact level of a fish farm, one month is enough time to detect changes influenced by the aquaculture effect in recolonisation. Seasonal effects are important in determining species composition and community structure in benthic habitats (Karakassis *et al.* 2013) and in benthic recolonisation, showing higher total abundance, S and H' in summer than in winter (Lu and Wu 2007). However, Zajac and Whitlatch (1982) found that species responses to disturbance were quite variable and that no seasonal pattern of recolonisation was observable. Further experimental studies would be necessary to define the sensitivity of EUs in different seasons, but summer or early autumn seem to be the periods of highest fish farming activity due to higher water temperatures. Longer periods of time could be chosen to detect 'press' impacts if needed, as previously noted. The time of deployment should also be test at different latitudes.

This study proposes the use of a new approach to the environmental management of fish farms, using pre-built EUs with selected defaunated sediment, thereby reducing the environmental variables that could affect field sampling. Using AMBI or MDS analyses with polychaeta assemblages shows clear results, with less interference of other variables. On one hand high taxonomic resolution is required, but on the other hand it is not necessary to study the whole macrofauna assemblage. EUs filled with sandy sediments make the difference more apparent in comparison to reference area EUs, and they are also more sensitive to detect changes in TFS and organic enrichment than muddy EUs. Even so, a better understanding of the effectiveness of EUs in environmental impact over other types of substrata is needed for a general use of these devices as a tool for marine monitoring programs.

# General Discussion

# Chapter 6

Photo: Pablo Arechavala López

# 6. General Discussion

Sustainable aquaculture is an essential goal if we wish to maintain the goods and services that marine environments provide (Frankic and Hershner 2003). The recent growth of aquaculture activities along our coastlines could potentially cause a negative impact in the ecosystem (Fernandes *et al.* 2001, Holmer *et al.* 2008, Olsen *et al.* 2008), but the sensitive management of these activities could largely minimise their impact (Primavera 2006). Good management procedures may also facilitate the recuperation of the ecosystem once the activity ceases; for example, if the carrying capacity of the sediments is not exceeded, they will be resilient enough to rapidly return to their original state (Huntington *et al.* 2006). However, if the sediments suffered repeated anoxia episodes, they may have difficulties to achieve full recovery (Conley *et al.* 2009).

The wastes of organic matter, mainly uneaten food (pellets or bait) and fish faeces, lead to an increase of organic enrichment, which could generate significant changes in the sediment biogeochemistry, with an extreme situation of anoxia (Dostat 2009). This situation produces one of the most serious changes: the shifting from aerobic to anaerobic respiration in microbial community (Holmer *et al.* 2005), that produces different toxic compounds for benthic organisms (Wang and Chapman 1999, Vaquer-Sunyer and Duarte 2010).

Nowadays, environmental management and monitoring have progressed significantly, with help of technology development, to evaluate these changes over the sediment. Many chemical analyses and biotic indices are available to detect the possible interactions in the benthic habitat. However, it is still possible to add some recommendations in three working lines: prevention measures, simplification of environmental monitoring plans and provision of new tools to reduce the spatial heterogeneity (Figure 6.2).

## 6.1. Sediment as key factor

In order to achieve sustainable aquaculture, avoiding to exceed the carrying capacity of the ecosystem, an appropriate management should ideally start at the planning stage, prior to the installation of the fish farm cages. A substantial number of papers have been published on the subject of site selection (as summarised in Sanchez-Jerez *et al.* 2016), aiming to find the most suitable areas for fish farm facilities, with a good balance between ecological, social and economic issues. The impact on the ecosystem depends, among other factors, on the resilience of the receiving environment (Huntington *et al.* 2006). For this reason, habitats with ecological value are avoided, such as *Posidonia oceanica* meadows or maerl beds (Barberá *et al.* 2003, Holmer *et al.* 2008, Karakassiss 2013). Hence, soft sediments are usually selected for these facilities, despite the fact that little is known about which types of soft sediment would be more suitable for aquaculture activities. The influence of grain size on biogeochemical processes has been studied (Chapter 4 and 5), and based on our results, sandy sediments typically showed less deleterious effects than muddy sediments (Figure 6.1). Moreover, sandy benthic communities have a more complex structure, with a larger number of species. A "complex" benthic macrofauna increases the prevalence of burrowing behaviour, leading to higher levels of sediment bioturbation, and thus promoting the bioirrigation of deeper layers of the sediment (Gingras *et al.* 2014). Bioirrigation and bioturbation enhance OM mineralization and nutrient recycling (Volkenborn *et al.* 2007). On the other hand, simple benthic macrofauna communities (often dominated by smaller opportunistic polychaetes) have lower bioirrigation, which results in slower mineralization rates (Heilskov *et al.* 2006). In summary, sediments with complex benthic structures usually have a higher resilience, a greater capacity to withstand fish farm waste without collapsing, and a higher ability to return to an original state once the perturbation ceases (Odum 1985).

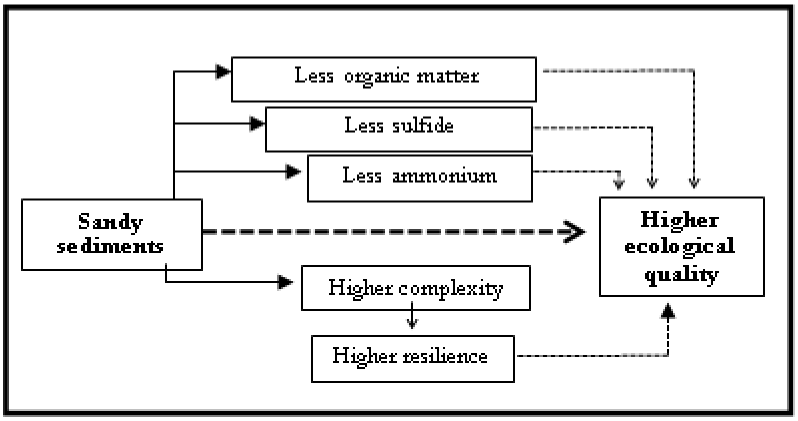


Figure 6.1. Effects of sandy benthic sediments under organic enrichment conditions.

In our studies sandy sediments were better able to maintain a minimum level of ecological quality under aquaculture facilities, preserving the ecosystem services more effectively. Consequently, granulometry, and especially the finest grain size in soft sediments, should be considered as a key parameter when selecting sites for fish farm cages (Figure 6.2).

### 6.2. Environmental Monitoring Programs (EMP) as a tool to achieve sustainability

After the Allowed Zone for Aquaculture is selected and the site is put into service for aquaculture production, EMP should be a compulsory requirement for a proper evaluation of surrounding marine environment ecological status (Sanchez-Jerez *et al.* 2016). To evaluate the level of alteration over the soft sediments, defining the balance between aerobic and anaerobic conditions, it is possible to use both physicochemical and biological indicators in EMP (Borja and Dauer 2008). With their results, administrators and managers should decide if it is necessary to take any measures to reduce the environmental impact. However, due to the complexity of marine ecosystems this is not an easy task. In the last decade, due to the implementation of the Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC), researchers have developed many different protocols and indicators to characterise and evaluate the ecological status of European coastal areas with high precision (Birk *et al.* 2012). This has caused some problems for managers due to the oversaturation of methodologies, because it is not possible to apply all of them for time and economic reasons. Clearly, better ecological status information is obtained from a greater number of variables and data, but such methodological diversity is not suitable for the successful implementation of the WFD (Birk *et al.* 2012). Therefore, there is a need to provide practical information and guidelines for managers to achieve a good balance between a representative quality status framework and a feasible set of physicochemical and biological variables. On the other hand, local administrators are responsible for the establishment of common EMP requirements, and there is no consensus between all of them, so each region has its own EMPs (Figure 6.2.).

### 6.3. Recommendations for environmental monitoring plans

An example of intent of EMP harmonisation is the proposal of EMP for the Spanish territory developed by the Ministry of Agriculture, Food and Environment, through the JACUMAR funding program. After 4 years of research, in 2012, an EMP was designed by an expert group with the aim of unifying all of these requirements, following a participatory approach (JACUMAR-EMP- Aguado-Giménez *et al.* 2012a). This was an exceptional step in the harmonisation of marine environmental management of aquaculture at European level. The ideal situation would be that, in some years, all the regional governments will implement this EMP proposal for monitoring fish farm facilities across the Spanish coastline. This change may seem inconvenient for the managers at the outset, but in a short period of time this proposal will allow to compare fish farms from different areas and obtain a long term data base. Also, it will result in a substantial reduction in costs, given that most facilities are currently carrying out more complex and expensive EMPs. Moreover, it will be easier to control all the impact of the fish farms at scale of hundreds of kms if they follow the same EMP, independently of the region.

Moreover, the JACUMAR-EMP was created using a dynamic approach, for which it is possible to modify the EMP if monitoring studies provide information that suggests some improvements. We use this approach in Chapter 2, in order to recommend some improvements for the JACUMAR-EMP. Overall, EMPs must deal with two topics: I) EMPs must balance economic and technological efforts with the acquisition of accurate information of the benthic quality status; II) EMPs have to deal with the spatial heterogeneity of the sea bottom.

### 6.3.1. Balance between cost/benefit for environmental monitoring plan design

Regarding the optimisation of data collection efforts, the EMP-JACUMAR already incorporates a reduction in the variables that are compulsory to measure. Sulphide concentration, typically measured as Total Free Sulphide (TFS), provides accurate information about the disturbance caused by aquaculture organic enrichment, as we have demonstrated in the Chapters 2, 3, 4 and 5. Therefore we recommend that TFS should be included in EMPs (Figure 6.2) (Katavić *et al.* 2005, Aguado-Giménez *et al.* 2015), and should be studied in comparison with reference areas. However, it is possible to reconsider two of the variables used: $\delta^{15}N$ and redox potential. The measurement of isotope $\delta^{15}N$ is a difficult and expensive technique, and did not provide useful information in our analyses in Chapter 2. Moreover, the isotopic signal may vary depending on the feed provided in the fish cages. The measurement of redox potential may also be reconsidered, as the measures show high variability due to potential "poisoning" of the probes (Wildish *et al.* 2005, MER 2008). Indeed, $\delta^{15}N$ and potential redox may be redundant if other variables can be used to detect the impacts of organic enrichment, such as sulphide concentration.

One of the positive proposals of EMP-JACUMAR is the use of one single group of organisms, polychaetes, at family level. The usage of lower taxonomic resolution seems to be a good approach for the optimisation of the effort (Figure 6.2), often referred to as taxonomic sufficiency. It has been shown in a range of environmental monitoring and impact studies, that the classification of samples to family level can provide a balance between sampling effort and loss of information (Warwick 1988, Gray *et al.* 1990, Beattie and Oliver 1994, Somerfield and Clarke 1995, Olsgard *et al.* 1997, 1998, Olsgard and Somerfield 2000, Olsgard *et al.* 2003, Bacci *et al.* 2009, de la Ossa-Carretero *et al.* 2011). However, some authors have stated that only a high level of mono-specificity of higher taxa may allow taxonomy sufficiency to work effectively (Terlizzi *et al.* 2003, Musco *et al.* 2009). Another problem with taxonomic sufficiency could derive from its application to benthic indices, as it may not be appropriate to assign the same tolerance level to a whole family (de-la-Ossa-Carretero *et al.* 2012).

As a result, taxonomic sufficiency remains one of the most controversial aspects regarding to monitoring programs and selection of indicators (Dauvin *et al.* 2003). Obviously, if all the species found were classified to species level, the amount of information would be huge, but this level of identification brings some disadvantages. Firstly, the fees of the taxonomists are high, because species-level identification requires a lot of time in the laboratory, including checking the available bibliography. Although polychaetes are one of the most-widely used taxa in environmental monitoring, there is currently no taxonomic guide for all Spanish species. The Spanish government has recently started a program to publish a set of taxonomical guides (including Polychaeta), but at the time of writing, only three volumes have been already published. In fact, the only way to accurately identify samples to species level is using a compilation of many publications, and if a specimen does not correspond to these guides, the taxonomists must check bibliographies from all over the world. The effort and time to train a taxonomist to the level required for these tasks can also be very long. Secondly, there are many species that require high-quality preservation, e.g. the Capitellidae family, in which all the setae must be present for a correct identification, or Maldanidae family, in which the pygidium is needed. Unfortunately, the samples taken for EMPs are sieved immediately after collection. This process can damage specimens, and it is common to lose part of the body, particularly setae (Bacci *et al.* 2009). As a consequence, the identifications can sometimes be doubtful. Therefore, monitoring studies based on higher taxonomic levels can reduce the risk of incorrect identifications, and consequently misclassification of ecological status (Pik *et al.* 1999, Mistri and Rossi 2001, Birk *et al.* 2012). However, species-level identification is usually required to apply some biotic indices. The use of AMBI is widespread in Spain, in which a list of species provides a value of the ecological status, as seen in Chapter 5. In that experiment, samples were carefully carried to the laboratory and carefully sieved with seawater. The identification was carried out with the help of an experienced taxonomist and without time pressure. Despite these efforts, the AMBI was conducted with a list of species in which 20% of specimens could not be assigned to any category, for a range of different reasons. When we were not sure about the species or the specimen was lacking some parts, they were not assigned to any category, or were clustered in genus or family level, and again they lost their category. Therefore, the final results were not based on all of the specimens we collected. It is unlikely that every EMP will be undertaken with sufficient care to preserve the animals in perfect conditions. Although the administration require the use of indices such as AMBI, it is important to consider that there is no guarantee of having a reliable species list to correctly apply the software, so the results of the EMP could potentially be interpreted incorrectly.

AMBI is one of the most complete indices based on the Pearson and Rosenberg (1978) paradigm, and the only one used in this thesis, but there are many others, some of which are based on AMBIs and therefore also on the Pearson and Rosenberg paradigm. MEDDOC (Pinedo *et al.* 2013) appears to adjust AMBI slightly, as the authors did not totally agree with the assignment of species to specific ecological groups. BOPA (Dauvin and Ruellet 2007) reduces the study of the benthic community to the groups Polychaeta and Amphipoda, but still requires a high taxonomic resolution. Later, Dauvin *et al.* (2016) used three indices, with three different levels of taxonomic resolution, BO2A (Benthic Opportunist Annelids and Amphipods- Dauvin and Ruellet 2007), BPOFA (Benthic Polychaete Opportunistic Families and Amphipods) and BPA (Benthic Polychaetes Amphipods ratio). In this study BPOFA was accepted as surrogate of the BO2A for assessing the ecological status of coastal waters, but it was only thought to provide a general idea with limited information. Another variant of BOPA was BOPA-FF (BOPA-Fish farming, Aguado-Giménez *et al.* 2015), which despite using the families of Chapter 3 to improve the index, concludes that the analysis of the polychaete assemblage at family level, alongside sulphides and granulometry, provides an appropriate strategy for fish farming monitoring, with more accurate information than the indices BOPA and BOPA-FF.

In the context of pollution monitoring, polychaete families have been tested for the measurement of increased organic matter (Gómez-Gesteira and Dauvin 2000). Polychaetes are interesting as a group because they include both tolerant and sensitive species (Chapter 3), and therefore, occur throughout gradients of pollution from undisturbed to heavily polluted areas (Rygg 1999, Tsutsumi 1990, Pocklington and Wells 1992, Del-Pilar-Ruso *et al.* 2009). Other taxonomic groups, such as crustaceans, echinoderms, and to a lesser extent, molluscs, are less tolerant (Rosenberg 1972) and often absent from the most disturbed parts of gradients (Pearson and Rosenberg 1978, Warwick and Clarke 1993). For that reason, the selection of polychaetes as the key taxon for the assessment of ecological status is appropriate for the monitoring of fish farms, i.e. the taxonomic surrogacy approach. Furthermore, polychaete assemblages using taxonomic sufficiency (identification to family level) provide accurate information on the changes in the sediment habitat, thus reducing time, effort and difficulties of identification to species level. Moreover, as polychaetes include families with both tolerant and sensitive behaviours, this information could provide a time and cost-effective approach for EMPs, as discussed in Chapters 2 and 3.

The usage of only the polychaete assemblage has been found to have good representativeness of the quality of the entire benthic assemblage. In four separate areas of Norway, there was a significant linear relationship between the number of polychaete species and the number of other species (Olsgard *et al.* 2003). Ferraro and Cole (1995) found that the costs of identification to genus, family and order level were respectively 23, 50 and 80% less than the cost of species-level identification. Moreover, when there is an anthropogenic impact, the overall balance in the benthic community is altered as the proportion of polychaetes is increased, generally to 35-50% of the total number of species and even higher proportions of total abundances (Olsgard and Somerfield 2000). So this rate would be a useful tool to assess the impacts on benthic assemblages, provided that there was available data prior to the impact event.

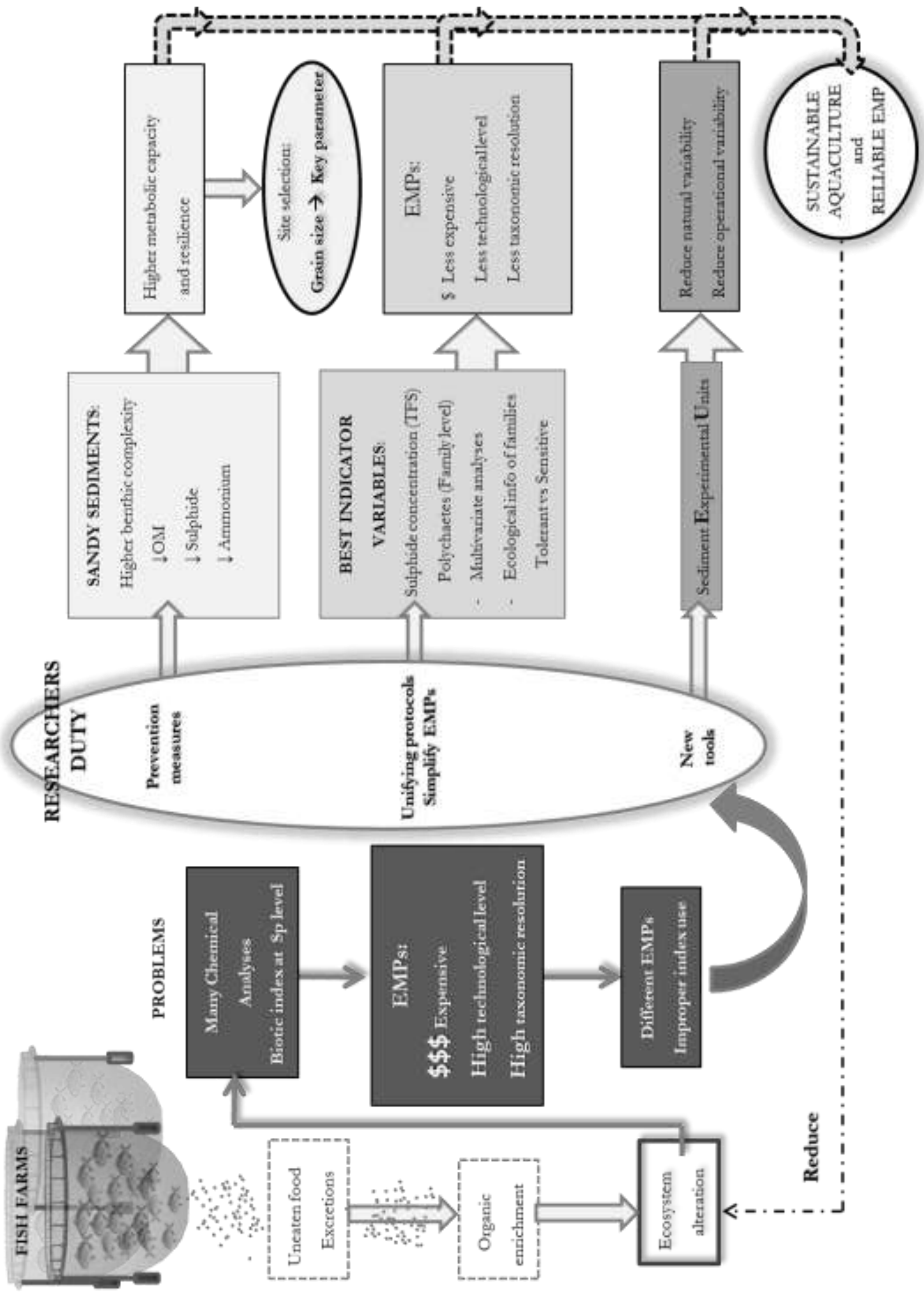


Figure 6.2. Conceptual model of possible improvements in environmental management and monitoring of marine aquaculture

### 6.3.2. Environmental monitoring plans and spatial heterogeneity

Patchy habitat and spatial heterogeneity in soft-bottom ecosystems are widely documented, as is their influence on the species composition and physicochemical variables that are needed to evaluate environmental quality status (Morrisey *et al.* 1992, Thrush *et al.* 1994, Stark *et al.* 2003, Fernandez-Gonzalez *et al.* 2013).

From a statistical point of view, replication at several temporal and spatial scales obtains representative samples over an environment with naturally-high variation. However, as we have seen at Chapter 2, in some situations the use of three nested sites within the main factor and three replicates may not be enough to eliminate or reduce this spatial variability. In order to enhance statistical power, a greater level of replication is highly advised, not only at a small scale (replicates) but also at higher-spatial scales (e.g. nested sites within zones) (Hurlbert 1984, Underwood 1997, Terlizzi *et al.* 2005), when univariate and multivariate results reveal spatial variability. The economic and working effort of greater replication may be compensated using a reduction in the environmental variables that must be measured.

In addition to natural patchiness, the methodologies used for sample collection can present another source of variability. Samples can be collected by divers directly over the bottom, using either a manual drag or directly with a bottle; or they can be collected from a boat using a Van Veen drag. Drags can collect greater or lesser quantities depending on the type of sediment, and sometimes can collect different quantities even with the same type of sediment; this is problematic when all samples are supposed to have the same amount of sediment. In fact, sediment fouling (e.g mussel valves) and litter from farming activities (nets, ropes, etc) can seriously complicate the use of these kinds of drags. In the end, this will result in greater sampling effort and higher quantitative variability between samples.

A different solution to reduce natural and anthropogenic variability is the usage of experimental units of recolonisation (Figure 6.2). The initial idea of using experimental units (Chapter 5) was to study processes related to grain-size effects on the recolonisation of soft sediments below the fish farm cages, and how an increase in organic enrichment could affect the structuring of benthic fauna. As a result of our observations, we started to appreciate the benefits of using pre-built experimental units in environmental management. The granulometry, initial organic matter and total surface of all experimental units were always constant, unlike some of the traditional methods, and patchiness of the habitat was also avoided. Hence, the utility and advantages of this method should be further studied in order to assess their usage on a broader scale. This approach has been already applied for defining water quality (De Pauw *et al.* 1986) or studying ecological processes such as fish recruitment (Ammann 2004) and it has been extensively used on fresh water monitoring programs (Barbour *et al.* 1999).

In summary, we propose a number of recommendations that could help to achieve sustainable aquaculture. Grain size of the underlying sediment should be a key parameter in the selection of sites for aquaculture facilities, being sandy habitats a better type of habitat for fish farming development. Shellfish farming, as it produces lower organic enrichment by pseudo-faeces, is not affected by the type of the bottom. Within EMPs, total free sulphides and polychaete assemblages at family level would provide accurate information on biogeochemical processes and the quality of the sediment, so they would be a good tool to assess the impacts of aquaculture activities. Moreover, these tools work effectively in controlled experimental units, which can reduce natural variability and offer the managers a new approach to observe the influence of aquaculture above the sea bottom. Regarding the encouraging results of these experimental units for finfish aquaculture, and in order to achieve a better assessment of environmental impacts, further studies are needed to test the experimental units as validate tools for EMPs for aquaculture.

### 6.4. Final conclusions

**Chapter 2**

1. Following the environmental monitoring plan of JACUMAR, multivariate analysis of polychaete assemblages and total free sulphides (TFS) show clearly the effects of the changes produced in the benthic habitat by organic enrichment due to fish farming.

2. The proposed environmental monitoring plan could be modified to optimise sampling effort. Redox potential and stable nitrogen isotope may be omitted in order to reduce costs and the technological requirements. In some situations, if the spatial heterogeneity of the sediment is likely to affect the statistical results, it would be beneficial to enhance statistical power by increasing spatial replication, in order to detect clear differences due to organic enrichment from aquaculture activities.

**Chapter 3**

3. At overall scale, after meta-analyses, the distribution patterns of polychaete families respond to combinations of physicochemical variables, mainly sulphide concentration and granulometry, which may be affected under fish farm conditions. As a result, polychaete abundance and diversity show significant alterations. Polychaetes increase their abundance in transitional situations, but decrease their abundance and diversity in advanced stage of pollution caused by organic enrichment.

4. Class Polychaeta, studied at family level, provides accurate information about the quality status of the environment, with both tolerant and sensitive families. Taxonomic sufficiency at family level and taxonomic surrogacy to polychaete assemblage seems to be a useful approach for environmental impact monitoring in fish farms.

5. Across the Western Mediterranean Sea, families Capitellidae, Dorvilleidae, Glyceridae, Nereididae, Oweniidae and Spionidae were tolerant to fish farm pollution, while families Magelonidae, Maldanidae, Nephtyidae, Onuphidae, Paralacydonidae, Paraonidae, Sabellidae and Cirratulidae were sensitive to fish farm pollution.

**Chapter 4**

6. Sandy sediments are more suitable for aquaculture facilities than muddy sediments, because their metabolic capacity is higher. Therefore, sediment grain size should be considered as a key parameter in the selection of sites for fish farming.

7. Macrofauna (simulated here using polychaetes) stimulate organic matter mineralization and nutrient recycling, increasing the metabolic capacity and resilience of the sediment.

8. There is an important difference on the benthic impact when comparing faeces from a carnivorous species, such as seabream with pseudo-faeces from a filter feeder as mussel. At low level of organic enrichment, as produced the mussel pseudo-faeces, there is no influence of the grain size in sediment metabolism.

**Chapter 5**

9. Polychaete assemblage colonisation, studied by multivariate analyses in the experimental units, clearly reflects the influences of aquaculture.

10. The index AMBI (performed only with polychaete assemblage) in the experimental units provided clear information about the environmental quality status of the sediment. Sandy and muddy sediments show the same level of pollution. However, AMBI is not recommended when more than 20% of specimens are not assigned to any category.

11. It is recommended to study the whole polychaete assemblage and not just focussing on the presence of certain species, since several species normally occurring in conditions stressed by organic enrichment also appeared in non-polluted areas, and vice versa.

12. Experimental units with sandy sediments are more sensitive to changes in sulphide concentration and organic enrichment than those using muddy sediments.

13. Pre-built experimental units with selected defaunated sediment seem to be a very useful tool for aquaculture monitoring, in order to reduce the effect of environmental variables that could influence the results.

# RESUMEN GENERAL

# CHAPTER 7

# 7. RESUMEN GENERAL

La mayor parte de la superficie de la Tierra está cubierta por el mar y, a su vez, la mayor parte del fondo marino está cubierto por sedimentos o sustrato blando, como son los fangos, arenas y gravas (Snelgrove 1999). Los sedimentos blandos están definidos como hábitats poco estructurados y están caracterizados por la movilidad y la inestabilidad de sus partículas (Peterson y Peterson 1979). Los sedimentos submareales están influenciados por muchas de las mismas fuerzas físicas y químicas que afectan a las zonas intermareales. Estas fuerzas incluyen corrientes, que erosionan y recolocan los sedimentos, determinando su profundidad y la composición del tamaño de grano, y los procesos fisicoquímicos que controlan la difusión de oxígeno y nutrientes en los sedimentos (Morrisey *et al.* 1998). A partir de ahora nos referiremos como sedimentos blandos solo a los de ambientes submareales. Las olas juegan un papel importante en la distribución y afección de los sedimentos hasta una profundidad de 100 m, pero su efecto disminuye exponencialmente con la profundidad y, por lo tanto la influencia dominante submareal en el transporte de sedimentos está constituida principalmente por las corrientes (Cater 2002). Por supuesto, hay muchas excepciones a este patrón, como sucede en áreas situadas alrededor de entradas a estuarios y bahías, donde las corrientes mareales están más concentradas, y cerca de costas expuestas al oleaje (Malvarez *et al.* 2001). Los sedimentos se distribuyen desde gravas gruesas (en áreas sujetas a una mayor acción del oleaje y de las corrientes) hasta fangos (típicos de áreas con poca energía de transporte de partículas) y limos y arcillas (que también forman parte de sedimentos profundos), dejando en medio de esta clasificación varios tipos de arena (Gray y Elliot 2009). Además, arenas y fangos pueden contener gravas que derivan de capas subyacentes más antiguas y conchas de moluscos que viven en el sedimento o son traídas por las corrientes. Los sedimentos blandos no suelen tener macrófitos, aunque en algunas zonas se desarrollan importantes praderas de fanerógamas y macroalgas.

Estos sedimentos, al igual que otros ecosistemas de áreas costeras, son susceptibles de impacto por actividades humanas a pequeña o gran escala (Nogales *et al.* 2011). La sobreexplotación de recursos naturales, las actividades productivas como la acuicultura o las plantas desalinizadoras, la introducción de especies exóticas, las emisiones de compuestos orgánicas e inorgánicos y aguas residuales son algunos de los factores que pueden alterar los procesos ecológicos naturales en sedimentos blandos. Algunas de estas actividades, como la acuicultura, se desarrollan sobre sedimentos blandos por su mayor resiliencia y resistencia frente a perturbaciones, pero estos impactos pueden tener un efecto negativo en el medio ambiente e interferir en el correcto funcionamiento del ecosistema si su capacidad de carga se sobrepasa (Rapport *et al.* 1985). En ecología, la resiliencia se refiere a la capacidad de un ecosistema de recuperarse tras una perturbación, en otras palabras, a la capacidad de absorber las presiones naturales y antrópicas (Odum 1994). Carpenter *et al.* (2001) definió tres características de resiliencia: 1) la habilidad del sistema para resistir a una alteración sin ser sobrepasado y por tanto mantener todas sus funciones, 2) la capacidad del sistema de auto-organizarse, y 3) la habilidad de aprender e incorporar alteraciones como mecanismo de adaptación. La resiliencia y la resistencia están influenciadas por el papel que juegan especies claves que mantienen las interacciónes entre los procesos biológicos, químicos y físicos (Townsend y Trush 2010).

Los sedimentos blandos son conocidos por proveer bienes y servicios del ecosistema (Erlich y Mooney 1983, Gray *et al.* 1999). Los bienes del ecosistema son recursos tangibles que se pueden extraer y utilizar por los humanos, como la comida o las materias primas. Los servicios del ecosistema son las capacidades del sistema ecológico para proporcionar a los humanos condiciones favorables para procesar material o aportar beneficios intrínsecos (Townsend y Thrush 2010), por ejemplo I) las relaciones tróficas: las algas micro-bentónicas que viven en el sedimento suponen un aporte importante a la producción primaria junto con el fitoplancton pelágico (Troell *et al.* 2005). Los organismos filtradores y depositívoros usan este fitoplancton y lo integran en la cadena trófica, transfiriendo esta energía a otros organismos bentónicos y epibentónicos, y estos a su vez a peces (Cloern 1982, Officer *et al.* 1982, Loo y Rosenberg 1989); II) el reciclado de nutrientes y la oxigenación de sedimentos procesados están relacionados con servicios de desintoxicación y eliminación de residuos, dado que los nutrientes son regenerados y los contaminantes degradados en el sedimento. Estos procesos están directamente regulados por organismos microbianos y por organismos bioturbadores, que tienen gran influencia en la oxigenación y el movimiento físico de los contaminantes (Henriksen *et al.* 1983, Pelegri y Blackburn 1995, Gray *et al.* 1999, Weslawski *et al.* 2004); III) la estructura del hábitat: los hábitats marinos proporcionan lugares para vivir a especies, prerrequisito para la disposición de los otros bienes y servicios (Townsend y Trush 2010); IV) el suministro de comida, el cual tiene una gran influencia en la sociedad debido a la importancia comercial y cultural, en áreas occidentales por el pescado y los mariscos y en Asia también por las algas. El sector acuícola también está involucrado, al depender del suministro de alimentos provenientes del fitoplancton y de harinas de pescado (Weslawski *et al.* 2004) y V) actividades recreativas como los deportes acuáticos o la pesca deportiva (Troell *et al.* 2005). Finalmente, los bienes y servicios de sedimentos blandos están relacionados con los ecosistemas adyacentes, como la columna de agua y las zonas costeras. El mantenimiento de estos bienes y servicios dependerá de la capacidad de resistencia y resiliencia de los sedimentos blandos y por lo tanto, de las características intrínsecas de cada tipo de sedimento, como por ejemplo, el tamaño de grano y la fauna asociada.

Respecto a los procesos biológicos, la fauna bentónica de los sedimentos blandos juega un papel fundamental en el mantenimiento de los bienes y servicios. Las comunidades de fauna bentónica son frecuentemente caracterizadas por el número de especies, abundancia y biomasa (Rosenberg 2001), las cuales dependen de la naturaleza del sedimento y de los procesos sedimentarios (Bellan 1985). Hay diferentes maneras de clasificar la fauna: los organismos pueden ser separados de acuerdo a su capacidad de movimiento, móviles, sedentarios o sésiles; y/o por su posición relativa respecto al sedimento, animales hiperbentónicos (organismos bentónicos que pueden realizar incursiones en la columna de agua), epibentónicos (organismos bentónicos que viven sobre el sedimento) y endobentónicos o infauna (organismos bentónicos que viven dentro del sedimento). Los taxones dominantes en los sedimentos blandos, ordenados por orden de abundancia son: poliquetos, crustáceos, equinodermos y moluscos. Estos organismos están sujetos a alteraciones físicas y biológicas que pueden variar de frecuencia e intensidad, así como de extensión espacial y temporal. La estructura del sedimento en estas comunidades, en otras palabras, la composición y la abundancia relativa de especies, y su estabilidad pueden variar notablemente en respuesta a estas alteraciones (Turner *et al.* 1995).

Una de las actividades que pueden afectar al ecosistema marino es la acuicultura. La definición dada por FAO (1990-2016) considera la acuicultura como el cultivo o cría de organismos acuáticos: peces, moluscos, crustáceos, plantas acuáticas, cocodrilos, tortugas y anfibios. El cultivo de estas especies implica algunas formas de intervención en el proceso de cría para aumentar la producción, como la siembra, la alimentación, la protección frente a predadores, etc. El cultivo también implica la propiedad individual o empresarial de los organismos que se cultivan. La actividad de obtener peces para consumo se denomina acuicultura cuando los organismos acuáticos recolectados por una persona física o jurídica también es la propietaria de los organismos durante el periodo de cría, mientras que cuando los organismos acuáticos son explotados por el público como un recurso de propiedad común, con o sin licencias apropiadas, se denominada pesca de captura. La acuicultura marina es aquella en la que el final del cultivo del organismo se realiza en agua de mar, ya sean fiordos, aguas costeras, aguas en alta mar o mares interiores dónde la salinidad normalmente excede los 20‰. Los estadios tempranos del ciclo de vida de estos organismos acuáticos pueden ser realizados en aguas salobres o incluso dulces.

Históricamente, los primeros comienzos de la acuicultura datan alrededor del año 2000-1000 a.C. en China. El acuicultor S.Y. Lin fue citado por Hickling (1962) como la primera persona en usar la carpa común (*Cyprinus carpio,* Linnaeus, 1758) para cultivo, aunque no hay documentos escritos que lo constaten, pues era una tradición popular que no se interrumpió con el paso del tiempo. En Europa es difícil de determinar con exactitud sus inicios, pero hay evidencias de que en el Imperio Romano hubo construcciones para el cultivo de peces. El gran desarrollo de la acuicultura Europea sucedió en el siglo XIX y alcanzó su máxima expansión gracias a los avances en biología y a las nuevas tecnologías (FAO-HAKI 1999). El cultivo de peces marinos en Europa ha sido intensivo desde 1988 (Read y Fernandes 2003). Las piscifactorías modernas en el Mediterráneo comenzaron en 1980 con la dorada (*Sparus aurata,* Linnaeus, 1758) y la lubina (*Dicentrarchus labrax*, Linnaeus, 1758).

En la actualidad, en la Unión Europea, los principales productos de acuicultura son los peces y los moluscos. La acuicultura de crustáceos, algas y otros invertebrados sigue siendo reducida. Concretamente en España, las especies con mayor producción son, por orden, los mejillones (*Mytilus galloprovincialis,* Lamarck 1819), la lubina (*Dicentrarchus labrax*), la dorada (*Sparus aurata*) y la trucha arcoíris (*Oncorhynchus mykiss,* Walbaum 1792). A excepción de la trucha arcoíris, las otras tres especies son cultivadas en el mar (APROMAR 2016). Los mejillones se cultivan en bateas, estructuras flotantes donde los mejillones se fijan y cuelgan de unas cuerdas principales unidas a grandes flotadores de plástico (Figura 7.1). Las doradas y las lubinas son cultivadas en jaulas flotantes, que consisten en un anillo flotante rígido que da soporte a la red en forma de bolsa (Figura 7.2). Hay tres tipos de instalaciones con jaulas flotantes: granjas costeras (a menos de 500 m de la costa y con una profundidad menor de 10 m), granjas fuera de costa (off-coast, alejadas hasta 3 km y con una profundidad entre 10-50

m) y granjas en mar abierto (offshore, a varios km de la costa y a una profundidad mayor de 50 m) (Holmer 2010).

Figura 7.1. Instalación de acuicultura de mejillones en Galicia (Foto: Pablo Sánchez)

FAO considera que la acuicultura contribuye al uso efectivo de los recursos naturales, a la seguridad alimentaria y al desarrollo de la economía, con un limitado y controlable impacto sobre el medio marino (FAO 2014).

Figura 7.2. Instalación de acuicultura de dorada y lubina en Guardamar (Foto: Pablo Sánchez)

Junto con el desarrollo de la acuicultura, las granjas marinas han sido estudiadas para entender qué aspectos del medio marino están siendo influenciados por dicha actividad. A continuación se resumen los principales riesgos de la acuicultura:

- Restos originados por la alimentación, entre los que se encuentran cuatro componentes principales: I) Comida no ingerida, que se produce durante la alimentación artificial, generalmente por un mal cultivo, enfermedad de los peces o condiciones ambientales inadecuadas (Figura 7.3). II) Comida no digerida, producida principalmente por bivalvos, los cuales no tienen mucho control en la ingesta, ingieren mucho más de lo que pueden procesar y eliminan microalgas intactas en forma de heces llamadas pseudo-heces. III) Compuestos indigeribles, debido a moléculas complejas que en el pienso están separadas en moléculas más pequeñas que pueden o no pasar a través de las paredes del intestino en la digestión. Estas moléculas que no pueden ser absorbidas, debido a su forma o tamaño y son rechazadas (heces). IV) Excreciones producidas por procesos fisiológicos, por los cuales las moléculas llegan disueltas al plasma sanguíneo pero son eliminadas, a pesar de haber sido degradadas. Son compuestos solubles en agua y eliminados a través de órganos específicos como las branquias (Dosdat 2009). Estos residuos producen un enriquecimiento orgánico que puede afectar a dos hábitats diferentes, al fondo marino, como se explica más adelante, y a la columna de agua. El enriquecimiento orgánico de la columna de agua podría derivar en eutrofización, resultando en un crecimiento algal excesivo, que puede tener otros efectos negativos como la reducción de la claridad del agua, asfixia física de la biota, o reducción extrema del oxígeno disuelto, por la descomposición microbiana de esa gran biomasa algal (Cloern 2001, Forrest *et al.* 2007). Una disminución importante del oxígeno disuelto en la columna de agua en piscifactorías de aguas marinas, solo ocurre cuando las jaulas están muy hacinadas o cuando se encuentran en áreas poco profundas con poca renovación de agua (La Rosa *et al.* 2002).

- Emisión de químicos: medicinas, desinfectantes y productos para evitar las incrustaciones de organismos en las estructuras son introducidos en el sistema marino a través de la acuicultura (Costello *et al.* 2001). Algunos de ellos pueden acumularse y persistir en el medio marino, llegando a producir serias consecuencias para la biota (Hansen y Lunestad 1992) e incluso bioacumulándose en la cadena trófica (Kiviranta *et al.* 2000).

- Problemas genéticos: el mayor riesgo genético de la acuicultura incluye la pérdida de diversidad genética dentro o entre poblaciones, y la pérdida de adaptaciones o capacidades (Waples *et al.* 2012, Segovia-Viadero *et al.* 2016).

- Incrustaciones o *fouling*: las superficies sumergidas en el medio marino se colonizan por organismos marinos, los más comunes son bivalvos, algas, hidroides y ascidias (Sarà *et al.* 2007, Fitridge *et al.* 2012), y también por organismos de la epifauna como los anfípodos (Fernandez-Gonzalez y Sanchez-Jerez 2014). Estas incrustaciones reducen la eficiencia de los materiales y equipos que se usan en el mar, por lo que el uso de productos para prevenirlas es muy común. Además, estas incrustaciones pueden competir por los recursos con los organismos cultivados y pueden incorporar predadores y enfermedades (Willemsen 2005).

- Escapes: un escape es definido como el proceso en el cual uno o varios peces dejan de estar en cautividad y se dispersan por el mar (Dempster *et al.* 2013). La definición se amplía incluso a huevos fertilizados tras la reproducción dentro de las jaulas (Jørstad *et al.* 2008). Los peces escapados tienen diferentes interacciones a distintos niveles tróficos, ya que pueden ser capaces de sobrevivir en estado salvaje y explotar los recursos naturales afectando a las relaciones predador-presa y a la competencia interespecífica (Toledo-Guedes *et al.* 2014). También pueden producir interacciones genéticas como ya se ha explicado anteriormente; interacciones patógenas, ya que los peces escapados pueden propagar parásitos y enfermedades (Arechavala-Lopez *et al.* 2013), e interacciones con pesquerías, los peces escapados pueden ser pescados suponiendo un ingreso extra por pesquerías profesionales (Izquierdo-Gomez y Sanchez-Jerez 2016).

- Animales salvajes: los peces son atraídos por las jaulas de acuicultura, y por tanto las piscifactorías pueden alterar la presencia, la abundancia, la permanencia y la dieta de estos (Sanchez-Jerez *et al.* 2011). Las aves marinas también utilizan las instalaciones de acuicultura como áreas de descanso y fuente de alimento, tratando de capturar los organismos cultivados. Con el fin de evitar estos ataques, se usan redes anti pájaros y otros dispositivos que pueden causar mortalidad en las aves (Quick *et al.* 2004). Algunos mamíferos marinos como los delfines también son atraídos por las instalaciones de acuicultura y, pueden quedar atrapados en las redes (Díaz-Lopez y Berna-Shirai 2007) o bioacumular químicos como antibióticos o antioxidantes.

Figura 7.3. Proceso de alimentación artificial en jaulas de acuicultura

Dada la expansión a lo largo de la costa y el asociado incremento de materia orgánica que llega al fondo marino de la acuicultura, se ha convertido en una gran preocupación medioambiental. Este enriquecimiento orgánico tiene una influencia directa sobre el sedimento, produciento cambios químicos, físicos y biológicos (Pearson y Rosenberg 1978). El metabolismo del sedimento durante el cultivo marino de organismos puede llegar a ser hasta 10 veces mayor de lo normal en áreas costeras (Holmer y Kristensen 1992). La materia orgánica que no es consumida por la macrofauna bentónica es degradada a compuestos inorgánicos a través de procesos bacterianos y diferentes rutas metabólicas con diferentes aceptadores como oxidantes (Canfield *et al.* 1993). Cuando hay oxígeno disponible, los procesos aeróbicos como la respiración heterótrofa y la quimiosíntesis aerobia son los más comunes en las capas superiores del sedimento (Holmer *et al.* 2005a). Las bacterias aeróbicas son capaces de mediar la secuencia de procesos para oxidar completamente el carbono orgánico particulado a $CO_2$ y $H_2O$ (Canfield 1994). Sin embargo, los sedimentos marinos son ambientes reducidos cubiertos solo por una capa óxica superficial, que va de milímetros a centímetros dependiendo de si son aguas someras y productivas o sedimentos oceánicos en condiciones oligotróficas (Reimers *et al.* 1986, Glud *et al.* 1994 Kristensen 2000). Cuando el oxígeno es consumido, un grupo de bacterias mutualistas realizan la descomposición anaerobia, ya que no hay ninguna bacteria anaerobia capaz de realizar la mineralización completa (Fenchel *et al.* 1998, Kristensen 2000). La producción de energía en la respiración con diferentes aceptores de electrones generalmente disminuye de acuerdo a esta secuencia: oxígeno ($O_2$), nitrato ($NO_3^-$) manganeso (MnIV), óxido de hierro (FeIII), sulfato ($SO_4^{2-}$) y dióxido de carbono ($CO_2$). La reducción del sulfato llega fácilmente a ser la ruta de respiración predominante (Christensen *et al.* 2000). Además, el enriquecimiento orgánico de los residuos de las piscifactorías estimula a las bacterias reductoras de sulfato más que otros tipos de fuentes de materia orgánica (Holmer *et al.* 2005b). El producto final de la reducción de sulfato es el sulfuro de hidrógeno ($H_2S$), que es altamente tóxico para la mayoría de la fauna bentónica (Wang y Chapman 1999, Vaquer-Sunyer y Duarte 2010). La acumulación de sulfuro de hidrógeno es contrarrestada por la oxigenación o precipitación de formas de sulfuro no tóxicas con $O_2$, $NO_3$, óxidos de Fe y Mn, que usan también $O_2$ en reacciones redox espontáneas o producidas por microbios (Thamdrup 2000, Schippers y Jørgensen 2002, Canfield *et al.* 2005). En la metanogénesis, que ocurre cuando no hay más sulfato para reducir, los electrones son transferidos desde la materia orgánica a $CO_2$, a través de intermediarios de $H_2$ (Canfield *et al.* 2005).

El otro proceso afectado por el enriquecimiento de la materia orgánica es el ciclo del nitrógeno (Casado-Coy *et al.* 2016). Este efecto ha sido ampliamente estudiado ya que la agricultura ha afectado al medio marino en mayor medida que la acuicultura (Paerl y Piehler 2008). Los bajos niveles de enriquecimiento orgánico proporcionan sustrato para desnitrificadores heterótrofos, que oxidan el carbono orgánico con $NO_3$ en el proceso de la nitrificación (Laursen y Seitzinger 2002). No obstante, a altos niveles de enriquecimiento orgánico, cuando la competición por aceptores de electrones aumenta y el medio ambiente se vuelve sulfhídrico, las rutas de mineralización del nitrógeno pueden cambiar de eliminación neta de nitrógeno a través de la desnitrificación o anammox a producción de amonio vía reducción de desasimilación del nitrato (Holmer y Kristensen 1992, Christensen *et al.* 2000).

Dado el número de procesos y hábitats que pueden verse afectados y para conseguir un desarrollo sostenible de la acuicultura, es necesario el establecimiento de un marco regulador, que considere aspectos ambientales importantes como la selección idónea de sitios para las instalaciones, la evaluación del impacto ambiental y la vigilancia o seguimiento ambiental (Sanchez-Jerez *et al.* 2016). Actualmente, la Directiva Marco del Agua (WFD, 2000/60/EC) y la Directiva Marco de Estrategias Marinas (MSFD, 2008/56/EC) establecen las directrices para definir los Objetivos de Calidad Ambiental a los Estados Miembros y desarrollar legislaciones nacionales para los ecosistemas marinos. En España, la ley de evaluación ambiental (Ley 21/2013, 9 de Diciembre) dictamina que las instalaciones de acuicultura, con una producción superior a 500 toneladas anuales, deben realizar una evaluación y seguimiento del impacto ambiental. Pero a partir de ahí, son los gobiernos territoriales los responsables de establecer el marco legal y los criterios para llevar a cabo la vigilancia. Esto ha dado lugar a un marco legal inconsistente dónde dependiendo de las leyes regionales, los programas de vigilancia ambiental incluyen unas medidas y protocolos u otros.

Con el fin de tratar esta cuestión, la Junta Nacional Asesora de Cultivos Marinos (JACUMAR) comenzó a trabajar en 1999 para homogeneizar los criterios y las metodologías utilizadas para evaluar los posibles efectos debidos a la acuicultura. Una década después, se publicó un documento con un plan de seguimiento ambiental general para toda España (Aguado-Giménez *et al.* 2012a). Este documento proporciona diferentes estándares de calidad ambiental (EQS) para los hábitats bentónicos y pelágicos. Estas directrices generales fueron un avance significativo para la aplicación de planes de vigilancia ambiental, dado que desde que la Directiva Marco del Agua fue publicada, surgieron muchos protocolos e indicadores diferentes y, como se ha mencionado anteriormente, diferentes normas regionales habían dado lugar a diversos tipos de planes de vigilancia ambiental. Sin embargo, era y es todavía necesario encontrar un buen balance entre la información requerida y el esfuerzo aplicado.

Algunos de estos nuevos indicadores son los bioindicadores. Un bioindicador es una especie o grupo de especies que reflejan el estado biótico o abiótico de un medio; representa el impacto de cambios ambientales en un hábitat, comunidad o ecosistema; es indicativo de la diversidad de una parte de un taxón o de la diversidad entera de un área (Gerhardt 2009). Hay indicadores positivos, oportunistas o especies tolerantes a la contaminación cuya abundancia se incrementa y su dominancia produce valores bajos de diversidad de macrofauna en las muestras, e indicadores negativos, especies sensibles o intolerantes, cuya abundancia disminuye (Rygg 1985, Warwick 1988). Uno de los primeros listados que incluyeron especies tolerantes fue el descrito por Pearson and Rosenberg (1978), una revisión sobre las especies usadas como bioindicadoras hasta dicho momento. También describieron la sucesión de la macrofauna en relación al enriquecimiento orgánico. Desde entonces, muchos autores han descrito diferentes especies y sus preferencias de hábitats o estrategias tróficas y muchos especies de la macrofauna han sido catalogadas como tolerantes o sensibles (Davis 1987, Méndez *et al.* 1998, Cañete *et al.* 2000, Belan 2003, Harkantra y Rodrigues 2004, Giangrande *et al.* 2005, Pagliosa 2005, Tomassetti y Porello 2005, Lee *et al.* 2006, Dean 2008). El uso de estos bioindicadores ha ido evolucionando a lo largo del tiempo. Por un lado, es posible buscar especies entre la macrofauna que den información según su presencia o ausencia. Por otra parte, se puede resumir la información en un solo número, dando lugar a un índice. Los índices de biodiversidad que primero se utilizaron estaban relacionados con la abundancia, como el índice de diversidad de Shannon (Shannon y Weaver 1963) y el índice de uniformidad de Pielou (Pielou 1966), o con el número de especies diferentes, como el índice de Simpson (Simpson 1949) o con la riqueza específica. Estos índices han sido ampliamente usados (Rosenberg *et al.* 2004) y además, en la última década, se han desarrollado alguno índices bióticos nuevos, añadiendo información sobre la sensibilidad (tolerante o sensible) de la macrofauna a distintas alteraciones, sobre todo por enriquecimiento orgánico, y resumen el estado ecológico en un solo valor (Aguado-Giménez *et al.* 2015). Este valor está asociado a una escala, desde muy bueno o saludable a severamente alterado, hecho que hace que sea mucho más fácil de entender para los gestores (Chainho *et al.* 2007). Los índices más conocidos en Europa y en zonas Mediterráneas son: AZTI Marine Biotic Index (AMBI-Borja *et al.* 2000), Benthic Quality Index (BQI-Rosenberg *et al.* 2004), Bentix index (Simboura y Zenetos 2002), BOPA (Dauvin y Ruellet 2007), M-AMBI (Muxica *et al.* 2007), BO2A (Dauvin y Ruellet 2009) y Mediterranean Occidental index (MEDOCC- Pinedo *et al.* 2015). Sin embargo, estos índices están en constante revisión, debido a las diferencias entre regiones y entre organismos según el tipo de alteración antrópica, por lo que ningún índice biótico es aplicable a escala global (Dauvin *et al.* 2007, Keeley *et al.* 2012a). Otra desventaja de estos índices es la alta resolución taxonómica necesaria, lo que requiere de taxónomos experimentados para cada uno de los taxones a identificar y, además mucho tiempo de identificación (de-la-Ossa-Carretero *et al.* 2012a). Una solución para reducir estos problemas es la suficiencia taxonómica, que consiste en identificar a niveles más altos la macrofauna, por ejemplo a nivel de familia, argumentando que no hay una pérdida sustancial de información para los estudios de impacto ambiental (Ferraro y Cole 1990, Bacci *et al.* 2009), más aún cuando se trata de una alteración por enriquecimiento orgánico. Además, estos niveles de identificación más altos, analizados con métodos multivariantes, dan información precisa sobre la influencia de las piscifactorías en el fondo marino, siendo más apropiados y estadísticamente válidos, que los índices bénticos univariantes (Aguado-Giménez *et al.* 2007, Callier *et al.* 2008, Quintino *et al.* 2012). Otra posible solución sería reducir el estudio de toda la comunidad bentónica a la comunidad de poliquetos (Aguado *et al.* 2015).

Los poliquetos han sido ampliamente estudiados sobre todo en sedimentos blandos (Crema *et al.* 1991, Elias 1992, Grall y Glémarec 1997, Olsgard y Somerfield 2000, Solis-Weiss *et al.* 2004, Giangrande *et al.* 2005, Domínguez-Castanedo 2007, Del-Pilar-Ruso 2015) y concretamente en el seguimiento ambiental de piscifactorías (Tomassetti y Porrello 2005, Lee *et al.* 2006, Dean 2008, Aguado-Giménez *et al.* 2015). No obstante, la mayoría de los estudios sobre la sensibilidad de los poliquetos describen niveles de tolerancia de especies concretas de poliquetos. Por lo tanto, hay poca información sobre tendencias generales de las familias de poliquetos. La clase Polychaeta contiene 11.688 especies aceptadas (hasta 2016) distribuidas por una compleja clasificación taxonómica con 87 familias aceptadas (WORMS-www.marinespecies.org). Otro millar de especies han sido nombradas y consideradas inválidas (Rouse y Pleijel 2001). Cabe destacar el gran esfuerzo realizado por parte de los taxónomos realizando descripciones y guías taxonómicas, así como la actualización de bases de datos de poliquetos y, haciendo más fácil para los ecólogos el uso de esta información. En el capítulo 8 de referencias, se añade una sección (8.1) dónde se refleja el listado de trabajos utilizados para realizar esta tesis, aunque no hayan sido propiamente citados en el texto.

Son muchas las razones por las que los poliquetos son adecuados para el seguimiento del impacto ambiental marino. Son considerados ubicuos y generalmente el grupo más abundante en las comunidades bentónicas, además del más rico en especies (Beesley *et al.*

2000, Dean 2008). Se hallan en un rango muy amplio de hábitats, desde aguas continentales, tanto saladas como dulces, hasta fondos oceánicos. Estos organismos muestran gran variedad de estrategias alimenticias y se pueden dividir en omnívoros, herbívoros, carnívoros, filtradores o suspensívoros, depositívoros de superficie y excavadores (Gambi y Giangrande 1986, Pagliosa 2005). Su ciclo de vida dura entre días y semanas y las tasas reproductivas son muy altas, lo que les permite responder rápidamente a cualquier alteración (Dean 2008). Entender la relación entre poliquetos y sedimento es muy importante, ya que la estructura del poblamiento de los poliquetos dependerá del tamaño de grano (Jansson 1967, Fresi *et al.* 1983, Gambi y Giangrande 1986). Finalmente, los poliquetos juegan un papel importante en la descomposición, subducción e incorporación de materia orgánica en los sedimentos y en su aireación (Kristensen y Kostka 2005, Heilskov *et al.* 2006).

### 7.1. Justificación y objetivo general

Tras el desarrollo de la acuicultura europea en los diferentes ecosistemas marinos y la implantación del marco legal y administrativo, la vigilancia del impacto ambiental ha mejorado mucho en la última década. Sin embargo, todavía es necesario homogeneizar protocolos y, más importante aún, hacerlos económicamente viables para los acuicultores y más fáciles de interpretar para los gestores. Los poliquetos son una herramienta precisa, pero todavía se puede profundizar más en el conocimiento de los procesos ecológicos relacionados con la alteración del medio por la acuicultura. En particular, hay una falta de información sobre cómo afecta la estructura del sedimento a los procesos biogeoquímicos en diferentes escenarios de enriquecimiento orgánico. Por consiguiente, el objetivo general de esta Tesis Doctoral es mejorar el conocimiento sobre la interacción entre distintos escenarios de enriquecimiento orgánico debido a la acuicultura y la estructura de la comunidad de poliquetos, teniendo en cuenta distintos tipos de sedimentos, con el propósito de proporcionar recomendaciones para los programas de seguimiento ambiental y contribuir de esta manera al desarrollo de una acuicultura más sostenible.

### 7.2. Aplicación del Plan de Vigilancia Ambiental para piscifactorías propuesto por JACUMAR, del papel a la práctica

Como hemos mencionado anteriormente, el plan de vigilancia ambiental de JACUMAR (Aguado-Giménez *et al.* 2012a) supuso un gran paso en la homogeneización de protocolos a nivel estatal diseñado en el año 2012. Sin embargo no hay estudios que proporcionen información acerca de su implantación o su viabilidad. Por ello se realizó un estudio de una granja marina de engorde de dorada y lubina según este plan de vigilancia ambiental y así analizar las ventajas y desventajas de las variables seleccionadas en dicho plan. Los resultados obtenidos mostraron que el plan de vigilancia ambiental de JACUMAR está formado por métodos y herramientas muy útiles para la vigilancia ambiental de la acuicultura. Sin embargo, se detectó heterogeneidad espacial en el análisis univariante de las variables experimentales y en el análisis multivariante de la comunidad de poliquetos. Esta variabilidad espacial puede ser debida a la fragmentación del hábitat y, por tanto, puede ser resuelta mediante el diseño experimental, por ejemplo, añadiendo replicación espacial para incrementar el poder del análisis estadístico. Por otra parte, en este capítulo se resaltó la posibilidad de reducir todavía más las variables a medir, por ejemplo el potencial redox y la concentración del isótopo $\delta^{15}N$, haciendo así el estudio más económico y viable, al reducir también el esfuerzo tecnológico que supone, por ejemplo, trabajar con isótopos estables. La concentración de sulfuros y la comunidad de poliquetos fueron las variables que mejor reflejaron la interacción de las jaulas de acuicultura con el sedimento. A pesar de que el análisis multivariante de la comunidad de poliquetos proporciona información detallada sobre el estado ecológico del sedimento, se podría completar con información ecológica sobre las familias de poliquetos, pudiendo evitar así posibles confusiones en los resultados cuando hay mucha disimilitud entre las muestras o en el número de familias. Cuando se detecte que la población está cambiando, sería más apropiado conocer si las familias que proliferan o desaparecen están catalogadas como sensibles o tolerantes.

### 7.3. Meta-análisis de los efectos de las piscifactorías sobre el poblamiento de poliquetos de fondos blandos

Dado que los poliquetos son una buena herramienta para la vigilancia ambiental, se realizó un estudio del comportamiento de la comunidad de poliquetos a nivel de familia en 10 piscifactorías españolas, 8 de ellas en el Mediterráneo y 2 en el Atlántico, para observar el efecto de las jaulas sobre el fondo marino. Dicho análisis se realizó mediante un meta-análisis de 20 familias de poliquetos, las cuales estaban presentes en las 10 instalaciones estudiadas. De las 20 familias estudiadas, seis fueron catalogadas como familias tolerantes, ya que resistían debajo de las jaulas o incluso aumentaban su abundancia de manera significativa respecto a las áreas de referencia: Capitellidae, Dorvilleidae, Glyceridae, Nereididae, Oweniidae y Spionidae. Ocho de las familias fueron clasificadas como sensibles, ya que tendían a reducir su abundancia o incluso desaparecer bajo el efecto de las jaulas: Magelonidae, Maldanidae, Nepthyidae, Onuphidae, Paralacydonidae, Paraonidae, Sabellidae y Cirratulidae. Las otras seis familias restantes se podían encontrar en algunas instalaciones en los controles y en otras debajo de las jaulas: Syllidae, Sigalionidae, Poecilochaetidae, Phyllodocidae, Lumbrineridae y Eunicidae. La identificación de los poliquetos a un nivel taxonómico más alto, como el nivel de familia, sigue siendo una buena herramienta en la vigilancia ambiental de piscifactorías. Además de la estructura de la comunidad de poliquetos se analizaron algunas variables ambientales como el porcentaje de limos y arcillas, el porcentaje de materia orgánica, la cantidad de sulfuros libres totales, sulfuros totales, nitrógeno total, carbono orgánico total y la composición isotópica de $^{15}N$. De todas ellas, la cantidad de sulfuros libres totales, el porcentaje de limos y arcillas y la composición isotópica de $^{15}N$ fueron las que más relación tenían con la estructura de la comunidad de poliquetos.

### 7.4. Efecto del tamaño de grano en el sedimento y la bioturbación en la descomposición de materia orgánica proveniente de la acuicultura

El tipo de sedimento juega un papel importante en la biogeoquímica del sedimento, por lo que se realizó un estudio con sedimentos de diferente tamaño de grano. A través de un experimento de mesocosmos, se observaron las diferencias entre un sedimento arenoso y un sedimento fangoso bajo dos niveles de enriquecimiento orgánico, un enriquecimiento leve con pseudo-heces de mejillones y un enriquecimiento alto con heces de jaulas de dorada. Para simular el efecto de la bioturbación y bioirrigación de los sedimentos a través de la fauna bentónica se añadieron al experimento poliquetos de la especie *Hediste diversicolor* (O.F. Müller, 1776). En este estudio se observó que dicho poliqueto estimuló la mineralización y el reciclado de nutrientes. Los sedimentos fangosos, que de manera natural acumulan mayores cantidades de materia orgánica que los sedimentos arenosos, presentaron una mayor tasa de reducción de sulfato en comparación con sedimentos arenosos. En condiciones de enriquecimiento orgánico leve, el tamaño del grano del sedimento no tuvo efectos en los flujos bentónicos (consumo de oxígeno del sedimento, $CO_2$ total, amonio, nitrato, nitrito y fosfato). Sin embargo, a mayores niveles de enriquecimiento orgánico, los sedimentos arenosos acumularon menos materia orgánica, menos sulfuro, y menos amonio que los sedimentos fangosos, mientras que los resultados de oxígeno consumido por el sedimento fueron similares.

### 7.5. Valoración de una nueva herramienta para evaluar el impacto bentónico de la acuicultura: unidades experimentales de colonización

Siguiendo la misma línea de investigación sobre la influencia del tamaño del grano, se realizó un experimento en el campo con unidades experimentales, para tratar de reducir el efecto de la heterogeneidad espacial a la hora de realizar un plan de vigilancia ambiental. Las unidades experimentales se llenaron con dos tipos de sedimentos diferentes, arena y fango, previamente defaunados. Se colocaron en dos áreas de referencia y debajo de dos instalaciones de engorde de dorada y lubina. Después de un mes, las unidades experimentales se recuperaron y se estudió la estructura de la comunidad de poliquetos a nivel de especie, para luego poder usar el índice biótico AMBI (AZTI Marine Biotec Index). También se realizó un análisis multivariante de las especies de poliquetos. La cantidad de sulfuros, el tamaño del grano y el porcentaje de materia orgánica fueron medidos para describir mejor los procesos de las unidades experimentales. Tanto el índice biótico AMBI como el análisis multivariante dieron información precisa sobre el estado ecológico de las unidades experimentales, detectando grandes diferencias con las unidades experimentales colocadas en las áreas de referencia. Las unidades experimentales de arena mostraron una mayor sensibilidad para detectar cambios debidos al enriquecimiento de materia orgánica que las unidades experimentales de fango. Las especies de poliquetos que mejor mostraban estos cambios fueron por un lado *Capitella capitata, Spiochaetopterus costarum, Capitella minima, Pseudopolydora pulchra* y *Aricidea (Strelzovia) claudiae* en las jaulas de piscifactoría; y por otro lado *Gallardoneris ibérica, Micronephtys stammeri, Sternaspis scutata* y *Ampharete lindstroemi* en las áreas de referencia. Las unidades experimentales pueden ser usadas como una herramienta fiable en la vigilancia ambiental de piscifactorías, pese a que estos eran unos estudios muy preliminares y sería necesario más pruebas para poder generalizar su uso.

### 7.6. Conclusiones

#### Chapter 2

1. De acuerdo con el plan de seguimiento ambiental de JACUMAR, los análisis multivariantes del poblamiento de poliquetos y los sulfuros libres totales (TFS) muestran claramente los efectos de los cambios producidos en el bentos por el enriquecimiento orgánico debido al cultivo de peces

2. El plan de seguimiento ambiental propuesto podría modificarse para optimizar el esfuerzo de muestreo. El potencial redox y el isótopo estable de nitrógeno ($\delta^{15}N$), se podrían omitir para reducir costes y equipamientos tecnológicos. En algunos casos, si la heterogeneidad espacial del sedimento pudiese afectar a los resultados estadísticos, podría ser beneficioso aumentar el poder de análisis incrementando la replicación espacial a diferentes escalas, para detectar diferencias claras debidas al enriquecimiento orgánico causado por las actividades de la acuicultura.

**Chapter 3**

3. A escala general, después del meta-análisis, los patrones de distribución de las familias de poliquetos responden a combinaciones de variables físico-químicas, especialmente a la concentración de sulfuros y a la granulometría, que pueden verse afectadas bajo las condiciones del cultivo de peces. Como resultado, la abundancia y diversidad de poliquetos sufre alteraciones significativas. Los poliquetos aumentan su abundancia en situaciones de transición, pero ésta, así como su diversidad, disminuye en etapas avanzadas de contaminación por enriquecimiento orgánico.

4. La clase Poliqueta, estudiada a nivel de familia, proporciona información precisa sobre el estado de calidad del medio marino, presentando familias tolerantes y sensibles. La suficiencia taxonómica a nivel de familia y la utilización solo de la comunidad de poliquetos suponen una buena herramienta para el seguimiento ambiental de piscifactorías.

5. En el Mediterráneo occidental, las familias Capitellidae, Dorvilleidae, Glyceridae, Nereididae, Oweniidae y Spionidae resultaron ser tolerantes a la contaminación de las piscifactorías, mientras que las familias Magelonidae, Maldanidae, Nephtyidae, Onuphidae, Paralacydonidae, Paraonidae, Sabellidae y Cirratulidae resultaron ser sensibles a este tipo de impacto.

**Chapter 4**

6. Los sedimentos arenosos son más adecuados para albergar instalaciones de acuicultura que los fangosos, debido a que su capacidad metabólica es mayor. Por lo tanto, el

tamaño de grano del sedimento debería ser considerado como un parámetro clave en la selección de emplazamientos para la acuicultura.

7. La macrofauna (simulada mediante el uso de poliquetos) estimula la mineralización de la materia orgánica y el reciclado de nutrientes, incrementando la capacidad metabólica y la resiliencia del sedimento

8. Existen diferencias importantes en el impacto sobre el bentos cuando se comparan las heces de una especie carnívora, con las pseudoheces de un filtrador como el mejillón. A niveles bajos de enriquecimiento orgánico, como el producido por las pseudoheces de mejillón, no hay influencia del tamaño de grano sobre el metabolismo del sedimento.

**Chapter 5**

9. La colonización del poblamiento de poliquetos, estudiado mediante el análisis multivariante en las unidades experimentales, refleja claramente la influencia de las jaulas.

10. El índice AMBI (usado solo con el poblamiento de poliquetos), en las unidades experimentales, proporciona información clara sobre el estado ecológico del sedimento Los sedimentos arenosos y fangosos muestran el mismo nivel de contaminación. Sin embargo, AMBI no es recomendable cuando más del 20% de los especímenes no se pueden asignar a ninguna categoría.

11. Se recomienda el estudio de la comunidad de poliquetos al completo, en lugar de centrarse en la presencia de ciertas especies indicadores, ya que bastantes especies que proliferan bajo condiciones de enriquecimiento orgánico, también aparecen en zonas no contaminadas y vice-versa.

12. Las unidades experimentales con sedimento arenoso, son más sensibles a los cambios en la concentración de sulfuros y enriquecimiento orgánico que las unidades con sedimento fangoso.

13. Las unidades experimentales con sedimento defaunado seleccionado se muestran como una herramienta útil para el seguimiento ambiental de piscifactorías, con el objetivo de reducir la variabilidad ambiental que afecta a los resultados

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# CHAPTER 8

Picture: Alexander Roslin

# 8. REFERENCES


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# Chapter 10

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