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Development and application of a marine biotic index for the evaluation of the influence of aquaculture activities on the benthic ecosystem in Mediterranean coastal areas

Dr Andrea Alberto Forchino

Guide Professor:

Marco Saroglia

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ABSTRACT

The project was oriented to the development of an innovative tool for a more complete evaluation of the effect of marine aquaculture on the benthic ecosystem in Mediterranean areas. Due to their high sensitivity, the attention was focused on the macroinvertebrates communities and on their use as descriptor of the marine soft bottom "health *status*". Therefore, the AMBI, an existing marine biotic index and its multivariate approach, the M-AMBI (Multivariate AMBI), already applied to detect impacts deriving from various human activities along European Atlantic coasts, were developed and tested in different Mediterranean areas. To achieve this goal, the development of AMBI software database was necessary, including an higher number of Mediterranean species. In fact, assigning the macrobenthic species to one of the five Ecological Groups defined by this index, related to species sensitivity to disturbance, the AMBI gives back a classification of the site based on the benthic community "health *status*".

Hence, trying to enlarge the dataset and in order to test AMBI in different scenarios, this study was carried out in three different Mediterranean regions: Sardinia (Western Mediterranean), Cyprus (Eastern Mediterranean) and Tuscany (Coastal Marine Transitional Ecosystem). In detail, five fish farms as three cases study were investigated, representing each a particular different environment. In order to validate the results, AMBI and M-AMBI were compared to other indices calculations including another biotic index: the BENTIX. The choice of this latter was due to the fact that, up today, BENTIX is the most widely used index for the Mediterranean regions, and it shares the base approach with the AMBI but it differs in structure.

In detail, for each fish farm, samples of sediment were collected and chemical (total nitrogen, total carbon, organic matter, water content), physical (granulometry) and biological analysis were carried out. Concerning this latter, all the organisms collected were counted and identified to the lowest possible *taxon*. The obtained data were used to calculate biological indices, including AMBI. At the end, 123 new species were added to the AMBI database, and this upgraded AMBI revealed good discriminating capability and higher sensibility when compared to BENTIX. The setting of correct reference conditions allowed the application of M-AMBI and this analysis led to a clearer comprehension of the quality *status* of the sampled stations. The obtained results placed the spatial limit of the impacts deriving from aquaculture up to 200 m from the sources and underlined the influence of site specific characteristics (e.g. see depth, current velocity and direction) on sedimentation process. So, this study confirmed the potentiality of AMBI in detecting effects of aquaculture on the benthic ecosystem and the development of this index database extended the possibility to use it also in Mediterranean areas.

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INTRODUCTIVE PART

1 Coastal areas: an overview

1.1 Definitions and importance of coastal areas

Coastal areas are commonly defined as the interface or transition areas between land and sea, including large inland lakes. Coastal areas are diverse in function and form, dynamic and do not lend themselves well to definition by strict spatial boundaries. Unlike watersheds, there are no exact natural boundaries that unambiguously delineate coastal areas (FAO, 1998). It has been suggested that a distinction be made between the terms "coastal zone" and "coastal area". The term "coastal zone" would refer to geographic area defined by the enabling legislation for coastal management, while "coastal area" would be used more broadly to refer to the geographic area along the coast that has not yet been defined as a zone for management purpose (FAO, 1998). In this study the term "coastal area" is used with this kind of meaning, including also off-shore areas located in front of the coast.

Coastal areas are characterized by an important socio-economic value, since many of the world's major cities are located in coastal areas and a large portion of economic activities are concentrated in these cities. These zones are areas of convergence of activities in urban centers and wastes generated from domestic source and by major industrial facilities. Thus, traditional resource-based activities, such coastal fisheries, aquaculture, forestry and agriculture are found side by side with activities such as industry, shipping and tourism.

Concerning the environmental aspect, coastal ecosystems are ecotonal between marine, freshwater and terrestrial environments and may exhibit properties of these systems as well as unique characteristics of their own. The mixture of fresh and salt water in estuarine areas provides many nutrients for marine life. Salt marshes and beaches also support a large variety of animals and plants crucial to the food chain.

1.2 State of coasts in Europe: main trends and main threats

The 1992 Earth Summit of Rio de Janeiro recognized in its Agenda 21 the need for environmental action for oceans and coastlines and committed coastal nations to the sustainable development of their coastal areas and implementation of integrated coastal zone management. According to the basic principles of sustainable development, all three dimensions of development - economic, social and environmental - need attention and should be treated together in a holistic way. On this approach is based also the European Water Framework Directive (WFD 2000/60/EC) that is focused on the maintaining and improving the *status* of the Nations Member waters. The ultimate aim of the WFD is to achieve, by 2015, a good Ecological Quality *status* (EcoQ) within all the European waters, by the elimination of priority hazardous substance and contribute to achieving concentration in the marine environment near background values for natural occurring substance. To do that, the WFD established that the implementation of an effective and coherent water policy must address, as a key component of water quality, the integrity of aquatic ecosystem. Consequently, the strategic importance of reliable, quantitative and directly comparable methods for assessing the integrity of coastal aquatic ecosystems on a large scale has promoted an expanding body of research focused on the field of bioindicators and biotic indices (see chapter 4).

Coastal ecosystems - coastal lands, areas of transitional waters, and near shore marine areas - are among the most productive yet highly threatened systems in the world. Between 1990 and 2000, Europe lost more coastal wetlands despite an already high wetland conversion rate during the previous decades (EEA, 2006). Other valuable ecosystems, such as coastal dunes and sea grass beds remain continuously under threat. Population densities along European coasts are higher and continue to grow faster than those inland. Populations tend to be concentrated in certain areas, most favourable for trade, marine industry or recreation. These areas are often the location of the most valuable coastal ecosystems (e.g. Mediterranean). There is widespread evidence that European coasts are a natural environment that attract socioeconomic development due to a range of reasons. This attractiveness introduces multiple factors related to changing land uses, which can lead to increased stress on both natural and human environments. The development-related loss of coastal systems, habitats and services has caused the most notable changes to coastal zones. Between 1990 and 2000, artificial surfaces in coastal zones increased in almost all European countries. Economic restructuring has been a driver for infrastructure development, which in turn has attracted residential sprawl. The highest increase in artificial surfaces (20-35%) has been observed in the coastal zones of Portugal, Ireland and Spain (EEA, 2006). At European level, more than 2,720 km^2 of agricultural land (especially mixed agriculture and pasture) and semi-natural and natural land were lost predominantly to artificial surfaces during the same period (EEA, 2006). Human pressures on coastal resources can compromise ecosystem integrity. Recent patterns of over-exploitation of key fish stocks in European regional seas have altered the structure of marine ecosystems (Naylor *et al.*, 2000). Other examples, involving increasing sand and gravel extraction for construction or beach nourishment, has the potential to disturb the sediment balance around a European coast already influenced by sediment trapping of river dams (EEA, 2006). Thus, there is growing evidence that Europe's coastal systems (including marine and terrestrial) are suffering widespread and significant degradation (e.g. loss of habitat, eutrophication, contamination, erosion, alien species). This poses a major challenge to policy makers and coastal managers. Land based sources of pollutants, but also other indirect sources, play an important role in the formation of coastal pressures. Coasts can support only a certain amount of activity without suffering environmental degradation (EEA, 2006). Due to the gradual expansion of different human activities, coastal zones have accommodated a number of different uses. Often these human activities lack longterm coordinated spatial planning. Consequently, unregulated growth has led to mixed land-use and large scale fragmentation of open space (Belpaeme and Konings, 2004). A schematic synthesis of the principal threats that could affect coastal areas is reported in Figure 1.1. Trying to face this problem, the EU has been designating extensive coastal sites through its Natura2000 network (both on land and sea) to protect the coast from further development. On the whole, Natura2000 sites cover more than 50,000 km^2, approximately 15% of the coastal zone (landwards and seawards). The protection of coastal zones can only be achieved through a much broader integrated approach, many actors from elsewhere in the same marine region, river basin or other parts of the hinterland must also be involved. However, up today, concrete integration actions usually occur at local level, in the context of detailed planning, problem solving and territorial management (EEA, 2006).

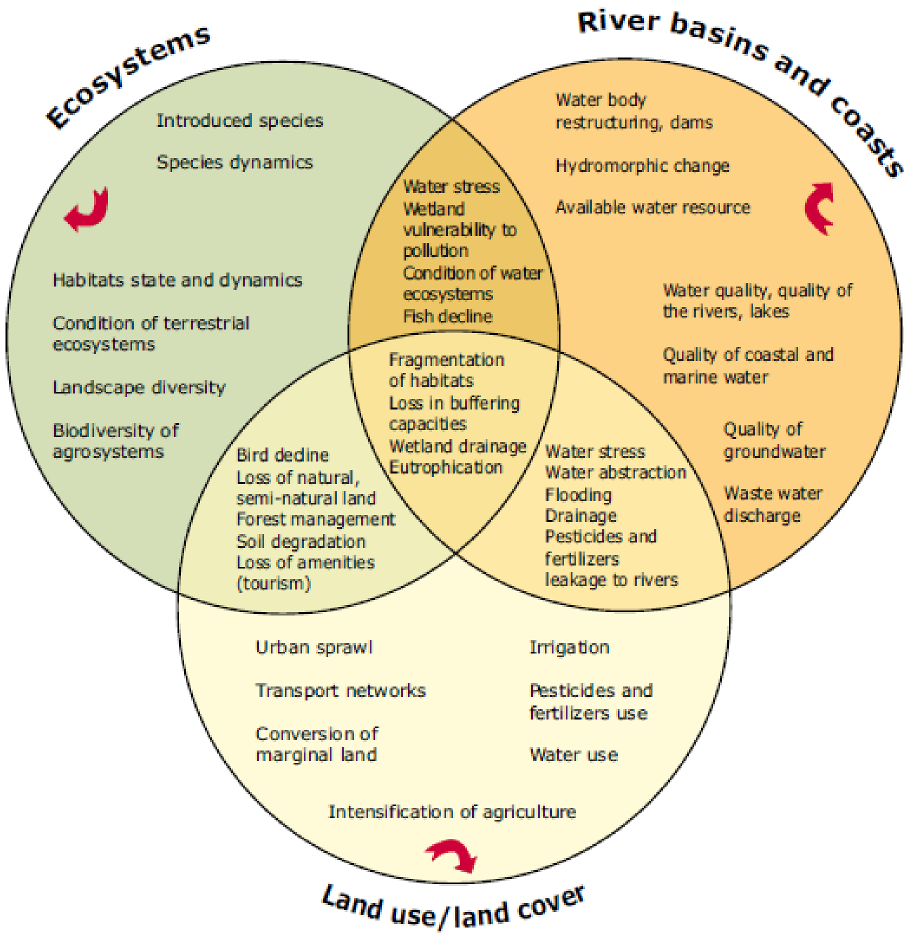

Figure 1. 1 Principal threats of European coastal areas (EEA, 2006)

2 Aquaculture and coastal areas

2.1 Global trends and farming typologies

Responding to the continuous decline in fishery harvests and in an effort to meet seafood consumption, aquaculture has become the world's fastest growing sector of food production, increasing nearly 60-fold during the last five decades (FAO, 2007). Currently, however, farmed marine species account for only 36% (3.2% for finfish) of the global shellfish and finfish aquaculture production (FAO, 2006) and provide only 11.5% (1.1.% for finfish) of all seafood products, inclusive of fisheries and aquaculture. In terms of quantity and value of products, Asia is the principal producer, followed by Europe (Figure 2.1).

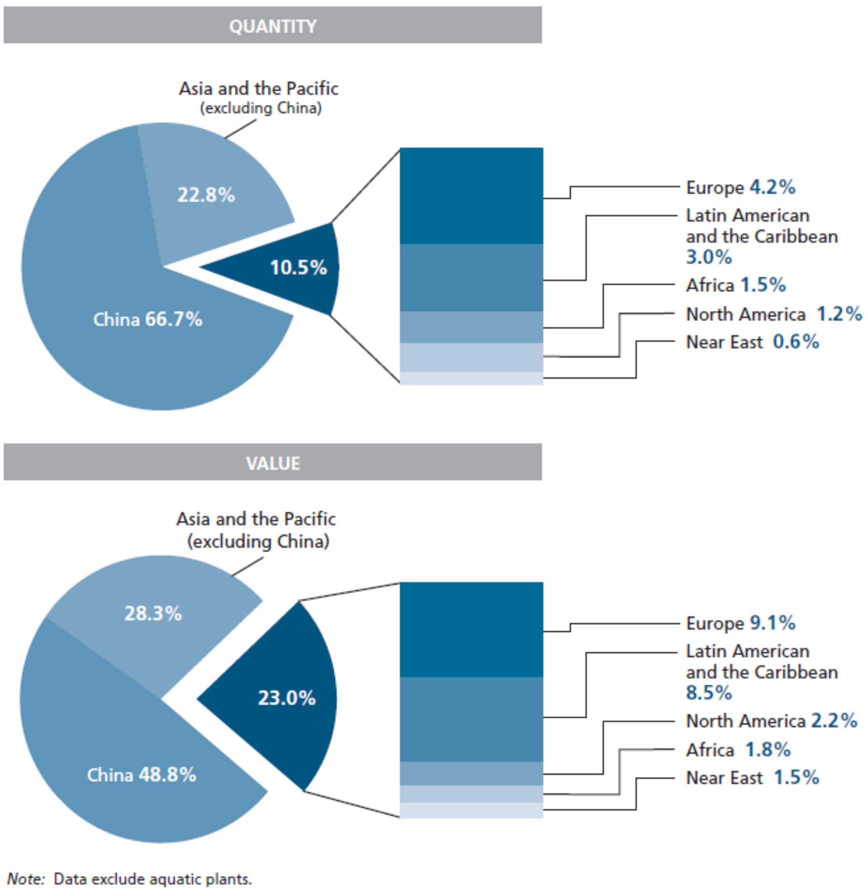

Figure 2.1 Aquaculture production by Region in 2006 (FAO, 2008)

Fish farm activities in coastal areas could be classified into two main farming typology: off-shore cage fish farming and land-base fish farming. In cage fish farming fishes are reared in cages located in the sea (Figure 2.2), while the land-based farming uses basins and ponds (Figure 2.3). Cages have developed a great deal from their humble origins and today there is an enormous diversity of types and design and they may be classified as shown in Figure 2.2. There are four basic types: fixed, floating, submersible and submerged. Fixed cages consist of a net bag supported by posts driven into the bottom of a lake or river. Fixed cages are comparatively inexpensive and simple to build, although they are limited in size and shape and their use is restricted to sheltered shallow size sites with suitable substrates. The bag of a floating cage is supported by a buoyant collar or, in some cases, a frame. This type is by far the most widely used and can be designed in an enormous variety of shapes and sizes to suite the purpose of the farmer. Floating cages are also less limited than most other designs in terms of site specifications. Some floating types are designed to rotate in order to control fouling. The much more widely used non-rotating floating types can be constructed with wide or narrow collars (Figure 2.4). The former are common on larger cages and serves as walk platform, facilitating many of the routine farm tasks. Most wide collars are designed to be rigid although some are flexible so that they may be used at more exposed sites. Some floating net bag designs, including early designs for flatfish culture, have a solid bottom (Hull and Edwards, 1979). Neither net nor rigid mesh bag submersible cages have a collar, but instead rely on frame or rigging to maintain shape. The advantage over other designs is that the position in the water column can be changed to exploit prevailing environmental conditions. Cages are typically kept at the surface during calm weather and are submerged during adverse weather or during a harmful algal event. While a number of submerged cages designs have been proposed, far fewer have gone beyond the design concept stage or indeed have been built or widely used. Despite the fact that cage designers and manufacturers have produced all sorts of designs in the past-half century or so, the range of cage types today is, if anything, smaller than it was a decade ago. Cost, always important has now become the overriding design criterion, particularly in the industrial-scale farming industries and this has led to uniformity in terms of shape, size and materials (Beveridge, 2004).

Concerning land-based fish farming, there are many types of systems and farm designs used to grow fish. These include: ponds, rectangle raceway tanks, circular (round) tanks, earthen tanks lined with plastics or clays and other forms of containment. Basic land-based fish farms involve the use of one or more types of tanks or ponds and generally have water piped in and out to maintain life support for the fish and to flush the tanks of waste products. Basic designs may also include mechanical aeration equipment for adding oxygen to water. In recent years, land based farms have advanced in technology to become more eco-friendly and to provide greater security and control of the farming process. The most advanced of these are known as water recirculation systems (RAS) designs. Advancements in design and technology are being driven in part by the need to develop alternative methods for aquaculture and by the demand for more fish and secure supplies.

Figure 2.2 Floating cage in Eastern Mediterranean Sea (Cyprus)

Figure 2.3 Ponds of a land-base fish farm in Tuscany (Italy)

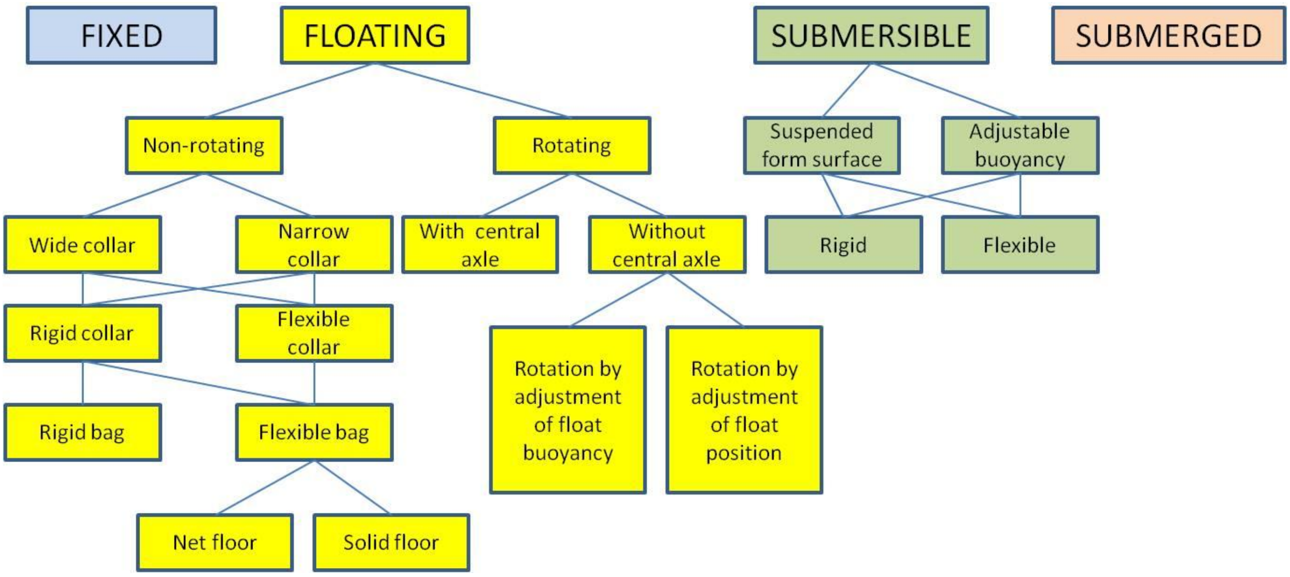

Figure 2.4 A classification system for cages (developed from Kerr *et al.*, 1980)

2.2 Sources of pollution

Among all the possible threats that could affect marine coastal areas, aquaculture activities could represent an important factor of risk, especially for Coastal Transitional Ecosystems (CTEs). Thus, these areas, due to their own transitional characteristic, appear to be more sensitive to natural and man-induced stress and so conscientious management practices are required.

Aquaculture, like other economic activities, uses and transforms resources into commodities valued by society, in this instance farmed fish, and, in so doing, produces wastes. Aquaculture activities release nutrients and chemicals into the marine environment (Naylor *et al.*, 2000; Gyllenhammar and Hakanson, 2005). Such particulate organic wastes could have effects on the water column in addition to settle onto the seabed and produce enriched sediments, which could lead to the deoxygenation of the bottom water and changes in the structure of benthic communities (Yokoyama *et al.*, 2006; Cole *et al.*, 2009). Wastes could derive from three main sources: uneaten food, fecal and urinary products (Figure 2.5).

A proportion of food thrown into a cage of fish is not eaten. Ingestion is dependent upon a sequence of events in which fish must first recognize that there is food present. They must be able to reach the food (strong currents, for example, may wash pellets out of the cage before they can be ingested) and be motivated (appetite, appearance) to take it into their mouths. Even at this stage, a food pellet may be rejected rather than swallowed if it feels wrong or contaminated (Thorpe and Huntingford, 1992; Smith *et al.*, 1995; Beveridge and Kadri, 2000).

As ingested material passes through the gut it is attacked by enzymes, the production of digestion being absorbed into the bloodstream and the undigested fraction being voided as feces. Metabolic breakdown products such as CO_2 and NH_4 and excess nutrients are passed out across the gills and in the urine. In addition, mucus and sloughed scales from caged fish, fouling organisms that have either become dislodged or have been discarded as a result of *in situ* net cleaning, mortalities and blood from harvesting operation, may be release into the environment (Beveridge, 2004).

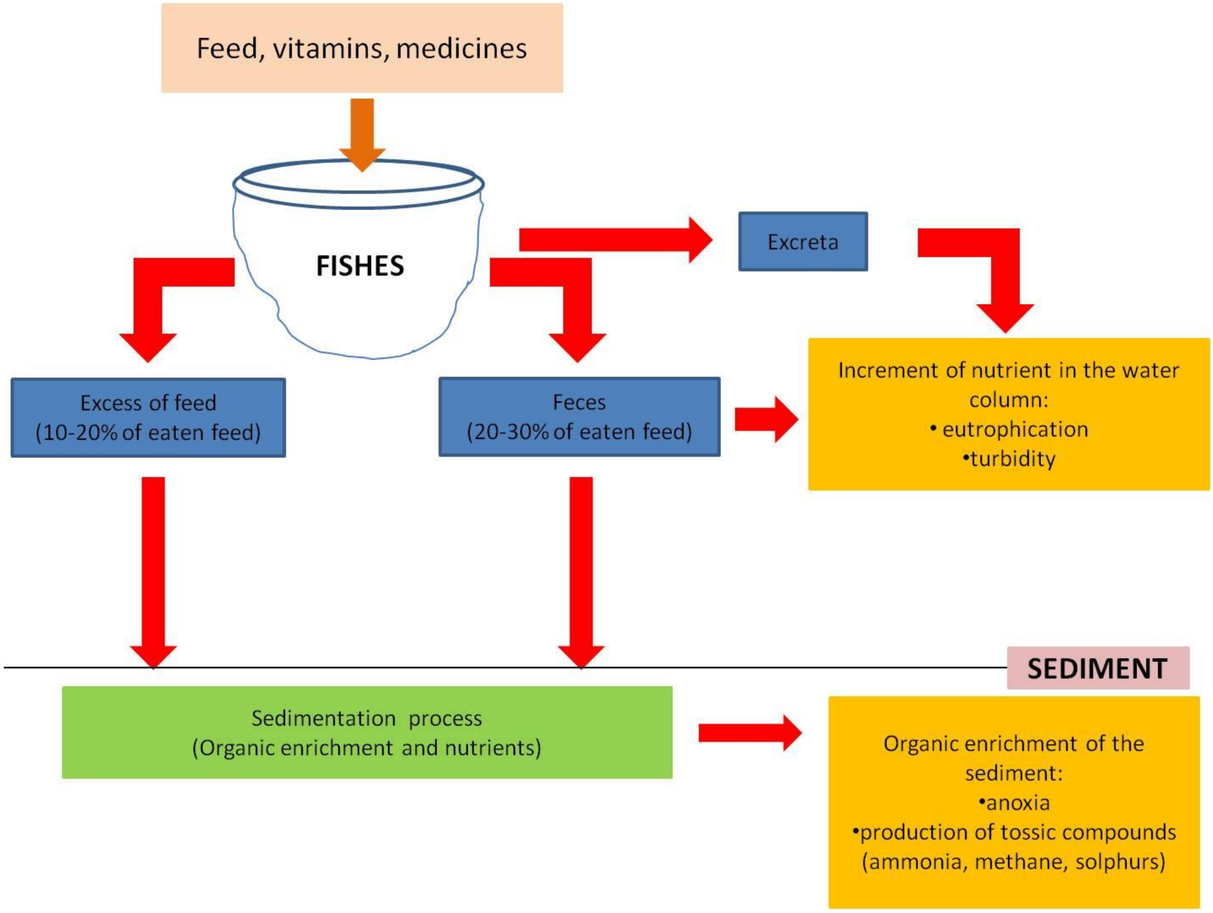

Figure 2.5 Scheme of the waste producted by cage aquaculture

2.3 Impacts on the water column, plankton, nekton and sediment

Excretory products are dispersed in the water column by currents while solids (uneaten food, feces) tend to settle towards the sea or lake bottom. During sedimentation, some of the uneaten food is consumed by fish (Carss, 1990; Johansson *et al.*, 1998) while some breaks down into fine particles (Stewart and Grant, 2002). Nutrients are solubilized, the quantities released depending upon the composition of feces and uneaten food, physical properties, temperature, depth of water and turbulence (Chen *et al.*, 2003). Nutrients are also released from sediment and it has been estimated that as much as 60% of total phosphorous and 80% of total nitrogen wastes end up in the water column (Hall *et al.*, 1992; Holby and Hall, 1992). The linkage between aquaculture activities and eutrophication, as indicated by increases in plankton and fish standing crop or productivity, is well documented in literature for freshwater basins (Costa-Pierce, 1996; Kelly and Elberizon, 2001) but not for marine waters. Thus, many studies have failed to find any influence on productivity in marine waters (Karakassis, 2001; Pearson and Black, 2001; Brooks *et al.*, 2002) while others have found only weak relationships between nutrient loadings and chlorophyll a (Wallin and Hakanson, 1991). This difference is principally due to the higher degree of movement and flushing of the marine water and so only highly enclosed, poorly managed sites can show signs of eutrophication.

Cage fish farming does not always result in changes in sediment chemistry or macrobenthic community ecology, because the degree of nutrient enrichment depends upon species being farmed, food, management, currents and depth (Beveridge, 2004). The extent of deposition is a consequence of the behaviour of organic particles in the water column and largely dependent on the nature of the site, water current regimes, and settling velocities of the released organic material. The degree and extent of such effects from fish farming have been previously investigated worldwide and it has been demonstrated that the impact on benthic environments is localized (Brown *et al.*, 1987; Gowen *et al.,* 1991). Water currents and eddies disperse these particles, and so the waste "footprint" on the seabed strictly depends on water depth and turbulence. In small amounts, this organic matter provides food for benthic animals and demersal fish, but when it accumulates on the seabed, it can block the supply of oxygen to burrowing animals and can drive an increase in oxygen consumption by micro-organisms.

The initial effect of adding large amounts of decomposable organic waste to marine sediments is increased metabolic activity by aerobic bacteria (Chàvez-Crooker and Obreque-Contreras, 2010). Their demand for oxygen results in localized hypoxia or anoxia phenomena, killing the most susceptible aerobic life forms (Gray *et al*., 2002). In the case of sediment in fish-cage footprints, much of the continuing metabolism then proceeds by anaerobic sulfate reduction (Holmer and Kristensen, 1992); simultaneously, the lack of oxygen inhibits aerobic nitrification and denitrification processes (Kaspar *et al*., 1988). Lack of sufficient oxygen leads to the death or migration of the sediment macrofauna responsible of bio-irrigation, and thus to a decline in aerated water within sediments and a further spread of anoxia. Upon the loss of bio-irrigation, pelagic-benthic coupling becomes reduced for these anoxic, azoic sediments. The net effect of organic enrichment in sediments is to move the ecosystem to the one dominated by bacteria, ciliates and meiofauna, where the trophic links to the next level of the food web are broken (Weston, 1990; Wildish *et al*., 2004). Under these conditions, the predominant bacteria are anaerobes, mainly sulfate reducers and methanogens (Wildish *et al*., 2004). Organic enrichment also can lead to an increased presence of pathogenic bacteria (Vezzulli *et al.*, 2002) and viruses (McAllister and Bebak, 1997). Such a deterioration in habitat often generates negative consequences for fish farming management too (Beveridge, 2003).

Finally, a further consideration must be taken into account. If aquaculture generates wastes that could have negative effects on the environment and on local biodiversity, at the same time, it generates dynamics that could damp this process. In fact, a factor that could play an important role in reducing the organic enrichment of sediment seems to be constituted by wild fishes. Wild fishes have been found to gather around aquaculture cages feeding on uneaten feed (Dempster *et al*., 2009) and recent studies suggest that they assimilate aquaculture wastes from the water column decreasing organic carbon, nitrogen and phosphorous sedimentation rates (Fernandez-Jover *et al.* 2007; Sanz-Làzaro *et al*., 2010).

3 Ecology of marine sediments

A significant synthesis of the scientific knowledge concerning the sediment biota is reported by Gray and Elliot (2010): “As the oceans cover 70% of the earth’s surface, marine sediments constitute the second larger habitat on earth, after the ocean water column, and yet we still know more about the dark side of the moon than about the biota of this vast habitat”. Thus, the aim of this chapter won’t be the exhaustive discussion of sediment ecology but the summary of the principal characteristics of this complex ecosystem.

3.1 Sediment characteristics and related environmental factors

One of the most important characteristic of the sediment is the **granulometry**. Concerning grain size the sediment composition depends on three main factors: settling velocity, which follows Stoke’s law[1], roughness velocity and threshold velocity. The roughness of a

[1] Stokes' law, is an expression for the frictional force - also called drag force - exerted on spherical objects with very small Reynolds numbers (e.g., very small particles) in a continuous viscous fluid. Stokes' law is derived by solving the Stokes flow limit for small Reynolds numbers of the generally unsolvable Navier-Stokes equations:

$$F_d = 6\pi\,\eta\,R\,V$$

where:

F_d is the frictional force acting on the interface between the fluid and the particle (in N),

- η is the fluid's viscosity (in [kg m^{-1} s^{-1}]),
- R is the radius of the spherical object (in m), and
- V is the particle's velocity (in m/s).

If the particles are falling in the viscous fluid by their own weight due to gravity, then a terminal velocity, also known as the settling velocity, is reached when this frictional force combined with the buoyant force exactly balance the gravitational force. The resulting settling velocity (or terminal velocity) is given by:[2]

$$V_s = \frac{2}{9}\frac{(\rho_p - \rho_f)}{\eta}\,g\,R^2$$

where:

- V_s is the particles' settling velocity (m/s) (vertically downwards if $\rho_p > \rho_f$, upwards if $\rho_p < \rho_f$),
- g is the gravitational acceleration (m/s^2),
- ρ_p is the mass density of the particles (kg/m^3), and
- ρ_f is the mass density of the fluid (kg/m^3).

sediment is important as rough sediments are more easily picked up by currents flowing over the sediment than the smooth particles. Threshold velocity is the force needed to pick up a particle when water flows over the sediment. The relationship among these three factors is reported in Figure 3.1. If sediments are very fine or if they are of a mixed composition they pack more tightly, so that it is harder for water movement to pick up the particles, hence the reverse inflection in the curve (Figure 3.1). Particles 0.18 mm in diameter are the easiest to move; particles coarser than this are difficult to pick up and transport because they are dense, whereas particles finer than 0.18 mm pack into a smooth bottom surface and are difficult to re-suspend (Gray and Elliott, 2010). With an increasing percentage of muds, the sediment become increasingly cohesive and thus requires an even greater force to re-suspend or erode the particles.

In general, coarse intertidal sediments drain fast and retaining little water or organic matter. They are therefore inhospital habitats, or at least inhabitated only by those species able to tolerate such mobile sediments, such polychaetes (e.g. Syllidae sp.) and fast burrowing venerid bivalves (Pastor de Ward, 2000). At the other extreme, very fine sediments such as mood, which have grains tightly packed together, may preclude the presence of a meiofauna inhabiting the pore spaces between grains (Pastor de Ward, 2000; Gray and Elliott, 2010). They have also poor water circulation and often a low oxygen tension, because there is only a small exchange of overlying oxygenated water, and only oxygen that diffuses into the sediments is rapidly used up by the aerobic bacteria and micro and meiofauna. In addition, a greater amount of organic matter settles out in the same area as fine muds, again increasing the oxygen demand and inducing changing in the biotic community (La Rosa *et al.*, 2001; Yoza *et al.*, 2007).

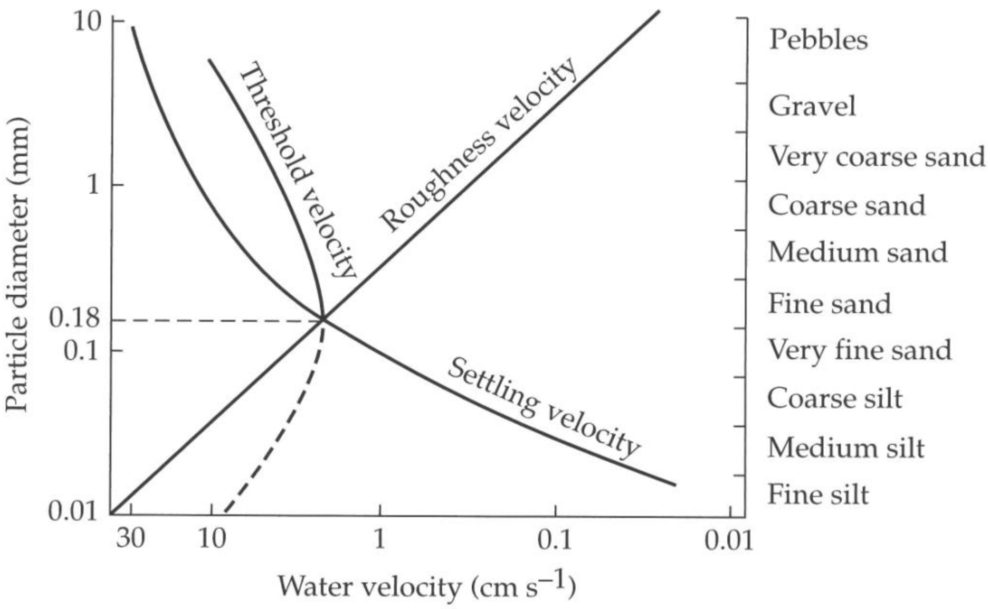

Figure 3.1 Key factor influencing mobility of sediment particles: settling velocity, roughness and threshold velocity (Gray and Elliott, 2010)

Others important characteristics that influence the water movement through the sediment are **porosity**, defined as the size of the available pore space and thus the amount of water being retained in a waterlogged sediment, and **permeability,** defined as the amount of water that can flow through the pores (Eleftheriou and McIntyre, 2005).

Concerning environmental elements, **light** is a key factor that affects intertidal and shallow marine areas. Because of the nutrients in the overlying water column and in the pore water, sediments have an abundance of benthic microalgae, which consist of unicellular eukaryotic algae and cyanobacteria that grow within the top few millimeters of illuminated sediments (McIntyre *et al.*, 1996). These organisms photosynthesize during light periods and the oxygen concentration in superficial sediments may be raised above that from simple diffusion process. At night the plants respire and the carbon dioxide is produced into the surface water and atmosphere. The depth of photosynthetic layer is determined by light penetration and contains cyanobacteria and photosynthetic eukaryotes (e.g. diatoms, dinoflagellates). Beneath this layer there is the "dark-blue-green layer" of filamentous cyanobacteria (*Phormidium* and *Oscillatoria*) which binds the sand grains together. Beneath this there is the "purple layer" of the purple bacterium *Chromatium* and chemolithic bacteria such *Beggiatoa* which oxidize sulfides to elemental sulfur (Cavanaugh, 1983). Surface sediments are prone to be disturbed by wave and current action and so the microphytobenthos may be spread evenly through the surface layer of the sediment (Stevenson, 1983). Thus, the top few millimeters constitute a zone of intense microbial and geochemical activity. In the bacterial assemblage below the “purple layer” the sediment is black and anaerobic; here the methanogenic fermenters and sulfate reducers dominate and under certain conditions methane and hydrogen sulfide could be released (Gray and Elliott, 2010). Fenchel and Finlay (1995) calculated that the complete mineralization of 1 kg of organic matter yields 570 g of hydrogen sulphide. A number of species that live in sediments can utilize sulfide as a source of energy. Bivalves of the *genus Thyasira* are good burrowers and are able to take up free sulphide deep in the sediment since they contain chemoautotrophic sulphur-oxidizing bacteria in their gills (Dando *et al.*, 2004). The fauna of the hydrothermal vents also derives its primary energy source from oxidation of sulphides by the use of chemosynthetic bacteria. These organism can be large: the tubeworm *Riftia* (Sibloglinidae), which has chemosynthetic symbiotic bacteria in its tissues, can be over 1.5 m long (Gage and Tyler, 1991; Hsu and Thiede, 1992).

3.2 Benthic community: general features

Before discussing benthic features in detail it is useful to define the component of the system. Benthic organism could be separated into the fauna and flora, and then according to their preference for hard and soft substrata, with the latter encompassing muds, sands, gravel, or even cobbles. Hard substrata include rock and hard, compacted glacial clay. Then, benthic organisms could be separated according to whether they are mobile, sedentary or sessile and their position in relation to the sediment. The latter separates organisms according to whether they are moving over the sediment (the mobile *hyperbenthic* animals), are on the sediment (the *epibenthos* – including the attached *epiflora* and *epifauna* and the mobile and sessile *epifauna*), or in the sediment (the *infauna*) (Gray and Elliott, 2010). Benthic organisms could be separated according to whether they occupy the intertidal zone, and can thus tolerate higher exposure to physical stressors (e.g. waves action), or are *sublittoral* (or *subtidal*). Subtidally, the *macro*- and *microflora* and those animals feeding directly on these will be restricted to the photic zone, the *infralittoral*, whereas the fauna also penetrate deeper. The next zone in depth is the animal-dominated *circalittoral* (Figure 3.2). Finally, according to the size it is possible to separate:

- **Microfauna** (<63 µm): ciliates, rotifers, sarcodines;
- **Meiofauna** (63-500 µm): nematodes, oligochaetes, gastrotrichs;
- **Macrofauna** (500 µm-5 cm): polychetes, amphipods, bivalves;
- **Megafauna** (>5 cm): echinoderms, decapods.

The ratios of the different dimensional categories depend on the sediment type but normally in a typical intertidal beach the microfauna, dominate numerically, but the macrofauna dominates in term of biomass (Gray and Elliott, 2010).

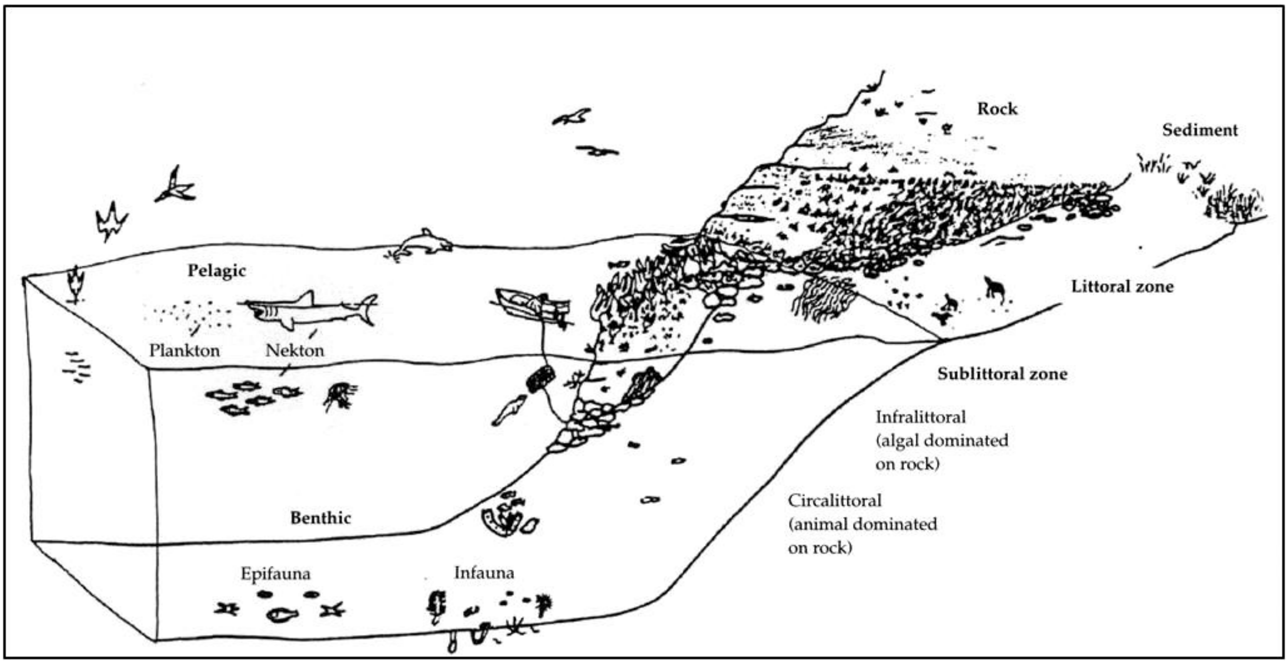

Figure 3.2 The components of the nearshore system(Hiscock, 1996)

3.3 Faunal patterns: a complex net of relations

The variables and processes which create marine biological communities is a set of interlinked relationships (Figure 3.3). Physicochemical variables such as water movements and sediment type set up the conditions which constitute a fundamental niche and under which the benthic organism colonize an area (the **environmental-biology relationships**, in Figure 3.3). Following this, biological interactions such as competition and predator-prey relationships modify the biological community structure and create the functioning (the **biology-biology relationship**, in Figure 3.3). Then, the biological benthic community can modify the physical structure such as through sediment turnover and changes to sediment chemistry (the **biology-environmental relationships**, in Figure 3.3). Finally, human influences are superimposed on these process (Elliott *et al.*, 2006).

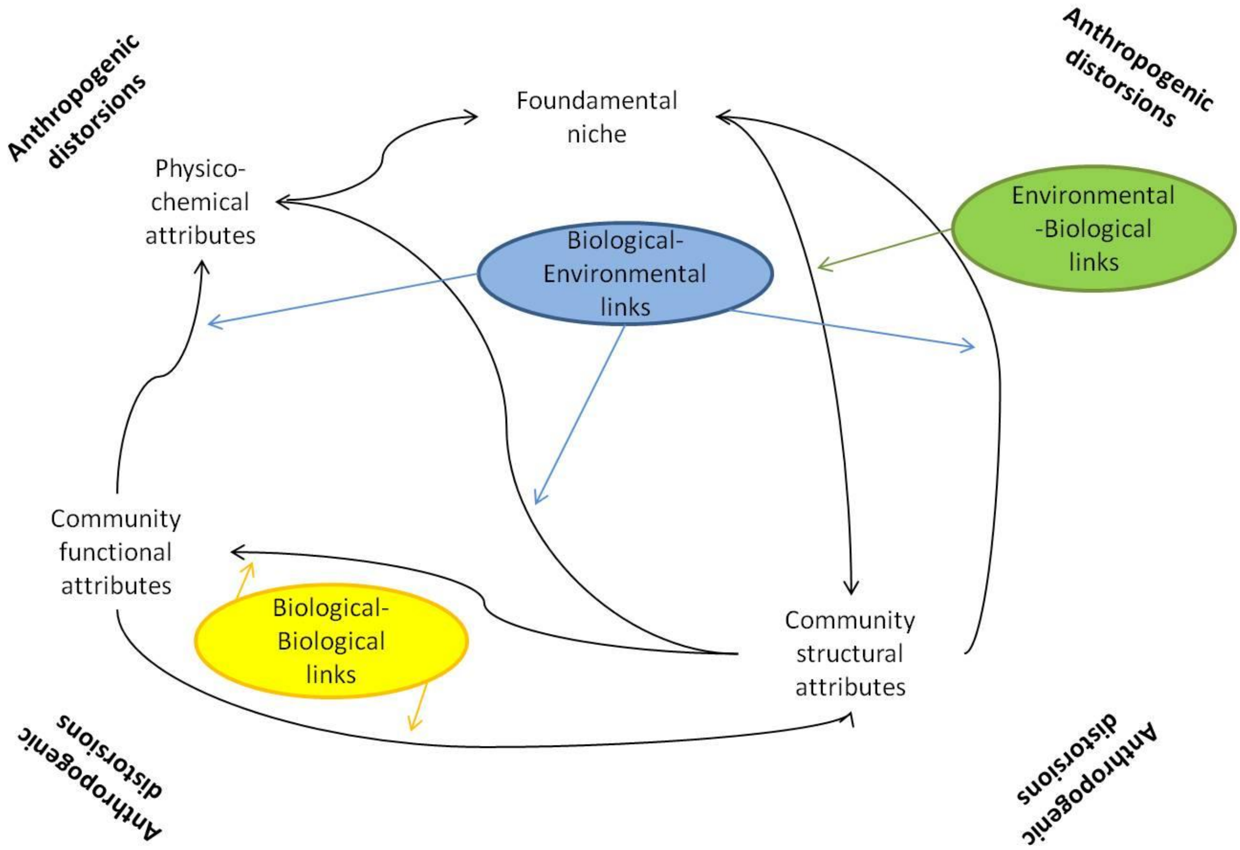

Figure 3.3 Benthic community forcing factors - a conceptual model of the main relationships (from Elliott *et al.*, 2006)

As described in chapter 3.1, the physical hydrodynamics is a key factor, which controls substratum type which, in turn, affects the biological features of these habitats (McLachlan, 1983, 1996). Within the biological niches created by the physical environment, biological factors such as predator/prey relationships operate. Furthermore, the biological components will also affect the physical conditions, e.g. bioturbating organisms rework and bind the sediment changing the properties of the substratum (Peterson, 1991). These interactions between the physical features and biota (**"environmental-biological links"**), the relationships between the biological components and processes (**"biological-biological links"**), and those whereby the biological processes modify the environmental conditions (**"biological-environmental links"**) produces several related features which can be used for defining the condition of the habitats. For example, the spatial extent and the tidal regime and elevation of the biotope complexes dictates the size of the primary consumer populations supported which in turn are prey for the fish and birds (Gray, 1981).

3.3.1 Environmental-biological links

Although the effect of one or more environmental factors acting singly or in conjunction with others is important, the primary factor controlling the dynamics of the intertidal sand and mudflats and the subtidal mobile sand banks is the **hydrophysical regime** (Elliott *et al.*, 1998). The interactions of all physical factors will determine the composition and density of the infauna (Eleftheriou and McIntyre, 1976). Species in the biotope complexes are somewhat protected against the sedimentary instability and variability in temperature, salinity, exposure and predation by burrowing (Eagle, 1973). Marine organisms have fundamental tolerances which dictate their large scale geographical distribution (Glemarec, 1973). On a regional scale, temperature tolerances will produce "*biogeographical zone"* (e.g. Arctic, Boreal, Lusitanian assemblages) and salinity tolerances will dictate the extent of distributions within freshwater-affected environments such as estuaries (McLusky, 1989). Unstable sediments support fewer organisms than stable ones and only those mobile species which can re-establish their position (Allen and Moore, 1987). Some species of macrofauna, in particular the crustaceans, are adapted to living in sediments exposed to heavy wave action mainly through their ability to burrow rapidly (Brown, 1983).

In general, decreasing exposure to wave action correlates with increases in abundance, species richness and biomass of polychaetes and a decrease in abundance of crustaceans (Dexter, 1990). Survival rates of organisms, such as sedentary polychaetes living in the sediment, decrease when surface sediments are disturbed daily although it is possible that small ones are simply relocated (Brown, 1982). Motile species such as *Scolelepis squamata*, however, are adapted to life in unstable sediments and survive through rapid burrowing (McDermott, 1983). Allen and Moore (1987) found correlations between community structure and the prevailing physical conditions including shore stability for both individual organisms and guilds. The relationships were more evident lower down the shore where other factors such as desiccation were less important.

Species diversity as well as overall community structure, is influenced by the habitat stability and sediment type. Coarse sediments, which are unstable and difficult to burrow into, are dominated by epifauna, while fine sediments are increasingly dominated by infauna. Many species are found in or on a range of sediment types, but others have a more restricted distribution (Wolff, 1973). The greatest diversity of macro-infaunal species is generally associated with poorly-sorted sands because they are physically heterogeneous, and thus have a large number of ecological niches, are reasonably stable and contain a supply of deposited organic matter (Elliott *et al.*, 1998). Sedimentary features influencing the distribution of feeding guilds (e.g. suspension and deposit feeding benthos (Sanders, 1958). Deposit feeders dominate over suspension feeders in areas with higher percentages of silt-clay. They feed on the bacterial and microphytobenthos film surrounding sand and mud particles and therefore tend to dominate mud flats and sheltered shores. The distribution of suspension feeders is greatly affected by sediment instability as muddy sediment and high turbidity clog the filtering organs. In addition, subtle changes in the relative proportions of sand, silt and clay will affect an organisms' ability to maintain a burrow (Meadows and Tait, 1989).

3.3.2 Biological-environmental links

The basic biological community established under the prevailing environmental conditions has the capacity to modify the sedimentary regime (**biomodification**). There are several categories of biomodification:

- by organisms with an ability to stabilize the sediment, (**biostabilization**) as shown on intertidal mud and sand flats, for example, by spionid tube beds (e.g. *Prionospio elegans*, by affecting boundary conditions), microphytobenthic mats (by mucopolysaccharide production), and eelgrass meadows (by sediment binding with rhizome production and by disturbing the sediment-water interface turbulence) (Gray and Elliott, 2010);
- by organism behaviour leading to **biodestabilisation**, which in turn may lead to increased erosion (**bioerosion**); this may result from excessive reworking (**bioturbation**) by mobile infaunal organisms (e.g. *Macoma balthica*) on mudflats (Orvain *et al*., 2006);
- by feeding behaviour increasing the supply of sediment from the water column to the seabed through the production of faeces and pseudofaeces (**biosedimentation**), for example by suspension feeders such as mussels (*Mytilus edulis*) on mudflats and cockles (*Cerastoderma edule*) on sandflats (Hertweck and Liebezeit, 1996).

Each of these processes modifies the sedimentary regime with the potential of increasing its heterogeneity and thus the number of niches available for colonization. For example, extensive reworking increases the depth of surface-phenomena such as oxygenated sediments as well as increasing rugosity (surface roughness). Surface roughness disrupts the sediment-water boundary conditions and the ability for organisms to settle although it may also increase erosion (Elliott *et al.*, 1998).

Heterotrophic marine organisms are predominantly deposit or suspension feeders. Deposit feeders may feed at the surface or at depth within the sediment, resulting in the production of faecal pellets and the movement of organic material from deeper within the sediment to the surface. The vertical and lateral movement of mobile deposit feeders causes the mixing and transport of particles, interstitial water and dissolved gases (Rhoads, 1974). In muddier areas the production of faecal pellets by deposit feeders are of a size which may be ingested or otherwise manipulated by other benthic invertebrates hence increasing sediment reworking. As a consequence, the degree of bioturbation tends to be greater in fine muds dominated by deposit-feeders than in coarse grained substrata (Rhoads, 1974).

The factors most highly correlated with bioturbation are feeding method and location in relation to the sediment-water interface, organism size and degree of mobility, population density, burrowing depth and the density and spacing between animal tubes (Rhoads and Boyer, 1982).

Many of these processes are population size and temperature dependent. In addition, Reichelt (1991) identified three main processes leading to bioturbation: feeding activity, burrow or tube construction and migration within the sediment column due to tidal and diurnal cycles.

Faecal pellets have higher deposition rates than their constituent particles and therefore settle out near the site of production. Deposit feeders may have a more quantitatively significant role in pelletization of the sea floor than suspension feeders or zooplankton (Rhoads, 1974).

3.3.3 Biological-biological links

This kind of links are principally constituted by:

- **predation:** the main predators in intertidal and subtidal areas are birds, fish and epifaunal crustacea such as crabs and shrimps (Meire *et al,* 1994);
- **competition:** the faunistic variation in these physically controlled environments reflects the species tolerance and sensitivity to those conditions. Competition between organisms occurs in response to a limitation of resources. Competition for space and food is unlikely to be a limiting feature in the high energy sedimentary environments (sandbanks). This is because the populations are small, due to the harsh conditions, and many organisms swim and feed in the water column at high tide and only shelter temporarily in the sediment at low tide (Peterson, 1991). Densities are kept low by the disturbance of sediment in high energy areas and so

there is probably no limitation of space (Peterson, 1991). In many marine, sedimentary communities, deposit and detritus feeders compete for food and suspension feeders compete for space (Levinton, 1979). Thus, the large populations inhabiting intertidal mudflats and, to a lesser extent intertidal sandflats, will have inter- and intra-specific competition for food. Because of this, resource partitioning may occur among certain deposit feeders to avoid competition as shown for the gastropod *Hydrobia* and the amphipod *Corophium* which ingest different size food particles (Fenchel, 1972). Interspecific competition may be relatively low in intertidal mud and sandflats because of the restricted community diversity.

- **recruitment and lifecycles:** most macrofauna are iteroparous in that they breed several times per lifetime. The fecundity is closely linked to the limited food supply with temperature changes an important controlling factor. Many polychaete worms, including *Nephtys* spp. and spionids, release eggs and sperm into the water where, after fertilisation, the larvae enter the plankton for a short time before settling to the substratum (Rasmussen, 1973). The passive movement of these stages again reinforces the importance of understanding the hydrographic regime to interpret the factors influencing the community structure.

3.4 Sensitivity to natural events

The assessment of the sensitivity of benthic communities to naturally occurring events is a difficult task due to the many important and measurable physical and biological features. Natural perturbations could be ascribed at four main categories: hydrophysical regime, seasonal changes, fresh water runoff and salinity, ecological relationships.

The **hydrophysical regime** is very variable and, while it is possible to consider "average" exposure, it is essential to recognize that for both these biotope complexes and particularly subtidal sandbanks, extreme conditions have profound effects, even when they only persist for a short length of time (Hiscock, 1983). Water movement due to wave action is the more erratic because it fluctuates considerably on a seasonal basis. Movements caused by tides and currents varies in regular patterns but it is not only the strength but also the type of movement that affects the distribution of marine organisms. Uni-directional, multidirectional and oscillatory movements each represents a different type of stress or confer particular advantages (Wood, 1987). Storm events will inflict extreme changes in wave action both in terms of strength and direction (Pethick, 1984; Carter, 1988). Increased wave action causes stress to the infauna by disrupting feeding and burrowing activities and reduces species richness, abundance and biomass. The infauna are sensitive to changes in sediment as many are adapted to burrow through certain grades of sediment (Trueman and Ansell, 1969). Coarse material is more difficult to burrow through and species have to be robust in order to survive the stronger currents/wave action in these areas (Gray and Elliott, 2010).

Changes in the hydrophysical regime and thus substratum will change the faunal composition of the biotope complex. Major changes in the former will produce mortality and reduce species richness. Although many species are capable of living in a variety of substrata, the species most affected will be those which are restricted to a particular grade of sediment.

Seasonal changes occur in subtidal community structure (Boesch, 1973) and environments that have characteristic seasonal patterns of species composition are relatively unstable and often “physically-controlled” (Sanders, 1968).

Intertidal sand and mudflats are sensitive to increased rainfall and thus an increased **freshwater input**. This may cause scouring of intertidal areas, changes in intertidal creeks and possibly a reduction in **salinity** in localised areas. Salinity is an important variable which influences the populations of intertidal and subtidal areas, especially in estuaries where it is the dominant factor (McLusky, 1989). On open coasts it is less important but it may have a significant local influence. The physiological effects of salinity change are well described (McLusky, 1989) and species in intertidal areas are adapted to tolerate changes in salinity by osmoregulation, reducing oxygen consumption and reducing metabolic activity to conserve energy (Brown, 1983) or by moving seaward if they are mobile. Thus salinity gradients over intertidal mud and sandflats will produce zonation in the fauna.

Each species is sensitive to changes in intra- and inter-specific interactions which will influence the development of the benthic communities and the stability and persistence of benthic communities is influenced by **ecological interactions**. The interactions between infaunal species may be in competition for space (Woodin, 1976) or as the result of survival, migration and recruitment patterns. For example, surface active *Nereis diversicolor* had a negative effect upon *Corophium* spp. (Jensen and Andrew, 1993). Species which are commensal, parasitic, symbiotic or epizoic depend on the presence of other species as hosts or partners. Environmental changes removing the latter will cause the species reliant on them to disappear. Community composition will be sensitive to synchrony (or otherwise) between the population dynamics of predators and the different prey species and species reproducing during times of minimal predator activity could significantly reduce the effects of predation (Eagle and Tyler, 1975; Banner, 1979). The community composition is sensitive to changes in food availability. For example, Buchanan and Moore (1986) found that a decline in quantities of organic matter changed the infauna of a deposit feeding community which is essentially food limited. In turn, this removed the competitive pressure from the other species and produced a period of instability as several species became dominant.

3.5 Sensitivity to anthropogenic activities: the organic enrichment

The effects of organic enrichment on sedimentary systems and their benthos is well documented and shows a consistent sequence of response - the **Pearson-Rosenberg model** (Pearson and Rosenberg, 1978).

As described in chapter 2.3, in essence, high organic inputs, coupled with poor oxygenation leading to conditions of slow degradation will produce anaerobic chemical conditions in the sediments. In turn, this increases microbial activity and reduces the redox potential of the sediments (Fenchel and Reidl, 1970). Ultimately, this increases the production of toxins such as hydrogen sulphide and methane. The changed status to anaerobiosis will limit the sedimentary macroinfauna in anoxic/reducing muds to species which can form burrows or have other mechanisms to obtain their oxygen from the overlying water.

Pearson and Rosenberg (1978) reviewed the effects of organic enrichment on benthic fauna and summarized them in a schematic figure (Figure 3.4). In 1986, Rhoads and Germano gave a very similar synthesis and final model of the response of the benthos to organic enrichment, consequently in literature often it’s referred to it as the Pearson-Rosenberg model (or paradigm) or, less commonly, the Rhoads-Germano model. Following this model, as enrichment of the organic content of the sediment increases, at first the deep-burrowing species such as decapods and echinoderms and the sensitive dwellers, the bivalve *Nucula* and the ophiuroid *Amphiura*, are replaced by a variety of transitional species. The redox discontinuity layer (RPD) shown as a broken line in Figure 3.4, moves closer to the surface. With increased organic matter loading, only opportunistic species such as the polychaete *Capitella* and *Chaetozone* are dominant. Finally, the redox layer touches the surface which become black and anoxic and only a few specialized sulfide-loving species as nematodes survive. In severe cases, the sediment surface is then covered by a layer of sulfur-oxidizing (sulfate reducing) bacterium *Beggiatoa* (Mubmann *et al.*, 2003). This successional model has been verified on numerous occasions; the species composition varies from location to location but the guilds of species found are similar. Hylland *et al.* (2006) recently expanded upon this model by using it as a conceptual basis for defining lower and upper thresholds in total organic carbon (TOC) concentrations corresponding to low versus high levels of benthic species richness in samples from seven coastal regions of the world. Specifically, it was shown that risks of reduced macrobenthic species richness from organic loading and other associated stressors in sediments should be relatively low at TOC values < about 10 mg g^{-1}, high at values > about 35 mg g^{-1}, and intermediate at values in-between.

Any nutrient stimulation of marine areas may be regarded as hypernutrification which, if not controlled, produces symptoms of eutrophication, defined as the adverse effects of organic enrichment (Scott *et al,* 1997). Such a symptom on intertidal sand and mudflats is an increased coverage by opportunistic green macroalgae, such as *Enteromorpha*, which will create anoxic conditions in the sediment below the mats, reduce the diversity and abundance of infauna and interfere with bird feeding (Simpson, 1997).

Changes in the species composition and density of benthic diatoms of an intertidal brackish mudflat diatom populations is also evident after organic enrichment (Peletier, 1996). This may be the result of the reduced densities of the macrofaunal diatom grazers *Nereis diversicolor* and *Corophium volutator* (Peletier, 1996).

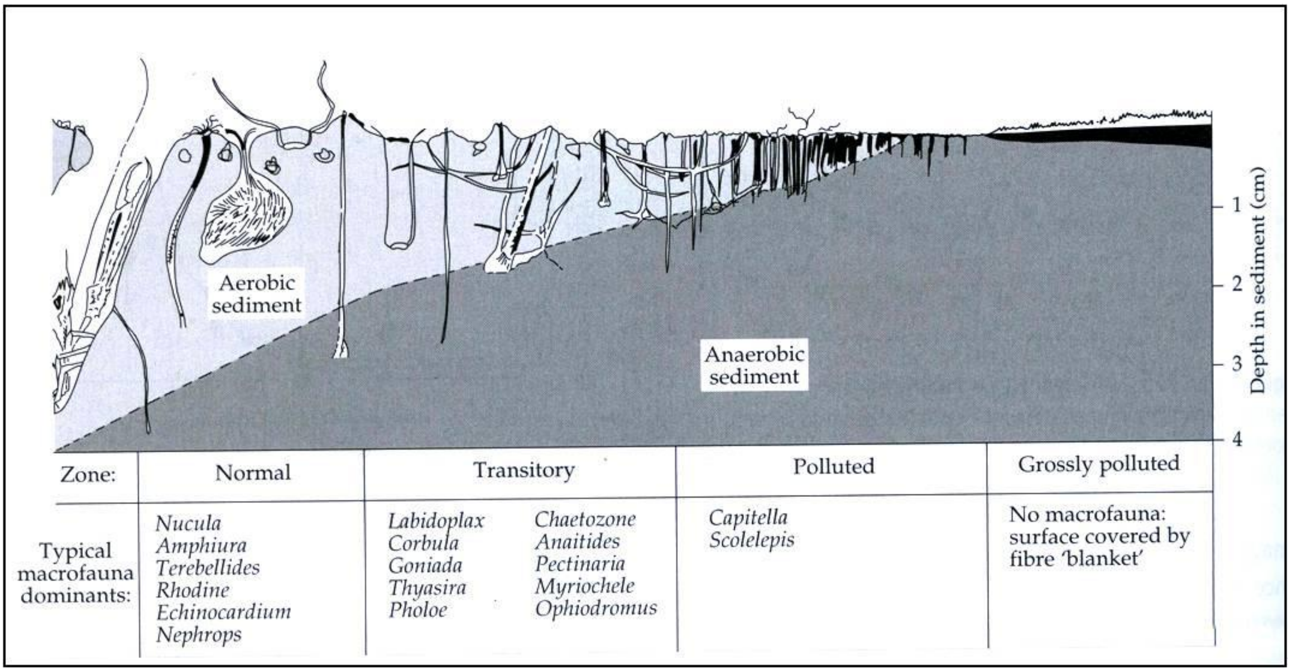

Figure 3.4 A schematic view of the effects of increasing organic enrichment on the fauna of soft sediments; the gradient increases from left to right (from Pearson and Rosenberg, 1978).

4 From the benthic communities to coastal water "quality status": the biotic indices

4.1 Biotic indices: general features and typologies

Assessing the quality of coastal waters is a crucial issue for society, being strictly linked to the individuation of corrected management strategies for these areas. In recent years, a lot of works have underlined the importance of performing this assessment using metric, comparable and transparent scales, internationally accepted and scientifically validated (Aguado-Gimenez *et al.*, 2007; Borja *et al.*, 2008, 2009; Martinez-Crego *et al.*, 2010; Tataranni and Lardicci, 2010). The concept of water quality has evolved into a much more holistic view for incorporating not only physical-chemical but also biological and ecological notions. Martinez-Crego *et al.* (2010), individualized the main characteristics that a biotic index should have as:

- **relevance to ecological integrity:** biological measures should be capable of reflecting the integrity of the entire ecosystem. Phytoplankton, aquatic flora, benthic invertebrate fauna and fish fauna are the most commonly proposed organisms for quality bioassessment program of coastal and estuarine waters;
- **broad-scale applicability:** a key feature of the different strategies for water management is their large spatial scale applicability, usually in the order of thousands kilometers;
- **early-detection capacity:** the early detection of environmental deterioration is necessary for several reasons, whether economic, practical, ethical or strategic. When required, management actions should be implemented in time to prevent serious ecosystem damage, avoiding prolonged recovery and/or costly remedial actions (Martinez-Crego *et al.*, 2010);
- **feasibility of implementation:** the bioassessment tools should be based on relatively widely distributed organisms, and should use standard protocols which do not present significant technical difficulties;

- **interpretability against reference conditions:** the definition of reference conditions against which to compare the current ecosystem *status* has become common practice, helping to harmonize the results. This definition depends on an unambiguous and non-arbitrary determination of system structure and function. "*Minimally or least disturbed condition*", "*historical condition*" and "*best attainable condition*" obtained by extrapolation of empirical models can be used as standards or benchmarks against which to compare the current condition (Martinez-Crego *et al.*, 2010);

- **linking ecosystem degradation to its causative stressors:** biological measures should be both sensitive to multiple stressors and, to a certain extent, specific enough to provide some clues about the possible causes of deterioration.

Consequently, in this last years, the design and implementation of bioindicators has become a major field in applied ecology, resulting in exacerbated market of biotic indices. More than 90 biotic indices are available in literature, and following Martinez-Crego *et al.* (2010) they could be divided in four main categories:

- **biotic indices based on functional and/or structural attributes of sentinel species (FSS):** sentinel species are usually selected for practical (e.g. ease of culture, well-known biology), ecological (e.g. species occupying critical trophic positions, especially sensitive) or economic reasons (e.g. species of economic relevance). These are expected to provide mechanistic alerts for the other components of the ecosystem (Cajaraville *et al.*, 2000; Rice, 2003).

- **biotic indices based on structural attributes at the community-level (SCL):** the sensitivity to environmental changes of biotic assemblage's taxonomic composition is widely recognized, and biotic indices based on this aspect are frequent in literature (Aguado-Gimenez *et al.*, 2007; Borja *et al.*, 2008, 2009; Martinez-Crego *et al.*, 2010). However, the targeted taxonomic group of species usually only encompasses a part of the whole organism assemblage; the most commonly used groups for this type of indices are benthic macroinvertebrates and phytoplankton

(Martinez-Crego *et al.*, 2010). Using this broad approach, different specific strategies have been applied. A first approach includes indices based on diversity values or other univariate expressions derived from the specific composition. For example, univariate measures based on the number of species (species richness, Margalef index), on species dominance or abundance distribution (Shannon index, Eveness index), or taxonomic separation between each pair of species (taxonomic distincteness index) have been successfully applied to determine the *status* of phytoplankton, benthic macroinvertebrates and fishes (Bellan-Santini, 1980; Karydis and Tsirtsis, 1996; Salas *et al.*, 2006; Alexandrova *et al.*, 2007). A second approach uses multivariate techniques to extract information about status from the matrix of species-samples, either qualitative or using adequate expressions of abundance. These indices have developed for the epiphytic community of seagrass leaves and for rocky shore, macroinvertebrate and fish communities (Hewitt *et al.*, 2005; Pinedo *et al.*, 2007; Martinez-Crego *et al.*, 2010). A third approach is based on the measure of the presence, biomass or abundance of indicator species or taxa of known sensitivity or tolerance to disturbance. This approach has been successfully applied on phytoplankton, macroalgae, seagrasses and macroinvertebrates. Generally, these indices are based on score for assessing the "biotic integrity" (Simboura and Zenetos, 2002; Ballesteros *et al.*, 2007; Wilkinson *et al.*, 2007; Borja *et al.*, 2008, 2009; Sfriso *et al.*, 2009; Martinez-Crego *et al.*, 2010). Two of the most widely used biotic indices, the **Azti's Marine Biotic Index (AMBI)** and the **BENTIX** belong to this category.

- **biotic indices based on functional attributes at the community-level (FCL):** biotic indices in this group are based on the assumption that, in addition to altering species functioning and taxonomic composition, human impact also affects the energy transfer between trophic levels and species interaction, or, more generally, ecosystem functioning. Under this broad notion, two approaches have been attempted: one focusing on trophic aspects, and the other on holistic expression of ecosystem condition derived from ecologically theory (Herrera-Silveria *et al.*, 2002;

Moreno and Laine, 2004; de Jonge, 2007; Reizopuolou and Nicolaidou, 2007; Pettinea *et al.*, 2007).

- **aggregative indices based on information gathered from different communities (ADC):** these indices are based on the aggregation of multiple biotic indices of the prevous types obtained from different communities. Tentatively, such indices have been calculated as the weighted sum, the average of the partial components, or by using multivariate ordination and ranking methods (Ferreira, 2000; Jordan and Vaas, 2000; Bricker *et al.*, 2003; Fano *et al.*, 2003; Paul, 2003).

4.2 The AZTI's Marine Biotic Index (AMBI) and its multivariate approach (M-AMBI)

Among the large number of benthic biotic indices proposed as ecological indicators in estuarine and coastal waters to determine natural and man-induced impacts (see chapter 4.1), the AZTI's Marine Biotic Index (AMBI), which was developed by Borja *et al.* (2000), has been applied successfully to different geographical areas and under different impact sources, including aquaculture (Borja *et al.*, 2004, 2008, 2009a; Aguado-Giménez *et al.*, 2007; Gamito and Furtado 2009; Tataranni and Lardicci, 2010; Munari and Mistri 2010). The AMBI was designed primarily to establish the ecological quality of European coastal and estuarine waters by examining the response of soft-bottom benthic communities to natural and man-induced disturbance in the environment (Muxica *et al.*, 2005). Hence, the AMBI offers a "disturbance or pollution classification" of a site, representing the benthic community "health" (Grall and Glémarec, 1997). Secondarily, it has been used for the determination of the Ecological Quality *status* (EcoQ) within the context of the European Water Framework Directive (Borja *et al.*, 2004). The AMBI is based upon ecological models, such Pearson and Rosenberg (1978) and the most novel contribution of AMBI has been a formula (1) to allow the derivation of a series of continuous values based upon the proportions of five ecological groups (E.G.) to which the benthic species are allocated:

$$\text{AMBI} = [(0 \times \%\ \text{E.G.I}) + (1.5 \times \%\text{E.G.II})$$
$$+ (3 \times \%\text{E.G.III}) + (4.5 \times \%\text{E.G.IV})$$
$$+ (6 \times \%\text{E.G.V})] / 100 \qquad (1)$$

with E.G.I being the disturbance-sensitive species, E.G.II the disturbance-indifferent species, E.G.III the disturbance-tolerant species, E.G.IV the second-order opportunistic species and E.G.V the first-order opportunistic species (Borja *et al.*, 2000). Several thresholds have been established over the scale of AMBI, based upon proportion among the various ecological groups (Borja *et al.*, 2000). The AMBI values and their equivalences are reported in Table 1.1.

Table 4.1 Summary of the AMBI values and their equivalences (modified from Borja *et al.*, 2000)

Biotic coefficient	**Dominating Ecological Group (E.G.)**	**Benthic community health**	**Site disturbance classification**	**Ecological *status***
0 < AMBI ≤ 0.2	I	Normal	Undisturbed	High *status*
0.2 < AMBI ≤ 1.2		Impoverished		
1.2 < AMBI ≤ 3.3	III	Unbalanced	Slightly disturbed	Good *status*
3.3 < AMBI ≤ 4.3	IV - V	Transitional to pollution	Moderately disturbed	Moderate *status*
4.3 < AMBI ≤ 5		Polluted		Poor *status*
5 < AMBI ≤ 5.5	V	Transitional to heavy pollution	Heavily disturbed	
5.5 < AMBI ≤ 6		Heavy polluted		Bad *status*
6 < AMBI ≤ 7	Azoic	Azoic	Extremely disturbed	

AMBI could be calculated using the specific software, freely downloadable from http://ambi.azti.es website (Figure 4.1, 4.2). Using this software, the assignation of the E.G. is automatic and referred to the species database present in the software (Figure 4.1). Due to the fact that the first applications of this index were made for European Atlantic regions, the first versions of the database contained prevalently Atlantic species and this has constituted a problem for the application of AMBI in the Mediterranean regions. However, in recent years, the increasing use of this tool to determine natural and man-induced impacts along Mediterranean coasts (Mirto *et al.*, 2009; Occhipinti-Ambrogi *et al.*, 2009; Prato *et al.*, 2009; Simonini *et al.*, 2009; Tomassetti *et al.*, 2009; Munari and Mistri, 2010) led to the development of the software database, and the present work want to be a contribution too.

Although AMBI can present some weaknesses in the inner part of estuaries or when the number of species is very low (Borja and Muxika, 2005), the addition of a multivariate species richness and Shannon diversity component to AMBI, called multivariate AMBI (M-AMBI) (Borja *et al.,* 2004; Muxika *et al.,* 2007), has allowed for a broader application within the European Water Framework Directive (WFD), in different countries (Borja *et al.,* 2007; 2009b). M-AMBI has been tested under different human pressures, and is being used increasingly (Bigot *et al.,* 2008; Borja *et al.,* 2008; Bakalem *et al.,* 2009; Prato *et al.,* 2009; Simonini *et al.,* 2009; Munari *et al.,* 2010; Tataranni and Lardicci, 2010). The use of this method requires the setting of reference conditions (Muxika *et al.,* 2007), specific for each type or habitat, which can represent a limitation when the number of habitats is too high (de Paz *et al.,* 2008; Teixeira *et al.,* 2008).
Following Borja *et al.* (2004) and Bald *et al.* (2005) the reference condition for a water body type is a description of the biological elements which corresponds totally, or nearly totally, to undisturbed conditions (e.g. with no, or with only a minor, impact from human activities). The objective of setting reference condition standards is to enable the assessment of the biological quality, against these standards. Type-specific reference conditions must summarise the range of possibilities and values for benthic communities, over periods of time and across the geographical extent of the type (Vincent *et al.*, 2002).
There are four options for deriving reference conditions (Borja *et al.,* 2004; Bald *et al.*, 2005):

(i) comparison with an existing "pristine"/undisturbed site (or a site with minor disturbance);

(ii) historical data and information;

(iii) models;

(iv) expert judgement.

Borja *et al.* (2004) have stated that one of the problems in deriving reference conditions in some areas arises from the absence of non-impacted areas or historical data. Hence, the use of “virtual” reference locations (as defined and proposed in Borja *et al.*, 2004), as an “expert judgement” approach, requires consideration. The use of “virtual” reference locations has been used successfully in the case of physical-chemical elements (Bald *et al.*, 2005) and benthic communities (Muxika *et al.,* 2007).

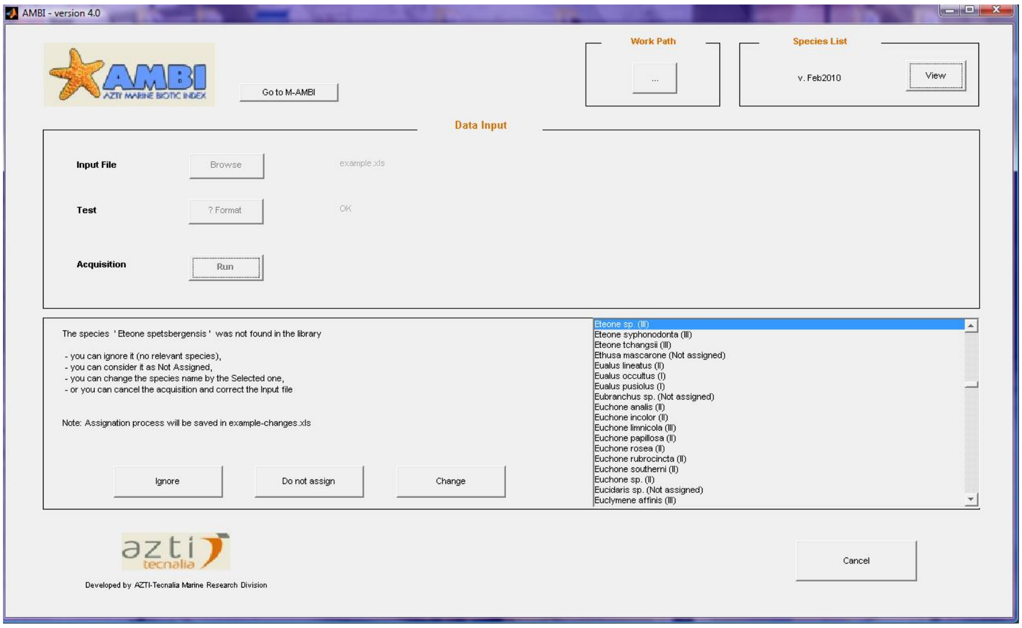

Figure 4.1 AMBI software screenshot: Ecological Groups assignation

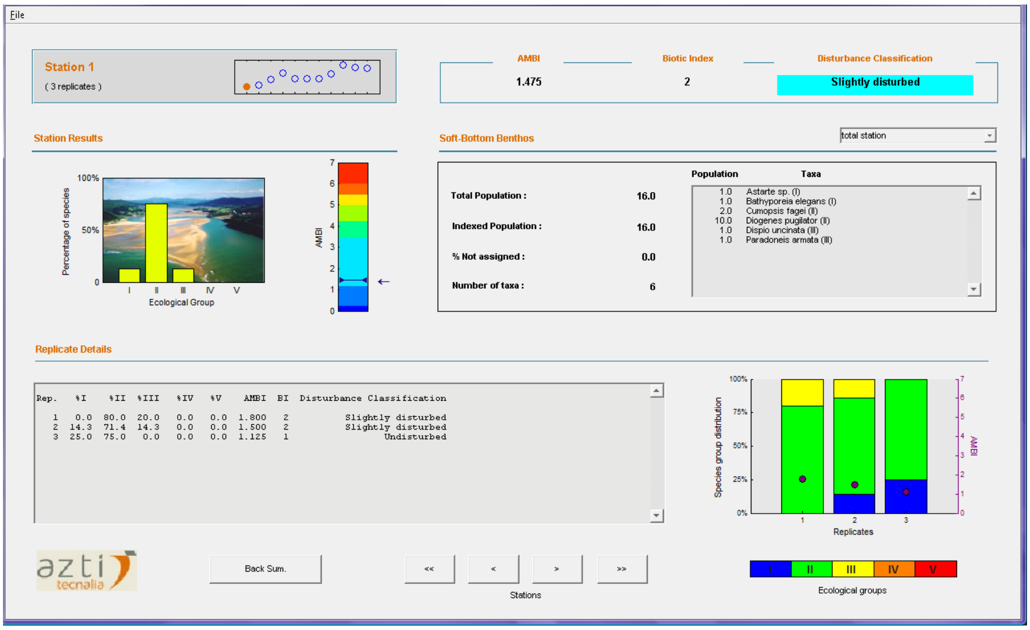

Figure 4.2 AMBI software screenshot: output; site classification and ecological groups relative percentages

5 Objective of the study

At the beginning of this study, among the plethora of biotic indices avaiable, none was completely suitable to detect impacts deriving from aquaculture activities in Mediterranean areas. In its recent formulation, the Azti's Marine Biotic Index (AMBI) presented a more complex and robust structure respect other biotic indices. Previous applications of AMBI along the European Atlantic coasts confirmed its discriminant capability, but these studies underlined the importance of the species database as well, suggesting that for the application of this index in the Mediterranean basin a further calibration was indispensable. Taking into account these considerations, the project was oriented to the development of the AMBI index in order to create an upgraded version more sensitive for the Mediterranean. To achieve this goal, the development of AMBI software database was necessary, including an higher number of Mediterranean species. Hence, trying to enlarge the dataset and in order to test AMBI in different scenarios, this study was carried out in three different Mediterranean regions: Sardinia (Western Mediterranean) (Chapter 7), Cyprus (Eastern Mediterranean) (Chapter 8) and Tuscany (Coastal Marine Transitional Ecosystem) (Chapter 9). In detail, five fish farms as three cases study were investigated, representing each a particular different environment. Moreover, in one of the cases study (Chapter 8) two similar fish farms (facilities, sizes) were compared, being operative at different bathymetric conditions.

So, the main objectives of this study were:

- to collect and identified the higher number of macrobenthic species, in order to include them into the AMBI software database;
- to test the new developed AMBI to evaluate the effects of aquaculture activities on the benthic ecosystem in different Mediterranean scenarios;
- to identify site-specific reference conditions in order to perform the M-AMBI analysis;
- to compare AMBI with both traditional ecological index calculations (e.g. Shannon index, Margalef index, Simspon, Pielou index) and with another biotic index, the

BENTIX; the choice of this latter was due to the fact that, up today, BENTIX is the most widely used index for the Mediterranean regions, and it shares the base approach with the AMBI but it differs in structure, with a formula that includes different weighting coefficient of each ecological group in relation to others. These comparisons were necessarily to validate the results.

6 Materials and methods

The analyses performed in this study were ascribable to three different categories: **physical-chemical**, **biological** and **statistical analyses**. The methods used for these analyses will be detailed described in this section, while in the following chapters only the related references will be reported.

6.1 Environmental measures

Dissolved oxygen concentration (mg l^{-1}), **oxygen percentage of saturation** and **temperature** (°C) were measured in water using Handy Gamma oxygen probe (Oxyguard). **Salinity** (‰) was measured using a manual refractometer (Mod. 106 ACT). **Redox potential** (Eh) was measured on the upper layer sediment, *in situ*, using an Orion platinum electrode model 9678BNWP (Thermo Scientific®). **Currents** data (direction and velocity) were obtained, when possible, by direct measure, using Sensor Data Current Meter (model SD 2000), otherwise elaborating data derived from local oceanographic institutes.

6.2 Samples collection

Depending to the characteristics of the site, sediment for biological, chemical and physical analyses were sampled in three different ways:

- using a Van Veen Grab sampler (sampled surface = 0.132 m^2) (Figure 6.1)
- hand collected by divers, using "sampling boxes" (15x30x8 cm; 3,600 cm^2)
- using a shovel (sampled surface = 3,600 cm^2)

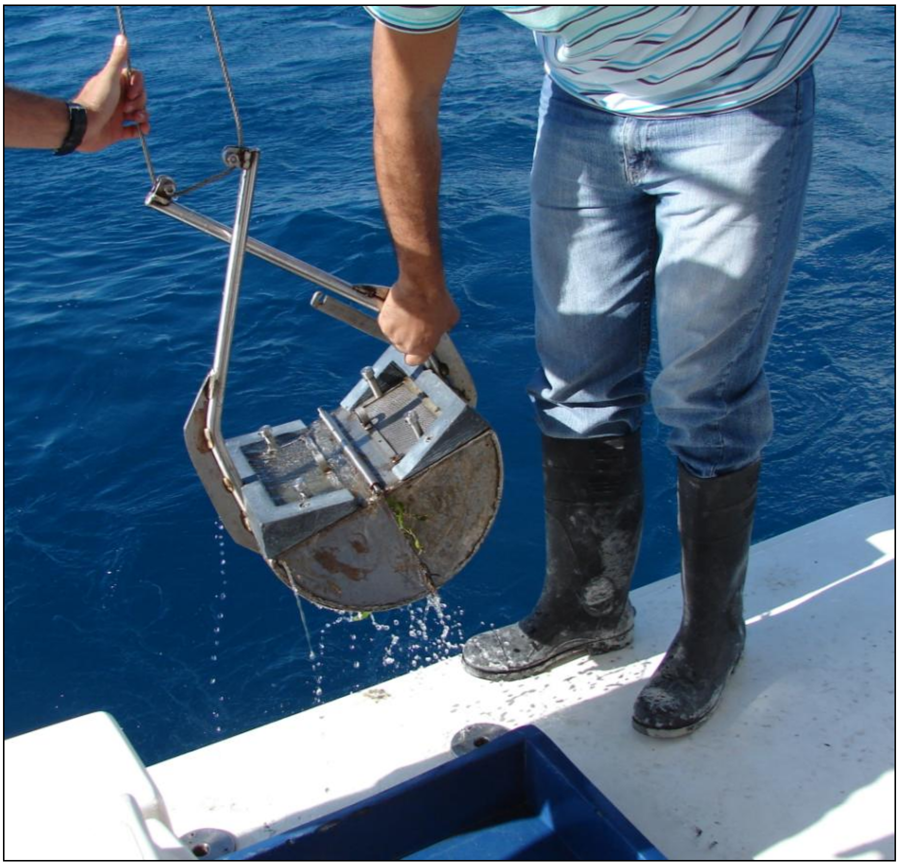

Figure 6.1 Van Veen Grab sampler used for sediment sampling

For each station, samplings were performed in triplicate. After the collection, sediment for chemical and physical analyses was frozen at -20° C and transferred in the laboratory. Sediment for biological analyses was sieved with a 0.5 mm mesh, fixed in 10% buffered formalin and transferred to the laboratory for further analyses.

6.3 Sediment analysis: abiotic parameters

Sediment grain size was assessed using a mechanical shaker by dry sieving through a tower of sieves. The sieves mesh ranged from 25 to 0.064 mm mesh and the whole set of sieved used is reported in Table 6.1. In order to remove the water content from the sediment, each sample (500 g) was dried in a stove at 110 °C for 24 h. Before performing the analysis each sieve was weighed, then the sediment was put in the upper sieve of the tower and the mechanical shaker set in action for 15 minutes. At the end of the shaking, the amount of sediment present in each sieve was determined by weighing. Sediment was classified in accordance with the Wentworth scale (Buchanan 1984): 64-2 mm *gravel*, 2-0.25 mm *sand*, 0.25-0.065 mm *fine sand* and <0.065 mm m*ud*.

Table 6.1 Sieves and respective meshes (mm) used for the sediment grain size analysis

Sieves	Mesh of the sieves (mm)
1	25
2	12.5
3	9.5
4	6.3
5	4.75
6	2.36
7	1.18
8	0.6
9	0.425
10	0.3
11	0.15
12	0.13
13	0.106
14	0.075

To determine the **percentage of water in the sediment (SWC)**, 500 g of sediment was dried in a stove at 60 °C until the weight was constant and the loss of weight in percentage represented the SWC. **The organic matter (OM)** was determined as the loss on ignition (LOI) after 5 h at 450 °C in a furnace, after that, sediment was burnt at 1000 °C to evaluate the **carbonate fraction** (Dean, 1974; Froelich, 1980).

The Carlo Erba Instrument EA1108 Elemental Analyzer (Carlo Erba Inst., Milan, Italy) was used to determine **Total Nitrogen (TN)**, **Total Carbon (TC)**, **Organic Carbon (OC)**, **Total Sulphur (TS)** and **Total Hydrogen (TH)**. This Elemental Analyzer is a commercially-available instrument comprised of a combustion furnace, gas chromatographic oven, and thermal conductivity detector. It can be configured to detect carbon, hydrogen, nitrogen, and sulfur simultaneously. The instrument is equipped with a pneumatic autosampler and a PC-based computer data system (Carlo Erba Eager 200). The analytical method uses one of two available furnaces to house a catalytic reactor tube. The reactor tube is packed with an upper part which functions as an oxidation catalyst (tungstic anhydride) and a lower portion which functions as the reduction reactor (elemental copper). After exiting the reactor tube, the gas-phase sample travels through a water trap (anhydrone), and then, into a packed chromatographic column. The sample components are separated by the column as CO_2, H_2, N_2, and H_2S. These species are detected by a thermal conductivity detector.

According to Olsen and Sommers (1982), the digestion by perchloric acid method was used to detect the amount of **phosphorous** present in the sediment **(TP)**. Following this method 30 mL of a 60% solution of perchloric acid ($HClO_4$) reacted with 2 g of sediment. Temperature was raised up until the boiling point in order to promote the separation of the phosphorous ($P\text{-}PO_4$) from the sediment. After a reaction with molybdenum blue the amount of TS was detected by spectrophotometry (λ = 400-490 nm).

6.4 Sediment analysis: biotic parameters

Faunal samples were sorted by hand into major *taxa* (*Polychaeta*, *Mollusca*, *Crustacea*, *Echinodermata*, *Sipunculida* and miscellaneous) and specimens were identified to the lower taxonomical possible level using stereoscopic microscope (mod. Aus Jena GSZ, Alessandrini Instrument) and specific manuals (Bouvier, 1940; Fauvel, 1923, 1927; Pruvot-Fol, 1954; Rose, 1933). For each sampling point biological indices were calculated:

- Margalef index (Margalef, 1958), calculated as

$$(S-1)/\ln N$$

  where S is the number of species observed and N the number of individuals;

- Shannon index (H’) (Shannon, 1948), calculated as

$$-\sum p_i \ln p_i$$

  where p_i is the relative abundance of each species;

- Simpson diversity index (D) (Simpson, 1949), calculated as

$$\sum (n_i / N)$$

where n_i is the total number of organisms of species i and N is the total number of organisms of all species;

- Pielou index (*eveness*, J') (Pielou, 1966), calculated as

$$H'/H'_{\max},$$

where H' is the Shannon index as defined above, $H'_{max} = \ln S$, and S is the number of species observed.

6.5 Qualitative biotic indices calculation: AMBI, M-AMBI and BENTIX

Even if in recent years, with the increasing use of this index, new *taxa* from around the world have been included (Muniz *et al.,* 2004; Borja *et al*., 2008), most of the species in the AMBI original species-list (www.azti.es), were from the European biogeographical area (Borja *et al.,* 2000). Thus, before running this analysis, it was necessary to assign the new Mediterranean macrobenthic species found in this study to one of the five Ecological Groups (EG) defined by Borja *et al.* (2000) (see chapter 4.2). The approach to assigning species not on the list was as follows:

(i) when bibliographic reference to sensitivity of the species was not found, but the same *genus* was present in the list, the new species were assigned to the same group;
(ii) occasionally, we contacted experts on certain macrobenthic *taxa* to assign species to groups.

Species for which enough information was unavailable to be assigned to a group, they were recorded as "not assigned". All the new assigned species are available in the new species-list (February, 2010) within the AMBI website (www.azti.es). Following species assignations, AMBI values were calculated using the free AMBI software version 4.0.

The **M-AMBI** was calculated by factor analysis (FA) of AMBI, species richness (as number of *taxa*) and Shannon diversity index values (for details, see Borja *et al.,* 2004; Bald *et al.,* 2005; Muxika *et al.,* 2007), using AMBI software (www.azti.es). The threshold values for the M-AMBI classification are based upon the European intercalibration (Borja *et al.,* 2007): "High" quality, >0.77; "Good", 0.53-0.77; "Moderate", 0.38-0.53; "Poor", 0.20-0.38 and "Bad",

<0.20.

BENTIX was calculated using BENTIX Add-In software Version 1.0 (2009 ©Hellenic Center for Marine Research, Institute of Oceanography). BENTIX index is based on the relative percentage of “sensitive” (GS) and “tolerant” (GT) species and its values range from 0 to 6 with high values reflecting poor ecological *status* (Simboura and Zenetos. 2002). An updated detailed species list with their respective group assignation can be found at http://www.hcmr.gr/english_site/services/env_aspects/bentix.html.

6.6 Statistical analysis

To evaluate significant differences ($p<0.05$) between the sampled stations the results were analyzed using the analysis of variance (ANOVA) and the Tukey test was applied for *post hoc* comparison. Statistical calculations were performed using SYSTAT® Version 10.2 (SYSTAT Software Inc., 2002). For benthos analysis the PRIMER 6 Software package (Plymouth Marine Laboratory; Clarke and Warwick, 2001) was used. For the biological data the cluster analysis was performed using Bray Curtis Similarity and the SIMPROF test was performed ($\alpha= 0.05$), while for chemical and physical parameters the Multi-Dimensional Scaling (MDS) analysis was applied using the Euclidean Distance; a fourth root transformation was applied to the data, prior to calculate distance, to normalize them.

CASE STUDY I: SARDINIA, ALGHERO BAY

7 Evaluation of the influence of off-shore cage aquaculture on the benthic ecosystem in the Alghero bay (Sardinia, Italy), using AMBI and M-AMBI.

The objectives of this study are: (i) to assess the impacts of aquaculture on benthic assemblages, using AMBI and M-AMBI indices (setting reference conditions for the later); and (ii) to compare observed and predicted AMBI values, taking into account hydrographical and managerial variables, using an offshore fish farm in Northwest Sardinia (Italy).

7.1 Study area

The study was carried out in a fish farm of 2.5 ha located in the Mediterranean Sea, in the Alghero bay (North Western Sardinia) (Figure 7.1a). The sea bottom is flat, with a mean water depth of 38 m. The sampling activities were performed during the month of September for two consecutive years, 2007 and 2008, at the end of the productive cycle of the farmed fish. About 116,000 seabass (*Dicentrarchus labrax*) and 380,000 sea bream (*Sparus aurata*) were being reared in 9 “tension-legs” REFA® pen cages. Cage volume was 800 m^3 (5 cages) and 2,500 m^3 (4 cages), and the fish density maintained very low, ranged from 0.4 to 4 kg m^{-3}. Fish were fed with commercially produced extruded pellets (Aller Aqua®; 42-56% dry matter (d. m.) protein, 18-21% d. m. crude fat, 7.5-12% ash, 0.5-2.5% d. m. crude fiber and 1.1-1.4% d. m. phosphorous) and the daily ratio ranged from 40 to 190 kg $cage^{-1}$, with a total daily average of 98 kg $cage^{-1}$. Total production of the farm was 99 t in 2007 and 99.3 t in 2008.

7.2 Sampling design

The main surface currents ran parallel to the bay perimeter, moving from SW to NE (APAT, 2008). Nevertheless, the water current speed and the prevailing direction were determined from July to December 2007 at four sampling stations along the vertices of the granted area (Figure 7.1c). Currents were measured at three different depths in the water column: surface, 10 m and 20 m depth, during 15 days at each meter position, with a time-span of 10 minutes, using a Sensor Data Current Meter.

During the month of September 2007, 8 stations were sampled: 4 stations located close to the cages (stations I, in Figure 7.1c) and 4 stations located away from the cages, in the direction of the four cardinal points (stations O, in Figure 7.1c). Taking into account the information from the surface current data and the current meter data, during September 2008 a transect of 4 stations was established, along the prevailing direction of the water current, at increasing distances from the cages (stations T, in Figure 7.1c). In both years, three replicates of sediment samples and three replicates of the macrofauna samples were collected at each station.

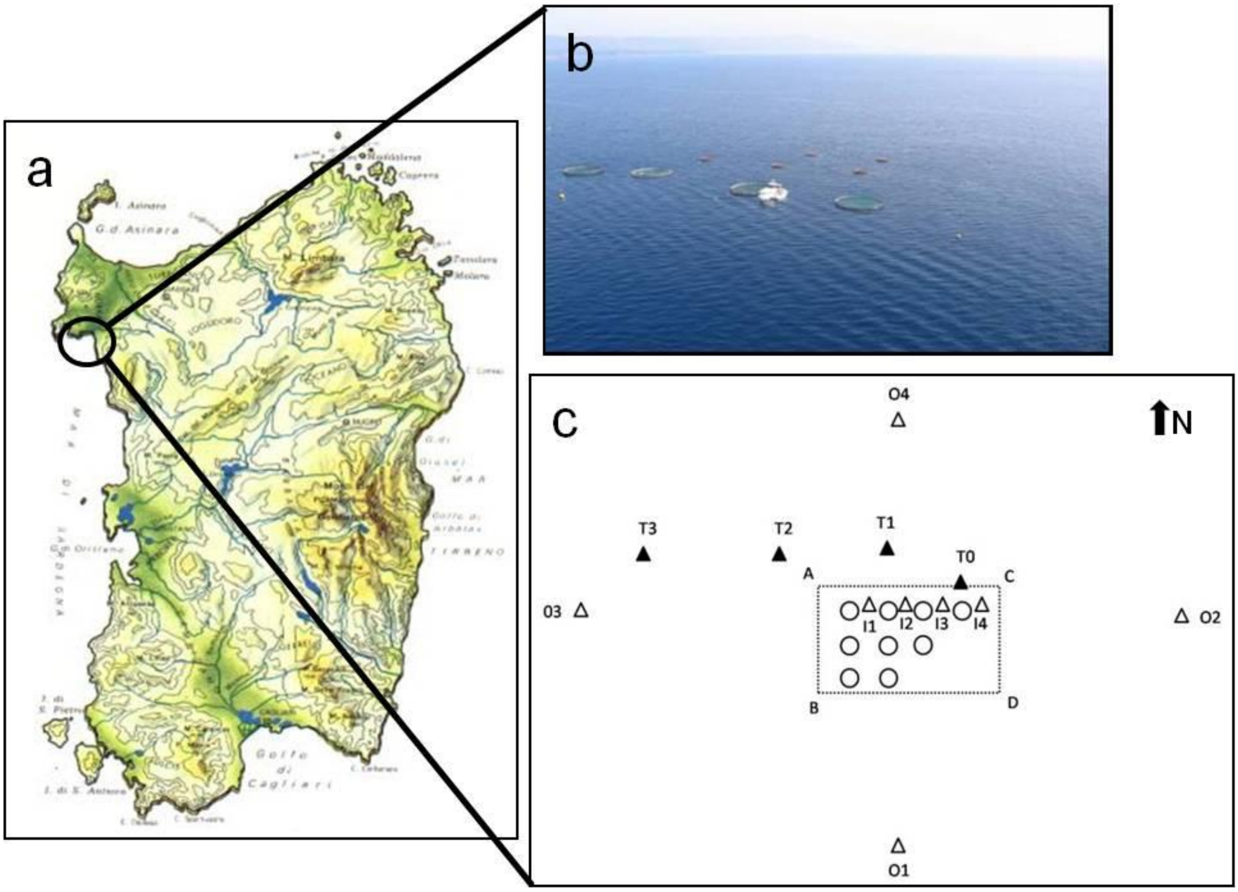

Figure7. 1 (a-b) Location of the study area in Alghero bay (northwest Sardinia, Italy); (c) sampling strategy and spatial disposition of the fish farm and the stations sampled in 2007 (I1, I2, I3, I4, O1, O2, O3, O4) and 2008 (T0, T1, T2, T3) surveys. Key: A, B, C, D: vertices of the fish farm granted area; O: cage; Δ: station sampled in the 2007; ▲: station sampled in the 2008.

7.3 AMBI and M-AMBI

According to Borja *et al.* (2009b), predicted AMBI values were calculated using the equation:

$$\text{Predicted AMBI} = 4.496 - (0.0486\ De) - (1.615\ C) + (0.000665\ P) - (0.593\ Di)$$

where De is depth at each sampling station, expressed as square root (m); C is the current speed, expressed as log (cm s^{-1}); P is the production expressed in tons yr^{-1}; Di is the distance of each station to the cages expressed in log (1+m). The predicted values calculated were compared to the observed ones in order to check the fitting of the observed values to the general model.

As M-AMBI needs setting bad and high reference conditions (see Muxika *et al.*, 2007), to compare with monitoring data, five different scenarios of high *status* were tested, including (i) those from the Italian Adriatic coast (Occhipinti Ambrogi *et al.*, 2009); (ii) those from a station (O2), in the opposite way of the prevailing currents; (iii) the lowest AMBI value and highest diversity and richness values from the area; and (iv) two more scenarios, increasing richness and diversity and decreasing AMBI, as a preventive measure, if the area is globally affected by the aquaculture activity. Referred to bad *status*, all of them were based upon azoic situation (diversity and richness equal to 0 and AMBI equal to 6).

7.4 Results

7.4.1 2007 survey: abiotic parameters

The average current speed and direction measured at the surface, at 10 m and at 20 m depth are reported in Table 4.1. The highest speed values were recorded for the surface layer (mean values ranging from 2.8 to 3.0 cm s^{-1}), decreasing with depth (range 1.5-1.8 cm s^{-1}, at the deepest layer). The prevailing currents within the Alghero bay had a northwest direction (Table 7.1).

Concerning the sediment characteristics, the mean percentage values are reported in Table 7.2. The percentage of fine sand was significantly higher in stations I1 and I2, compared to I4. For the rest of parameters, no significant differences were found between the stations located close to the cages (stations I), indicating the substantial homogeneity of the sediments. The MDS analysis applied to the abiotic parameters confirmed this homogeneity, showing all I stations clustered close together (Figure 7.2a). On the contrary, no homogeneity was found among the O stations that cluster separately in two different groups: the first one formed by O3 and O4 and the other one by O1 and O2 (Figure 7.2a).

Considering I_{1-4} as the average value calculated for each parameter putting together all the I stations, an ANOVA with Tukey *post hoc* comparison was carried out to investigate the differences between I_{1-4} and the O sites. The mean percent SWC value calculated resulted significantly higher for I_{1-4} than for O4. Significant differences were found for the percentage of OM between $I_{1-4,}$ O1 and O4, and also for the carbonate, for which the mean value recorded for I_{1-4} resulted significantly different from O1, O2 and O4 (Table 7.2). Concerning grain size, the percentage of gravel calculated for I_{1-4} resulted similar to those found in O3 and O4, but this value was significantly lower than those calculated for O1 and O2. For the sediment sampled close to the cages, the prevailing grain size fractions were represented by sand and fine sand and for I_{1-4} their percentage resulted significantly different from all the O stations (Table 7.2). The mud percentage calculated for I_{1-4} was significantly different from O1, O2 and O4.

7.4.2 2007 survey: biotic parameters

The richness was significantly higher in O1, O2 and O3 stations. No significant differences were found between the other stations, being the lowest richness recorded at station I3 (Table 7.3). Concerning density the lowest mean values were calculated for the stations I3 (573 N m^{-2}) and I4 (535 N m^{-2}). The density in these stations is significantly different from O1 and O2, for which the highest mean values were recorded (1,785 and 1,618 N m^{-2}, respectively). For all the stations, *Polychaeta* was the most abundant group, with values ranging from 58.9% (station I2) to 73.1% (station O3), except for O4 where *Crustacea* represent 66.7% of the total. The cluster analysis and the SIMPROF test applied to the biological data (Figure 7.3a) showed two principal groups that clustered separately: one is represented by the stations located close to the cages (stations I) and the other one by the stations located far from the cages (stations O), except the station O4, which appeared grouped with the stations close to the cage, even if in a separated cluster.

7.4.3 2008 survey: abiotic parameters

The mean values for the chemical and physical parameters analyzed in 2008 are reported in Table 7.2. The SWC percentage was similar in T0, T1 and T2 (values around 36%). Lower values were recorded for T3 site, being these differences significant (Table 7.2). The OM percentage calculated for T0 resulted significantly lower than T1, T2 and T3 (Table 7.2). Carbonate in T0 and T1 appeared significantly different from T2 and T3 (Table 7.2). The redox potential varied among the stations and the maximum value was recorded at T3 station. The stations T0 and T1 showed similar values, while lower values were recorded at T2, being this station significantly different from T3 (Table 7.2). The TN mean value was higher for T0 and T1, and these stations appeared significantly different from T2 and T3 (Table 7.2). The maximum values for the TC were measured at T3 station, being significantly different from all the other stations. There were significant differences in TC between T3 and the other sites. The highest values for the OC were recorded at T1 and T3 sites, while lower values were recorded at T0 and T2. The mean TS values recorded at the sampling sites ranged between 0.063 and 0.163%. No significant differences in OC and TS values were registered at any sampling site (Table 7.2). For the TP, the recorded concentrations, stations T1 and T2 appeared significantly different from the other stations (Table 7.2). By analyzing the sediment grain size, no significant differences were registered among the stations for the percentage of gravel (Table 7.2). Concerning the percentage of sand, the stations T0 and T3 resulted significantly different and the mean values recorded at these stations were significantly different from the other stations. A predominance of fine sand and mud was observed at T0, T1 and T2 stations, being the mean value recorded at T3 significantly lower. The mean percent value recorded at T0 for mud resulted significantly different from the other stations (Table 7.2). The MDS analysis applied to the abiotic parameters (Figure 7.2b) showed that T1 and T2 appeared to be the most similar stations, and they formed a larger cluster with T0, while T3 showed a major spatial distance from all the other sampled station.

7.4.4 2008 survey: biotic parameters

Concerning richness, the highest mean value was calculated for T3 and this station appeared significantly different from T0 and T2. The station T2 was also characterized by the lowest density and it resulted significantly different from the other stations. For all the stations, except for T3, the dominant group was *Polychaeta*, with a relative abundance that decreased as the distance from the cages increased (T0: 81.2%, T1: 81.3%, T2: 48.4%, T3: 18.3%). In turn, an opposite trend was found for *Mollusca* (T0: 6.6%, T1: 12.9%, T2: 22.6%, T3: 45.2%). The cluster analysis showed two different groups (Figure 7.3b), one represented by the T3 station and the other by the remainder of the stations. The SIMPROF test applied showed that both groups were significantly different.

Table 7.1 Mean, maximum and minimum speed and mean direction of the currents recorded at three different depths at the four vertices of the fish farm granted area.

Site	Meter position (bottom: -33 m)	Mean speed (cm sec^{-1})	Max speed (cm sec^{-1})	Min speed (cm sec^{-1})	Mean direction (Magnetic degree)
	surface	3.0±7.6	30.2	0.1	281.1±57.2
A	midwater (-10 m)	2.1±2.5	7.5	0.1	310.3±48.4
	near bed (-20 m)	1.6±1.6	5.1	0.1	260.6±97.6
	surface	2.9±7.7	30.4	0.1	279.1±57.9
B	midwater (-10 m)	1.7±1.6	4.2	0.1	317.3±28.3
	near bed (-20 m)	1.8±1.6	5.1	0.1	259.8±94.4
	surface	2.8±7.6	30.2	0.1	3.6±86.8
C	midwater (-10 m)	2.0±2.2	7.6	0.1	315.3±40.1
	near bed (-20 m)	1.8±1.7	5.1	0.1	269.6±99.6
	surface	2.9±6.4	24.9	0.1	296.4±24.5
D	midwater (-10 m)	1.4±1.4	3.4	0.1	322.3±29.9
	near bed (-20 m)	1.5±1.6	5.1	0.1	267.6±104.6

Table 7.2 Mean and standard deviation (s.d.) values of the chemical and physical parameters analysed in the stations sampled in 2007 and 2008. I_{1-4} is the mean value of the stations I1 to I4 (for explanations, see text).

Stations		SWC (%)	OM (%)	Carbonate (%)	Eh (mV)	TN (%)	TC (%)	OC (%)	TS (%)	TP (mg g^{-1})	Gravel (%)	Sand (%)	Fine sand (%)	Mud (%)
I1	Mean	30.98	2.67	37.18	-	-	-	-	-	-	0.68	4.28	90.54	4.50
	s.d.	2.57	0.17	0.57	-	-	-	-	-	-	0.11	0.92	0.39	0.85
I2	Mean	31.97	2.82	37.05	-	-	-	-	-	-	0.87	4.84	89.30	4.99
	s.d.	0.27	0.37	0.40	-	-	-	-	-	-	0.30	0.53	0.28	0.46
I3	Mean	31.77	2.40	36.48	-	-	-	-	-	-	0.63	6.53	88.96	3.89
	s.d.	3.88	0.09	0.86	-	-	-	-	-	-	0.17	0.75	0.99	0.10
I4	Mean	32.04	2.36	36.18	-	-	-	-	-	-	1.87	7.45	87.20	3.49
	s.d.	3.23	0.20	0.69	-	-	-	-	-	-	0.95	0.38	0.69	0.14
I_{1-4}	Mean	31.68	2.56	36.72	-	-	-	-	-	-	1.01	5.77	89.00	4.22
	s.d.	2.46	0.29	0.75	-	-	-	-	-	-	0.68	1.45	1.36	0.73
O1	Mean	25.98	3.24*	44.94*	-	-	-	-	-	-	11.38*	88.36*	0.23*	0.02*
	s.d.	0.99	0.71	0.16	-	-	-	-	-	-	1.80	1.79	0.08	0.00
O2	Mean	26.07	2.90	44.67*	-	-	-	-	-	-	16.56*	83.06*	0.3*	0.08*
	s.d.	5.64	0.68	0.37	-	-	-	-	-	-	1.63	1.59	0.04	0.03
O3	Mean	26.84	2.27	36.99	-	-	-	-	-	-	1.43	10.93*	84.66*	2.98
	s.d.	4.76	0.26	0.99	-	-	-	-	-	-	0.30	0.65	0.74	1.61
O4	Mean	25.65*	1.73*	32.7*	-	-	-	-	-	-	1.97	14.93*	76.65*	6.46*
	s.d.	0.26	0.28	0.76	-	-	-	-	-	-	0.74	2.21	1.15	1.32
T0	Mean	36.72	2.99^{b}	34.96^{c}	151.67	0.035^{h}	9.3	0.69	0.092	0.41^{n}	2.58	7.46^{q}	70.72	19.24^{u}
	s.d.	1.03	0.06	0.4	20.21	0.004	0.08	0.28	0.051	0.1	2.55	0.52	2.42	0.63
T1	Mean	36.19	3.9	34.35^{c}	154.33	0.035^{h}	9.16	0.93	0.063	0.49^{o}	0.77	5.07^{r}	70.65	23.51
	s.d.	1.58	0.33	0.33	16.92	0.007	0.39	0.49	0.045	0.09	0.07	0.12	0.88	1.05
T2	Mean	35.79	4.03	33.48^{d}	115.33^{f}	0.032^{i}	9.09	0.51	0.163	0.35^{p}	1.10	4.47^{r}	69.89	24.54
	s.d.	0.65	0.24	0.3	26.27	0.004	0.41	0.45	0.091	0.11	0.34	0.85	0.91	1.13
T3	Mean	28.09^{a}	4.36	41.05^{e}	214.67^{g}	0.029^{l}	11.18^{m}	0.85	0.141	0.43^{n}	3.46	91.67^{s}	4.29^{t}	0.58^{v}
	s.d.	0.5	0.11	0.17	58.39	0.001	0.03	0.1	0.06	0.11	0.57	0.97	0.31	0.11

Key: Sediment Water Content (SWC), Organic Matter (OM), Redox potential (Eh), Total nitrogen (TN), Total Carbon (TC), Organic Carbon (OC), Total Sulphur (TS), Total Phosphorus (TP). * : significant differences ($p<0.05$) between I1-4 and O sites, in 2007; lower case letters: significant differences ($p<0.05$) between the locations sampled in 2008.

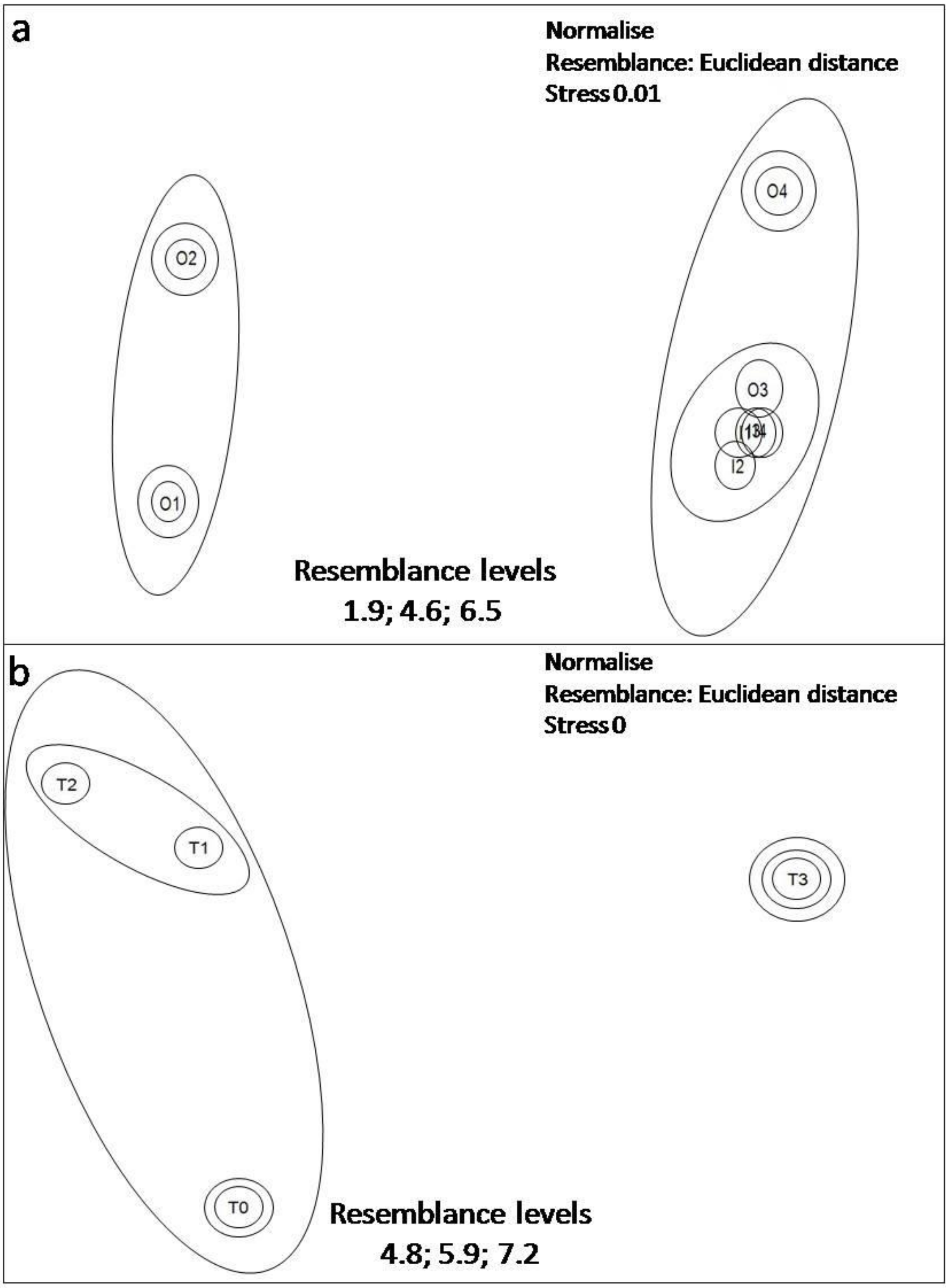

Figure7. 2 Multi-Dimensional Scaling analysis (MDS) derived from physical and chemical data (see Table 2), recorded in 2007 (a) and 2008 (b) surveys.

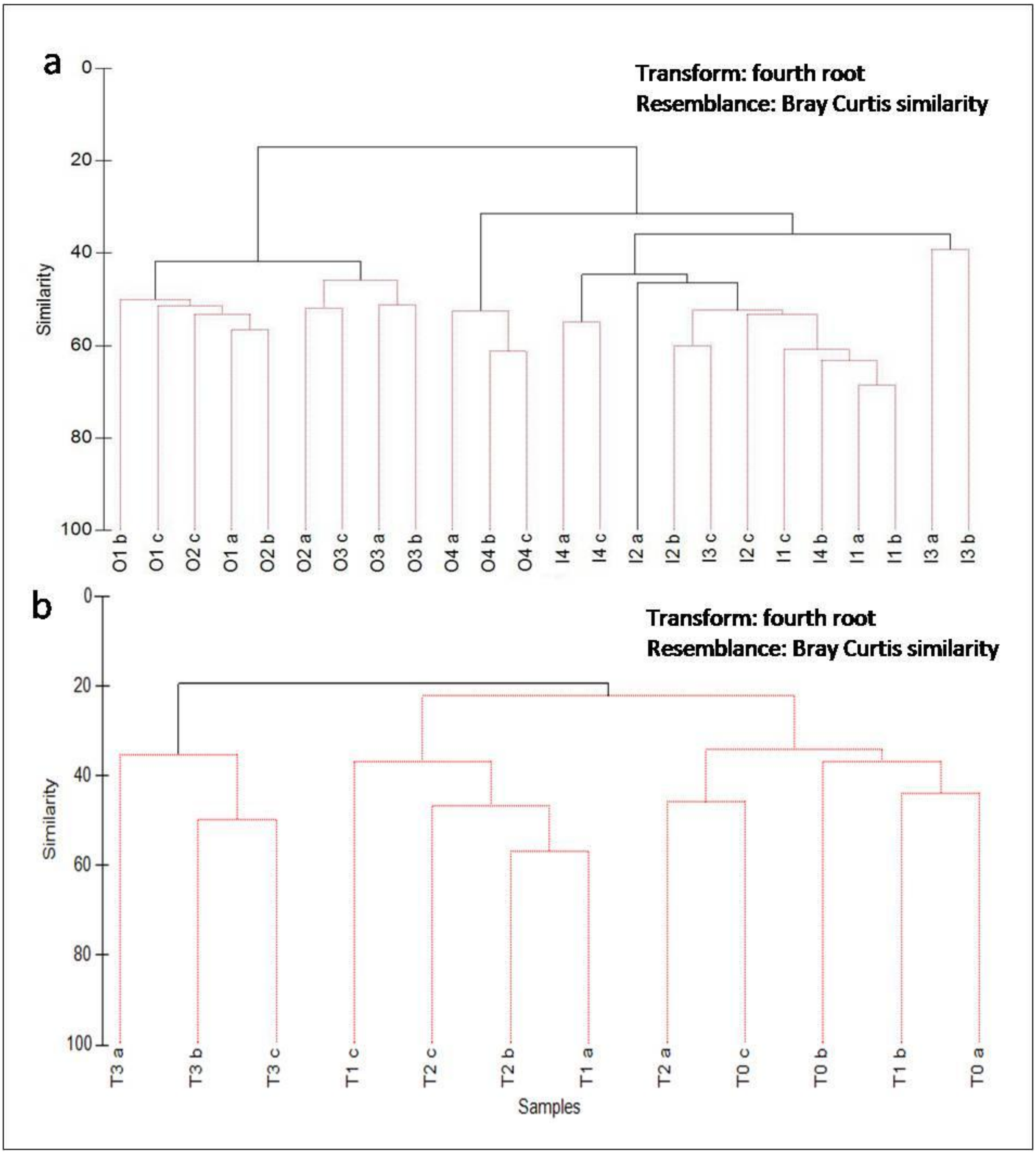

Figure7. 3 Cluster analysis performed on the fauna samples collected in 2007 (a) and in 2008 (b) surveys. The Bray Curtis Similarity and the SIMPROF test were applied.

7.4.5 AMBI and M-AMBI

For the 2007 survey, the highest AMBI values were recorded for the sites close to the cages, with a maximum value in I3 station (3.4), classified as "*Moderately disturbed*", dominated by first-order opportunistic species (EG V) (Table 7.3). All the other stations showed lower AMBI mean values, being classified as "*Slightly disturbed*" (dominated by indifferent, EG II, and/or

sensitive species, EG I) except O4 that was "*Undisturbed*", with EG I (sensitive species) being dominant (Table 7.3). The AMBI values showed a decreasing gradient in 2008, from T0 to T3. The stations T0 and T1 were classified as "*Moderately disturbed*" (values of 3.8), with a dominance of EG IV, while T2 appeared as "*Slightly disturbed*" (codominance of groups EG I and EG III), and T3 as "*Undisturbed*", with dominance of EG I (Table 7.3).
The relationship between observed and predicted AMBI values is shown in Figure 7.4; 8 out of the 12 stations fit within the 95% confidence limits for the predicted AMBI. Stations close to the cages show higher values than those far away from them, in both surveys. There is a clear gradient of AMBI values north-northwestwards (Figure 7.5).

On the other hand, as M-AMBI reference conditions have not been set for the area, 5 different scenarios were designed. The global picture is quite similar in the 5 cases, although the final status classification varies slightly among the scenarios (Figure 7.6). The results obtained using as reference conditions lower AMBI values and higher diversity and richness values than those really measured, produce the same results in both cases (Figure 7.6a,b). These reference conditions are conservative, taking into account the probable alteration of the area, after several years of the farm operation. The use the reference conditions from the Adriatic Sea produces high M-AMBI values (5 out of the 12 cases >1; Figure 7.6c), showing that those conditions are too lax for the area. The results found using O2 station as reference (Figure 7.5d) or using as reference conditions the lowest AMBI value and the highest diversity and richness values calculated for the area (Figure 7.5e) are quite similar. In general, excepting case c (Figure 7.6c) the remainder of the cases shows the same *status* picture: the stations located close to the cages in 2007 showed moderate to good *status*, being those far from the cages in good-high *status*. In 2008 a clear gradient was detected from poor *status* close to the cages to good *status* far away (Figure 7.6).

Table 7.3 Results of the AMBI calculation for each station sampled in 2007 and 2008, showing the distance from the cages, the percentage of each Ecological Group (EG %) and mean AMBI values. For each sampled stations mean abundance (number of individuals per square meter), mean richness and mean diversity are also reported. Key: EG I: sensitive species; EG II: indifferent species; EG III: tolerant species; EG IV: second-order opportunistic species, EG V: first-order opportunistic species.

	Stations	Distance from the cages (m)	EG I(%)	EG II(%)	EG III(%)	EG IV(%)	EG V(%)	Mean AMBI	Mean Abundance (N m^{-2})	Richness	Diversity
2007	I1	20	51.1	19.6	14.6	3.5	11.1	1.64	1,040	53	4.36
	I2	20	17.5	20.2	11.5	29	21.8	3.19	929	50	4.18
	I3	20	25.8	8.8	5.1	18	42.4	3.4	573	34	3.54
	I4	20	32.2	20.6	7.5	13.6	26.1	2.23	535	40	4.34
	O1	719	27.5	56.5	14.5	1.2	0.3	1.36	1,785	105	5.33
	O2	863	23.7	58.5	15.3	2.1	0.3	1.47	1,619	104	5.42
	O3	1138	17.7	64.1	11.7	6.2	0.4	1.61	1,495	99	5.33
	O4	729	67.8	10	20.8	0.9	0.5	0.84	1,169	43	3.46
2008	T0	20	9.4	5	7.2	76.8	1.7	3.82	3,017	25	1.82
	T1	149	16.4	2.3	3.9	64.8	12.5	3.85	2,317	27	2.47
	T2	230	30	10	30	10	20	2.83	517	18	3.89
	T3	799	71.6	8.4	11.6	7.4	1.1	0.94	3,283	43	4.32

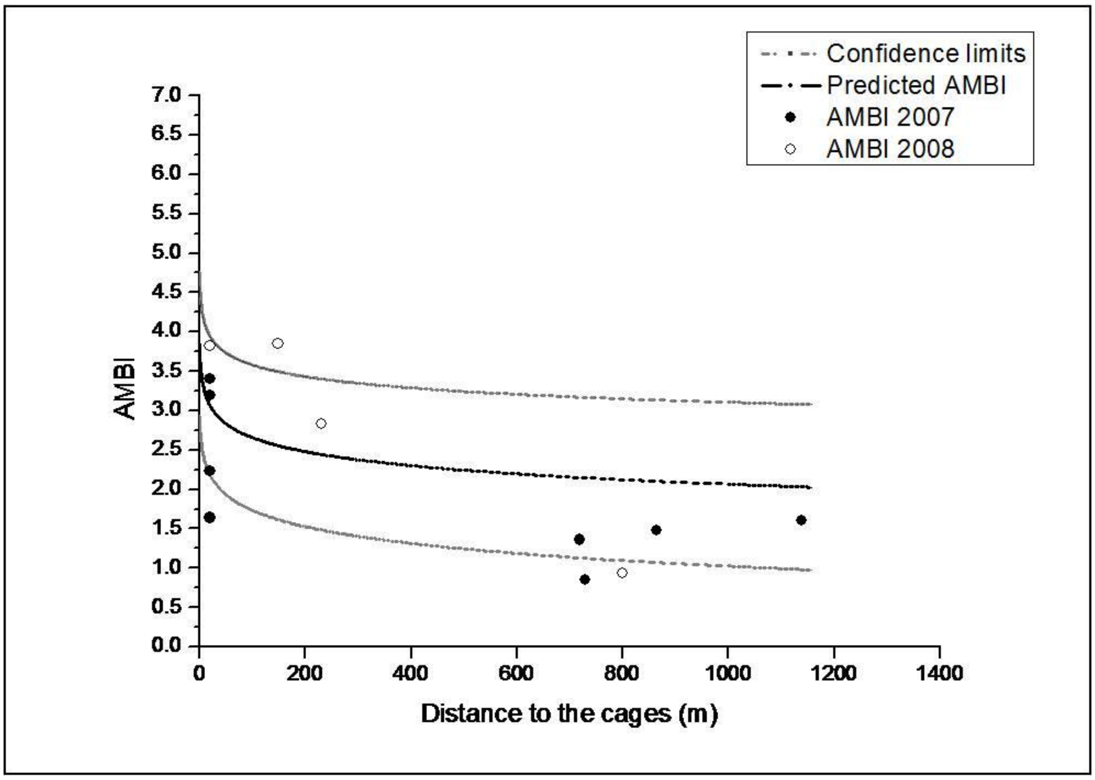

Figure 7. 4 AMBI values, predicted by a multiple regression analysis using depth (m), current speed (cm^{-1}), fish farm production (t $year^{-1}$) and distance to the cages (m), as independent variables (see Borja *et al.,* 2009b), compared with the AMBI values observed in 2007 and 2008. Confidence limits (95%) of the predicted values are included.

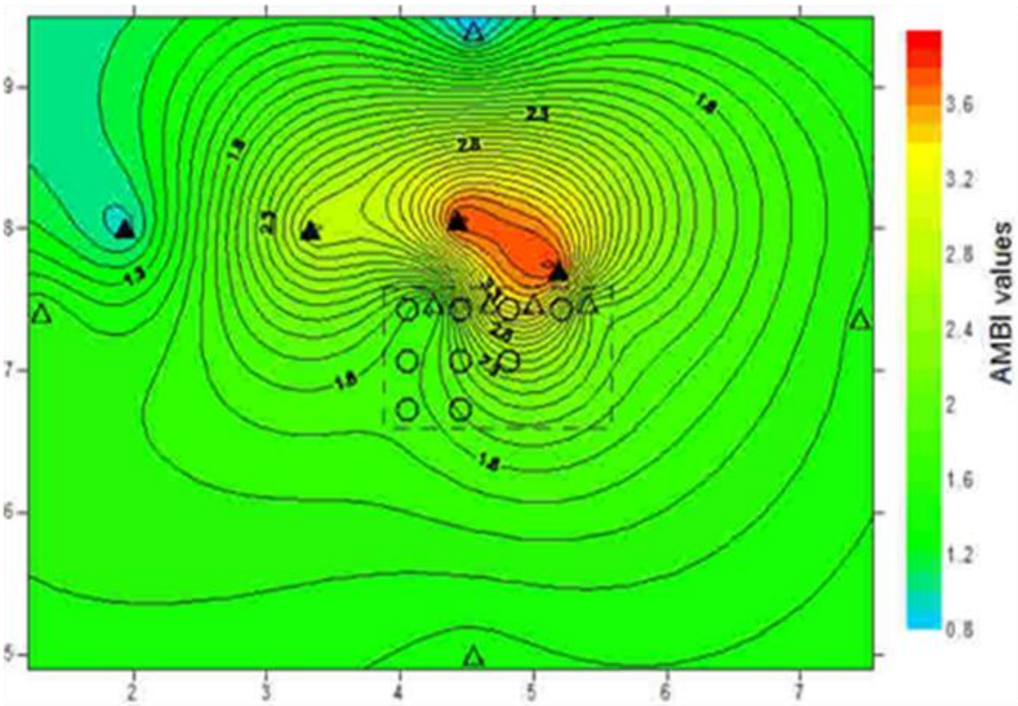

Figure 7. 5 Contour map created using the AMBI values calculated for each station sampled in 2007 and 2008 surveys.

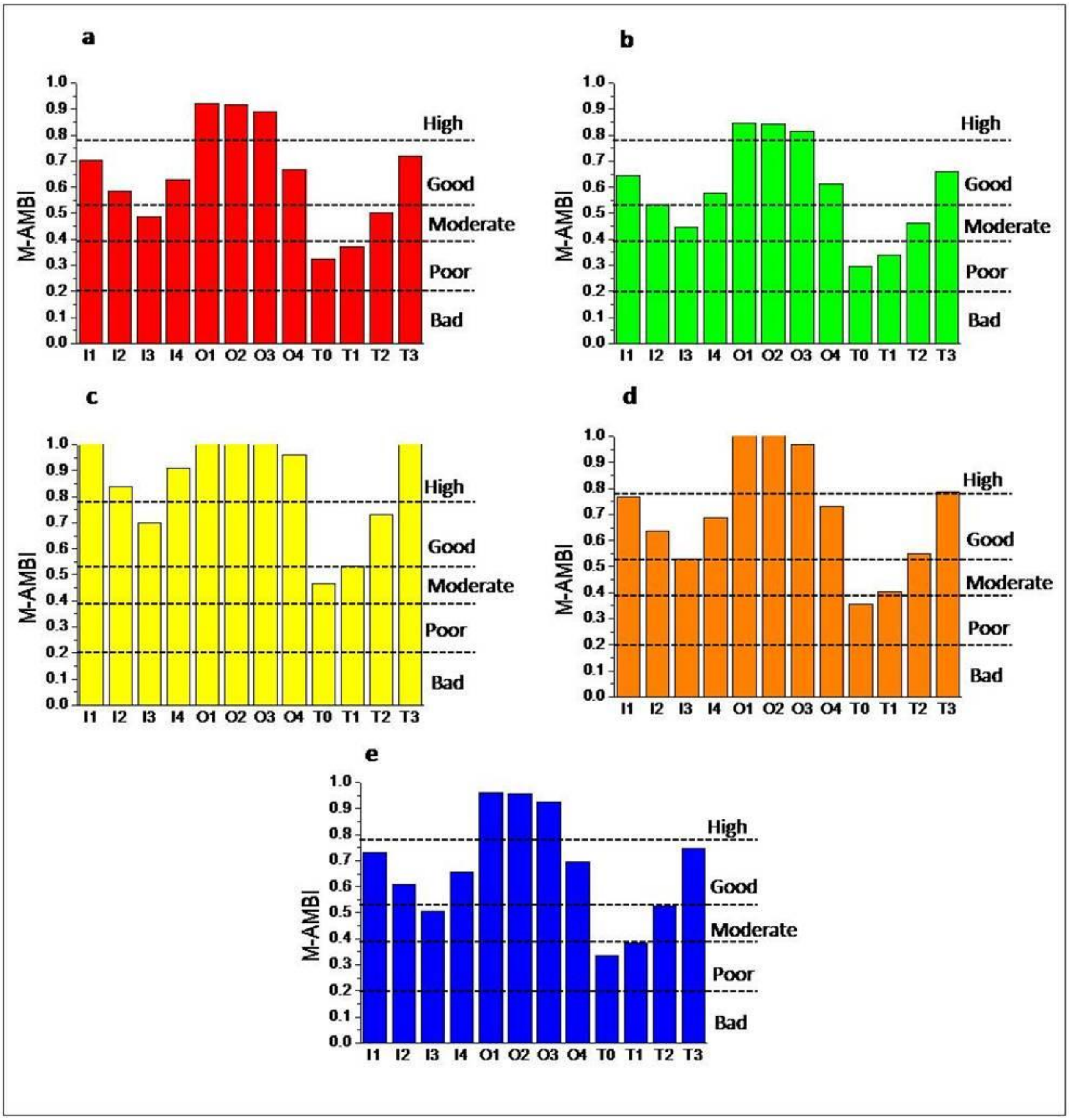

Figure 7. 6 M-AMBI results using different reference conditions (high *status*): (a) AMBI 0.5, richness 110, diversity 5.5; (b) AMBI 0, richness 120, diversity 6; (c) AMBI 0.5, richness 30, diversity 4 (used as reference conditions in the Italian Adriatic coast (Occhipinti-Ambrogi *et al*., 2009)); (d) AMBI 1.475, richness 104, diversity 5.42 (data from station O2); (e) AMBI 0.84, richness 105, diversity 5.42 (lowest AMBI value and highest diversity and richness from the area). Bad *status* values were: AMBI = 6, diversity and richness = 0.

7.5 Discussion

Investigating causal relationships between environmental stressors and effects on marine biota is a major issue in recent times (Adams, 2005). These stressors or pressures (*sensu* the WFD, Heiskanen and Solimini, 2005) need to be evaluated to assess the ecological *status* of marine waters. Hence, paradigmatic responses of marine benthic communities have been detected and assessed using indicators such as those used here (Borja *et al.*, 2009d). One of the increasing pressures in Mediterranean coastal waters is aquaculture, and benthic indices such as AMBI and M-AMBI have been used in assessing their impacts (Aguado-Giménez *et al.*, 2007; Bouchet and Sauriau, 2008; Callier *et al.*, 2008, 2009; Carvalho *et al.*, 2006; Muxika *et al.*, 2005; Sanz-Lázaro and Marín, 2006; Tomassetti *et al.*, 2009). However, sometimes these investigations report contradictory results in farm impacts, and some studies demonstrate that the absence of hydrodynamics or husbandry practices in their analyses can produce these erroneous or contradictory interpretations in the assessing indices results (Borja *et al.*, 2009d).

In the case of Alghero bay, both approaches used in assessing the benthic *status* (AMBI and M-AMBI) and both surveys conducted in consecutive years are consistent with the sediment physico-chemical alteration within the area. Hence, there is a local impact of the fish farm on the benthic ecosystem, limited to the area close to the cages, and mainly in the prevailing currents direction, which is north and northwestwards, producing a gradient of impact, higher close to the cages in this direction. The limited perturbation of this area agrees with the results reported in other studies of fish farms in the Mediterranean, which show disturbed areas within 20-30 m from the cages (Karakassis *et al.*, 2000; La Rosa *et al.*, 2001; Mirto *et al.*, 2002) and until 50 to 300 m from the cages (Cancemi *et al.*, 2003; Porrello *et al.*, 2005; Yokoyama *et al.*, 2006). Similar patterns have been described in other countries worldwide, with effects between 35 and 200 m (Edgar *et al.*, 2010; Gao *et al.*, 2005)

In a recent investigation in Tasmania, Edgar *et al.* (2010) identified four main indicators of farm impact: (i) redox potential at the sediment surface, (ii) redox potential at 4 cm depth, (iii) the proportional abundance of Capitellids, and (iv) the bivalve/mollusc ratio. At some extent, indicators (iii) and (iv) are included in the AMBI index, being redox potential also a good predictor for AMBI (Nickell *et al.*, 2009). Hence, it seems that there are general enrichment factors around fish farms which explain the response of univariate biotic indices, such as AMBI, making them useful in assessing benthic impacts.

However, when using multivariate methods, such as M-AMBI, there is a clear need to determine adequate reference conditions to assess the status. Hence, using M-AMBI, 3 out of the 5 reference condition cases coincide in the final *status* classification in all the stations (Figures 7.6a, 6b, 6e). Taking into account this classification and calculating mean and standard error values for different structural parameters, for the stations with coincident *status* classification, a clear gradient pattern is shown (Table 7.4). Hence, the *status* improves with increasing distance to the cages, with non-acceptable (<good *status*, *sensu* the WFD) within 125±105 m away of the cages, being the most degraded situation within the first 84 m, and the highest quality situation farther than 907±123 m. The gradient is shown by decreasing AMBI values and opportunistic species (groups IV and V) percentages, and increasing richness, diversity and sensitive-indifferent species contribution (groups I and II). The degradation pattern is similar to that described in other studies, and is influenced by the currents pattern and the hydrographical characteristics of the area and the production of the farm (e.g. Borja *et al.*, 2009b; Giles, 2008; Kalantzi and Karakassis, 2006).

The comparison between the observed and predicted AMBI values in Alghero bay is in general in agreement with the model proposed by Borja *et al.* (2009b). However, there are 4 values out of the confidence limits predicted in the AMBI curve (Figure 7.4). These differences could be related to the current speed recorded at Alghero bay (1.8 cm s^{-1}), which is smaller than the range (2.4 to 14.1 cm s^{-1}) reported by Borja *et al.* (2009b). However, the most probable explanation is the small production of the farm (only 99 t). In this way, the fact that the ecological *status* in 2008 is worst than in 2007, indicates that the time of the farm activity is another important factor in the benthic health, as highlighted by Borja *et al.* (2009b).

In order to perform the M-AMBI analysis, the setting of the reference conditions is required (Muxika *et al.*, 2007). As underlined by Teixeira *et al.* (2008), this represents a critical point for the correct evaluation of pressures on benthic communities, within the WFD. The reference conditions for a water body type are a description of the biological elements which corresponds totally, or near totally, to undisturbed (pristine) condition (Muxika *et al.*, 2007). There are four options for deriving reference conditions: (i) comparison with existing "pristine"/undisturbed site (or site with minor disturbance); (ii) historical data and information; (iii) models; and (iv) expert judgment (Bald *et al.*, 2005; Borja *et al.*, 2004). The setting of reference conditions to be used in Italy is particularly problematic (Occhipinti-

Ambrogi *et al.*, 2009). This problem principally regards the lack of information in the literature and the oceanographical characteristics of the Italian peninsula. The number of studies on the application of M-AMBI to the benthic impact assessment along the Italian coasts is increasing in recent times (Forni and Occhipinti-Ambrogi, 2007; Munari and Mistri, 2010; Occhipinti-Ambrogi *et al.*, 2009; Pranovi *et al.*, 2007; Prato *et al.*, 2009; Simonini *et al.*, 2009; Tataranni and Lardicci, 2010; and Tomassetti *et al.*, 2009). However, most of these studies have been carried out in transitional waters (mainly in lagoons) or in the Adriatic Sea. In very few cases, reference conditions used in the Italian coasts are included (Occhipinti-Ambrogi *et al.*, 2009), making the comparison of these reference conditions to those used in Alghero bay impossible. Another important factor that must be considered is that the reference conditions change naturally with ecoregion, water body type and habitat (Borja *et al.*, 2009c). This is particularly clear for Italy, which is characterized by the presence of extremely long coast with variable features; hence, selecting the same reference conditions for all the regions could be unrealistic (Occhipinti-Ambrogi *et al.*, 2009).

In agreement with Bald *et al.* (2005) and Borja *et al.* (2004) our approach in setting the reference conditions for Alghero bay includes sites with minor disturbance (sites located in the opposite way of the prevailing currents in the zone) combined with the expert judgment and the test of reference conditions reported in literature for the Italian coast. Similar approach was used by Tomassetti *et al.* (2009) in another Italian farm, in Apulia. The reference conditions proposed by Occhipinti-Ambrogi *et al.* (2009) for the Adriatic coast were not appropriate for the Alghero bay, because of the low values of richness and diversity reported by the authors, which results in M-AMBI values >1 in most cases. From the values corresponding to the structural parameters in high *status* (see Table 7. 4) it seems adequate to use the reference conditions chosen in Figure 7.6e, for the Sardinian coastal zone of Italy.

Table 7.4 Mean and standard error values of some structural parameters, for the stations classified in the same ecological *status*, using M-AMBI, within Alghero Bay, showing also the distance to the cages. EG: Ecological Group.

Status	Number of stations	AMBI	Richness	Diversity	EG I-II (%)	EG IV-V (%)	Distance (m)
Poor	2	3.8±0.01	26±1	2.1±0.33	16.6±2.15	77.9±0.6	84±64
Moderate	3	3.1±0.28	26±8	3.7±0.18	37.3±2.7	45.2±15.2	125±105
Good	4	1.8±0.44	45.8±2.44	4.1±0.17	63.8±8.09	23±9.48	318±183
High	3	1.5±0.07	102.7±1.86	5.4±0.03	82.7±0.68	3.5±1.57	907±123

7.6 Conclusions

The present study showed that AMBI and M-AMBI could be useful tools in detecting benthic impacts from fish-farm activity in Sardinian coasts. The use of these indices shows a relationship between the benthic health *status* and the important role of the water currents in the dispersion of wastes. Hence, the use of equations comparing observed and predicted values of these indices (i.e. AMBI) allows a better understanding of these relationships. The identification of appropriate reference conditions to be used in Alghero bay allowed an adequate M-AMBI calculation and a discrimination of the ecological *status* of the stations. This allows visualizing the gradient of impact within the area, in terms of benthic indices and structural parameters of the community. As discussed above, taking into account the caution that must be adopted in choosing the appropriate reference conditions, the reference conditions proposed for the Alghero bay could be considered in further studies in the Sardinian region.

CASE STUDY II: CYPRUS, AKROTIRI BAY

8 The use of AMBI, M-AMBI and BENTIX to evaluate the effects of aquaculture activities in Akrotiri bay (Cyprus): a comparison among different approaches.

The objectives of this study are (i) to investigate the effects deriving from the activity of two off-shore fish farm located in the Akrotiri bay (South-west Cyprus) on benthic assemblages using AMBI and BENTIX indices; (ii) set adequate reference condition for this area in order to apply the M-AMBI calculation; (iii) to compare the information derived from these indices in order to evaluate differences and validate the results.

8.1 Study area

The study was carried out in two off-shore fish farms located in the Akrotiri bay, Cyprus (Eastern Mediterranean Sea) (Figure 8.1). The two fish farms differed one another for their structural and management characteristics (e.g. number of cages, total annual production) and for environmental factors (e.g. water currents, sea depth). At the moment of samplings, “Kimagro fishfarming Ltd.” reared *Dicentrarchus labrax* and *Sparus aurata* for a total biomass of about 971 tons in 31 floating cages (diameter = 22 m, net depth = 8 m) displaced on a surface of about 9 hectares. The other fish farm, “Blue Island fish farm Ltd.”, covered an area of about 18 hectares, and counted 38 floating cages (11 cages: diameter = 22 m, net depth = 10 m; 12 cages: diameter = 20 m, net depth = 10 m; 15 cages: diameter = 16 m, net depth = 10 m) in which *Dicentrarchus labrax* and *Sparus aurata* were reared for a total biomass of 1,039 tons. For both the fish farms, fishes were feed with commercial extruded dry pellet with a monthly average of 208.33 and 219.41 tons for Kimagro and Blue island respectively. The sea bottom is sandy and the depth under the cages is of 14 m for Kimagro and of 30 m for Blue island.

8.2 Sampling strategy

Sampling activities were carried out in November 2009 and for both the fish farms 3 stations were sampled (K1, K2 K3 for Kimagro and B1, B2, B3 for Blue island in Figure 8.1). The sampled stations were located on a transect leading out from the cages towards the direction of the prevailing currents (South-West). The distance from the cages was 50 m, 219.2 m, 341.2 m for K1, K2 and K3 respectively and 50 m, 146.08 m, 186.73 m for B1, B2 and B3 respectively.

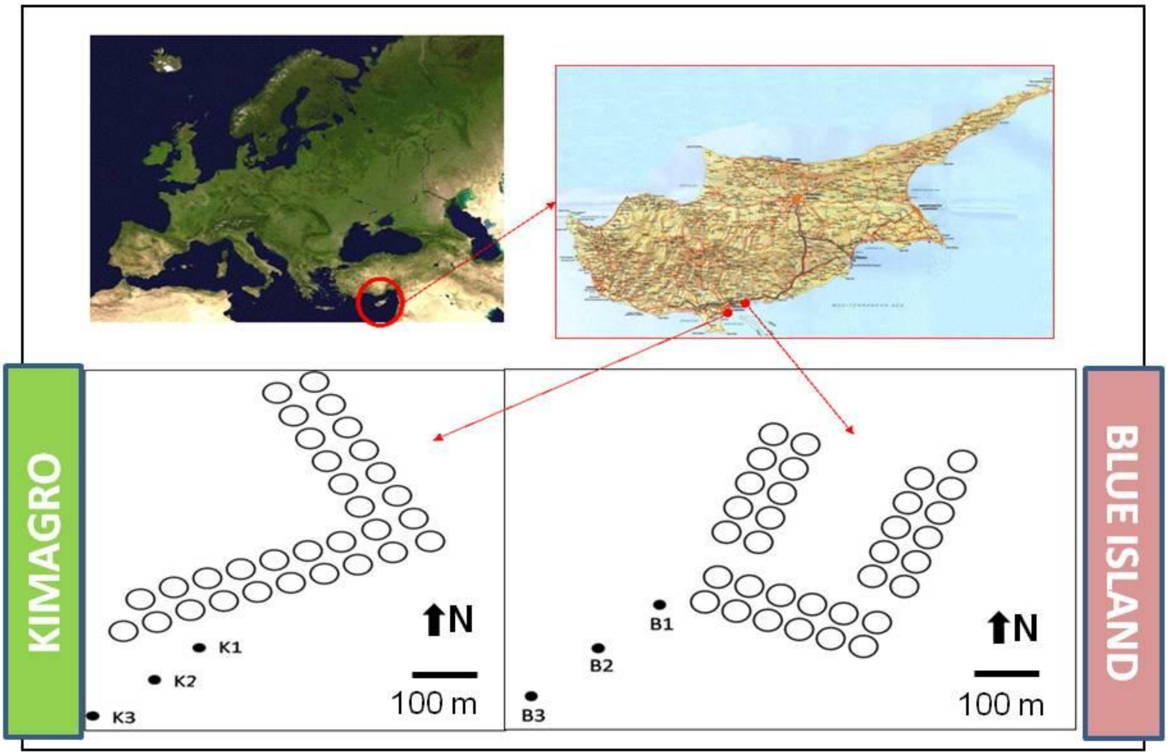

Figure 8.1 Location of the study area in Akrotiri bay (Cyprus, Eastern Mediterranean Sea); sampling strategy and spatial disposition of the stations sampled for Kimagro Ltd. (K1, K2, K3) and for Blue Island Ltd. (B1, B2, B3).

8.3 Results

8.3.1 Abiotic parameters

Information on sea temperature, salinity and water currents (velocity and direction) were obtained in collaboration with the Cyprus Oceanography Center (www.oceanography.ucy.ac.cy) and the mean annual values (November 2008 – November 2009) are reported in Table 8.1. Concerning currents, the higher mean values were found on the sea surface while at 5 m depth the velocity slightly decreased (Table 8.1). Even if for Blue Island the surface current presented a mean direction of 48 degrees, for both the sites the deeper currents had a South-West direction, indicating that the most relevant sedimentation processes should occur in this direction.

The results of chemical and physical analyses of the sediment are reported in Table 8.2. Referring to Kimagro, the mean SWC (%) value calculated for K3 (18.58±1.16) resulted significantly lower than K1 (23.82±1.16) and K2 (25.52±1.65) and the same was found for TC (%) with mean values of 4.76±0.32 for K3 and 6.06±0.73 and 5.69±0.10 for K1 and K2 respectively (Table 8.2). No significant difference was found among the stations concerning OM% and TH%, while total nitrogen resulted not detectable due to its scarce amount (Table 8.2). The granulometric analysis underlined the heterogeneity of sampled sediment, and each station resulted significantly different from the others for grain size classes composition (Table 8.2). In particular, the higher percentage of gravel was found for K2 (25.71±0.41) and this station was characterized by the higher percentage of mud (10.03±0.76) too. The sediment in K1 and K3 resulted composed by an higher percentage of sand (74.04±0.40 and 75.92±0.18 for K1 and K3, respectively) (Table 8.2).

Referring to Blue Island, the higher SWC percentage was found for B1 with mean value of 75.37±1.87 and this station resulted significantly different from B3, where the lowest mean percentage was recorded (57.28±0.93) (Table 8.2). No significant difference was found among the stations concerning OM% and TC% and, as previously reported for Kimagro also for Blue Island the Total Nitrogen percentage resulted not detectable. The lower mean TH % value was found for B2 (0.55±0.03) and this station resulted significantly different from B1 (0.67±0.06) and B3 (0.65±0.05) (Table 8.2). Also for Blue Island, the stations appeared heterogenic concerning grain size. Thus, if any significant difference was not found among the stations for mud mean percentage, B1, B2 and B3 resulted significantly different concerning the coarser grain size classes. In particular, the higher percentage of gravel was recorded for B3 (61.17±0.44) while for B1 and B2 mean values were lower (31.53±0.13 and 36.95±0.32, respectively) (Table 8.2). The situation was vice versa concerning the sand, and for this fraction the lower mean value was recorded for B3 (30.82±0.34) while B1 and B2 showed higher mean values (60.59±0.61 and 54.57±0.33) (Table 8.2). The Multidimensional Scaling Analysis (MDS) as synthesis of chemical and physical results is reported in Figure 8.2.

Table 8. 1 Mean annual values (November 2008 – November 2009) for sea water temperature (°C), salinity (‰), current velocity (m s^{-1}) and direction (magnetic degrees). Parameters recorded in collaboration with the Cyprus Oceanography Center.

		Temperature (°C)	Salinity (‰)	Current velocity (m s^{-1})	Current direction (magnetic degrees)
Kimagro	Depth (m) 0	20.67	39.19	0.093	255
	Depth (m) 5	20.48	39.19	0.084	240
Blue island	Depth (m) 0	21	39.21	0.108	48
	Depth (m) 5	20	39.2	0.085	251

Table 8. 2 Mean and standard deviation (s.d.) values of the chemical and physical parameters analyzed for the stations sampled for Kimagro and Blue Island.

Stations		SWC (%)	OM (%)	TN (%)	TC (%)	TH (%)	Gravel (%)	Sand (%)	Mud (%)
K1	Mean	23.82	1.41	n.d.	6.06	0.56	20.1[a]	74.04[d]	5.85[g]
	s.d.	1.16	0.51	n.d.	0.73	0.10	0.39	0.40	0.07
K2	Mean	25.52	1.67	n.d.	5.69	0.57	25.71[b]	64.27[e]	10.03[h]
	s.d.	1.65	0.46	n.d.	0.10	0.03	0.41	1.10	0.76
K3	Mean	18.58*	2.00	n.d.	4.76*	0.47	15.74[c]	75.92[f]	8.35[i]
	s.d.	1.16	0.30	n.d.	0.32	0.06	0.47	0.18	0.30
B1	Mean	75.37[a]	1.56	n.d.	4.27	0.67	31.53[c]	60.59[f]	7.89
	s.d.	1.87	0.98	n.d.	0.65	0.06	0.13	0.61	0.48
B2	Mean	65.42	1.67	n.d.	2.77	0.55*	36.95[d]	54.57[g]	8.49
	s.d.	2.46	0.36	n.d.	0.73	0.03	0.32	0.33	0.19
B3	Mean	57.28[b]	1.50	n.d.	3.20	0.65	61.17[e]	30.82[h]	8.01
	s.d.	0.93	0.14	n.d.	0.88	0.05	0.44	0.34	0.13

Key: Sediment Water Content (SWC), Organic Matter (OM), Total Nitrogen (TN), Total Carbon (TC).
* : significant differences ($p<0.05$); lower case letters: significant differences ($p<0.05$) between the stations

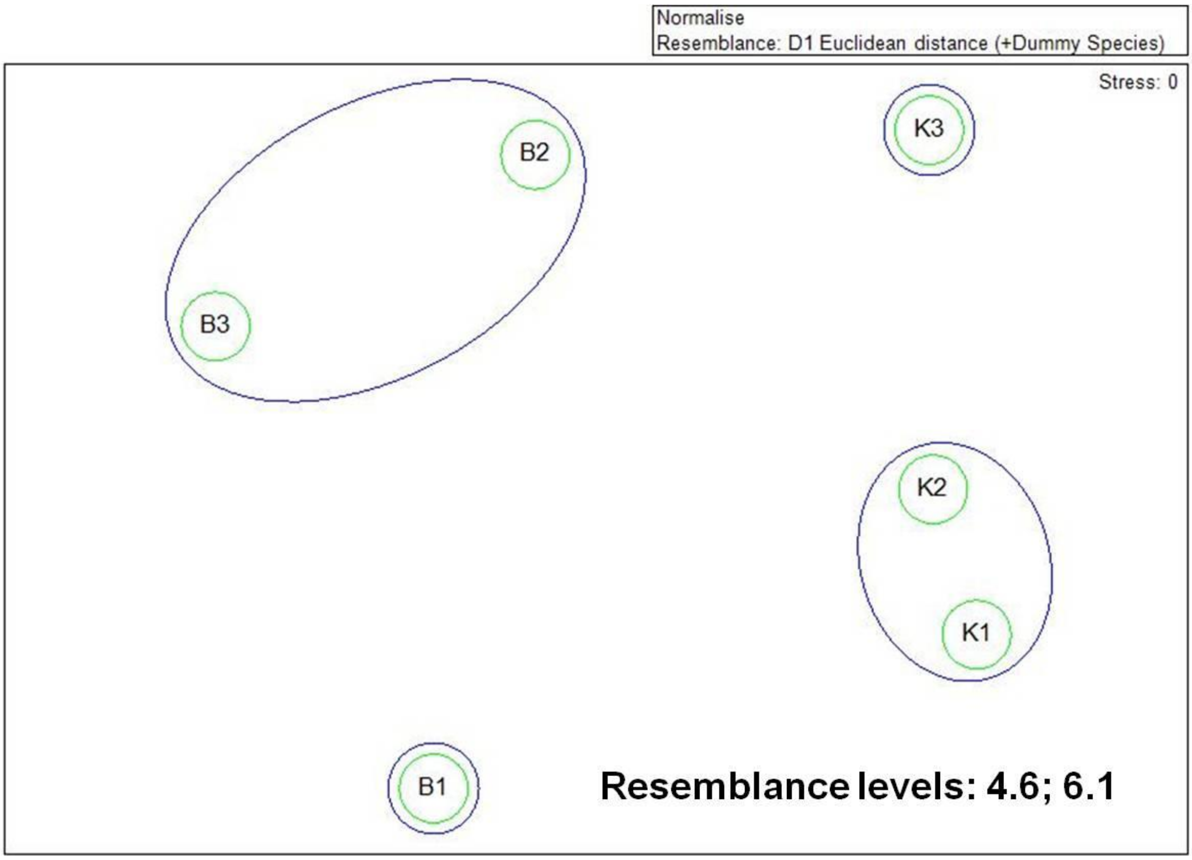

Figure 8. 2 Multidimensional Scaling Analysis (MDS) performed using chemical and physical results obtained for Kimagro and for Blue Island.

8.3.2 Biotic parameters

A total number of 1.651 individuals belonging to 78 different *taxa* and a total number of 1.089 individuals belonging to 121 *taxa* was found for Kimagro and Blue Island, respectively. For both the fish farms, no significant difference was recorded among the stations concerning number of *taxa* and number of individuals per square meter (Table 8.3). However, patterns seemed different between the two fish farms. In fact, for Kimagro the higher number of individuals per square meter (N m^{-2}) and *taxa* were found for site located far from the cages, in particular K2 for number of individuals (1.618±494.77) and K3 for number of taxa (36.33±4.73) while for Blue Island, B1 showed the higher mean values (1.641±685.36 and 43.67±12.01 indivduals per square meter, and number of *taxa* respectively) (Table 8.3). Concerning Kimagro, K3 resulted significantly different from the other two stations for Shannon, Simpson and Pielou indices mean values and in this site the benthic community resulted to be more complex and well structured (Table 8.3).

Table 8. 3 Mean values and standard deviation (s.d.) for number of taxa (S), number of individuals per square meter (N m^{2-1}), Shannon index (H'), Simpson index (D) and Pielou index (J') calculated for Kimagro and Blue Island.

Stations		S	N m^{-2}	H'	D	J'
K1	Mean	29.33	1452.02	2.19	0.77	0.66
	s.d.	7.02	297.52	0.19	0.06	0.06
K2	Mean	29.33	1618.69	1.90	0.66	0.56
	s.d.	3.06	494.77	0.23	0.06	0.05
K3	Mean	36.33	1098.48	3.03*	0.93*	0.84*
	s.d.	4.73	191.20	0.20	0.02	0.05
B1	Mean	43.67	1641.41	2.66	0.82	0.72
	s.d.	12.01	685.36	0.41	0.13	0.15
B2	Mean	40.33	1035.35	3.16	0.93	0.85
	s.d.	5.86	108.82	0.34	0.04	0.06
B3	Mean	33.00	805.56	3.00	0.93	0.86
	s.d.	4.36	118.42	0.08	0.01	0.02

These results were confirmed by both AMBI and BENTIX index calculation (Table 8.4-5). Thus, AMBI calculation showed a decreasing gradient as the distance from the cages increases and the lower mean value recorded for K3 (AMBI = 2.29) resulted significantly different from the values found for the other two stations (AMBI = 3.05 and AMBI = 3 for K1 and K2, respectively) (Table 8.4). BENTIX index calculation led to similar results and K3 resulted significantly different from the other stations with an higher mean value (Table 8.5). Concerning Blue Island, no significant differences resulted from the quantitative indices calculation, even if the lower mean values were calculated for B1 (Table 8.3). Both AMBI and BENTIX calculations seemed to underline that the station characterized by higher "quality" should be B2, that presented the higher mean AMBI values (AMBI = 1.84) and the lowest mean BENTIX value (BENTIX = 3.43). Concerning AMBI calculation this station appeared significantly different from B1 (AMBI = 2.5) and B3 (AMBI = 2.47) while the differences found using BENTIX index were not significantly. Even if with some slightly differences the trends of these two indices appeared similar (Figure 8.3).

Table 8. 4 AMBI calculation: Ecological Groups (E.G.) relative abundance (%) and mean AMBI values calculated for the stations sampled for Kimagro and Blue Island.

AMBI

Stations	E.G. I (%)	E.G. II (%)	E.G. III (%)	E.G. IV (%)	E.G. V (%)	Mean AMBI value
K1	11.1	9	61.2	3.9	14.7	3.05
K2	7	9.7	65.6	8.6	9.1	3.00
K3	24.5	21.4	39.8	5	9.2	2.29*
B1	15.9	21.8	48.5	2.7	11.1	2.50
B2	27.8	32.7	32.7	1.3	5.4	1.84*
B3	15.9	26.7	41.2	7.8	8.4	2.47

Table 8. 5 BENTIX calculation: Confidence Level of the computation, relative abundace (%) of sensitive (GS) and tolerant (GT) groups and mean BENTIX values calculated for the stations sampled for Kimagro and Blue Island.

BENTIX

Stations	Confidence Level	GS % (sensitive)	GT % (tolerant)	Mean Bentix
K1	☑	15.22	0.84	2.59
K2	☑	0.13	0.85	2.51
K3	☑	0.32	0.61	3.12*
B1	☑	21.34	0.75	2.78
B2	☑	0.38	0.56	3.43
B3	☑	0.28	0.63	2.95

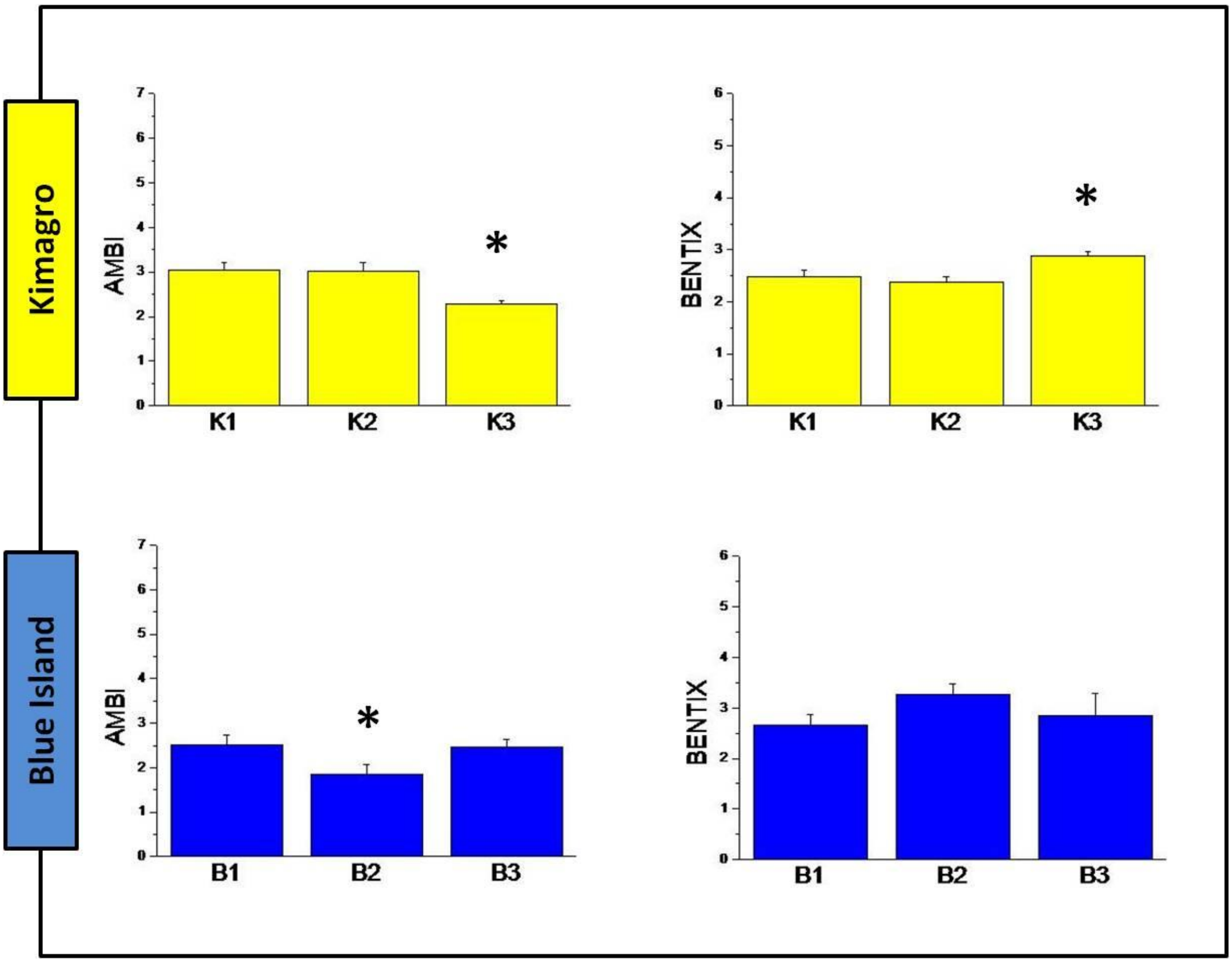

Figure 8. 3 AMBI and BENTIX index calculated for Kimagro and Blue Island. *: significant difference (p<0.05)

To perform the M-AMBI calculation the setting of the reference condition was necessary. In order to find a correct reference condition to apply. For such purpose, data from previous samples were utilized, being kindly provided by the Ministry of Agriculture, Natural Resources and the Environment of Cyprus and in particular in collaboration with the Department of Fisheries and Marine Research, Marine Environmental Division. This Division provided historical data about two stations, Lady's Mile and Zigy, traditionally used as reference condition for Kimagro and Blue Island, respectively. Thus, AMBI, diversity and richness mean values were calculated for Lady's Mile (AMBI = 1.83; diversity = 5.23; richness = 108) and Zigy (AMBI = 1.65; diversity = 5.63; richness = 100) and from them the reference conditions were derived (HIGH: AMBI = 1.6; diversity = 5.7; richness = 110; BAD: AMBI = 6; diversity = 0; richness = 0). The result of M-AMBI calculation is reported in Figure 8.4. For both the fish farms, all the stations were classified as *"Good" status*, except K2 *("Moderate" status*). However, Blue Island stations showed higher M-AMBI values (B1:0.76; B2: 0.81; B3: 0.70) than Kimagro ones (K1: 0.56; K2: 0.54; K3: 0.74) suggesting the presence of more disturbed benthic communities in these latters.

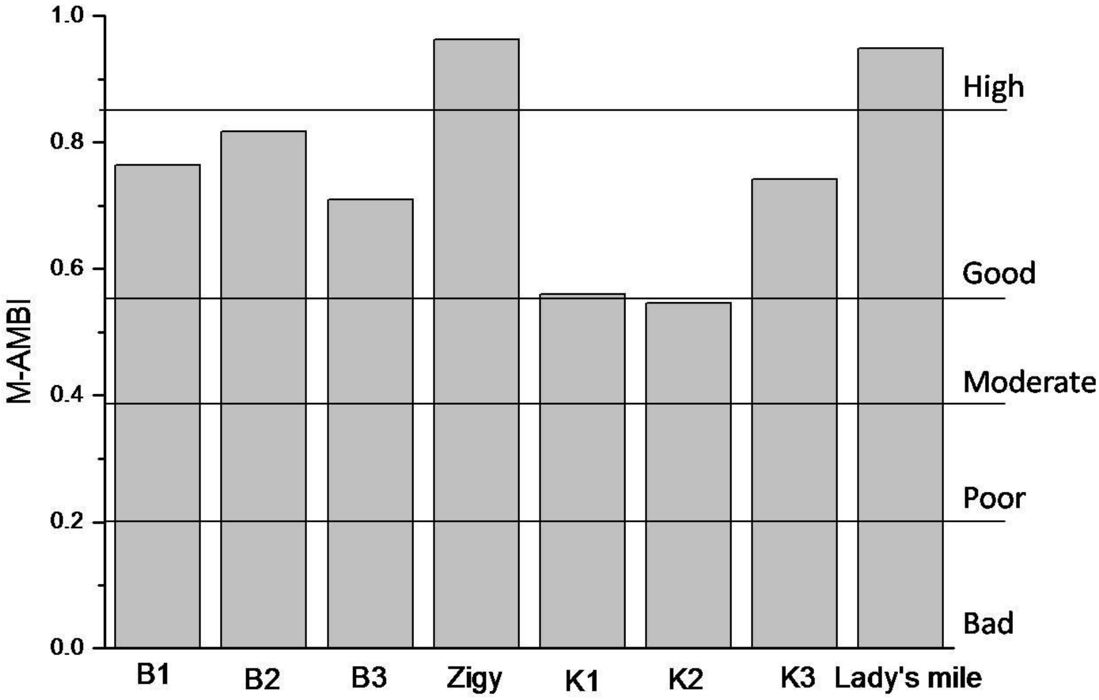

Figure 8. 4 M-AMBI calculation for Kimagro, Blue Island and respectively reference sites (historical data). Reference condition: High: AMBI = 1.6; diversity = 5.7; richness = 110; Bad: AMBI = 6; diversity = 0; richness = 0.

8.4 Discussion

The use of biotic indices to assess the quality *status* of benthic ecosystems is fundamental especially in areas, where, due to their own peculiar characteristics (e.g. oligotrophy, transitional ecosystems), the calculation of traditional ecological indices fails. A practical example is represented by this study. Thus, being the eastern Mediterranean Sea an oligotrophic area (Siokou-Frangou *et al.*, 2002) the effects of organic enrichment are as evident as difficult to interpret. In relation with the performed samplings, the number of individual per square meter showed a clear gradient among the stations, and the density increased according to the distance from the cages, except for Kimagro, where the higher density was recorded for K2 (Table 8.3) However, the traditional ecological indices calculation did not led to clear and exhaustive results. If for Kimagro, these calculations put in evidence significative differences among the stations, for Blue Island no significant difference were found and the results appeared more complicate to be interpreted. Trying to avoid these kind of problems and to get more complete information on the health *status* of bethic communities, the application of biotic indices such as AMBI, M-AMBI and BENTIX is needed. As recently summarized by Simboura and Argyrou (2010) the performance of each biotic index in a certain area depends on the structure of the index, which includes the weighting coefficient of each ecological group in relation to others. It is the design and structuring of each method that shapes the final assessment. Previous studies suggested that eventual discrepancies in these two indices results could be ascribed to differences in: (i) the weighting of tolerant and sensitive groups of species in the formulae; (ii) the scaling of boundary limits among classes; (iii) the arrangement of the "tolerant" species, which are weighted separately in the AMBI, whereas the BENTIX method required all tolerant species to be weighted equally; (iv) the attribution of the species to the ecological groups (Simboura and Zenetos, 2002; Simboura, 2004; Occhipinti-Ambrogi and Forni, 2004; Simboura and Reizopoulou, 2007, 2008).

In the present study the application of these two indices to detect aquaculture impacts in Akrotiri Bay led to similar results (Figure 8.2) but AMBI seemed to be more sensitive than BENTIX, highlighting one significant difference among the stations that BENTIX did not discriminate (Figure 8.3). This similarity of trends between the two indices is supported in literature by various authors that compared AMBI and BENTIX in different Mediterranean areas (Forni and Occhipinti-Ambrogi, 2004; Simonini *et al.*, 2009). However, in some cases these indices did not work in the expected ways (Simboura, 2004; Gomez-Gesteira and Dauvin 2005; Muxica *et al.*, 2007 Simboura and Reizopoulou,. 2008) and problems coming out from the comparison of these two indices. In particular, depending from the study area problems of overestimation with AMBI (Tataranni and Lardicci, 2010) or of underestimation with BENTIX (Simboura and Argyrou, 2010) were found. The M-AMBI analysis, comparing the sampled stations with reference conditions, amplified differences among the stations and provided a more complete information about the *status* of the sites (Figure 8.4). Even if, in general, the area appeared not seriously compromised and all the stations being classified in “Good” or “Moderate” conditions, the worst situation regarded Kimagro. Concerning this fish farm, the gap between the sampled stations and the reference conditions appeared higher than the one found for Blue Island and K1 and K2 resulted the most compromised stations, appearing borderline between Good-Moderate *status* (Figure 8.4). These results could be put in relation with the different environmental characteristics of the two areas where the fish farms are located. The principal difference concerned depth, which measured 14 m for Kimagro and 30 m for Blue Island. Depth within hydrodynamic regime is a key factor influencing sedimentation processes (Gray and Elliott, 2010). Hence, being Kimagro and Blue Island characterized by current with similar speed (Table 8.1), more spatially limited sedimentation processes are expected to occur where depth is lower. This fact is reflected also in differences in sediment composition between the two areas. Thus, the sediment sampled for Blue Island showed higher percentages of coarse fraction (gravel) while for Kimagro higher percentages of sand were found (Table 8.2). An analogue consideration could be done for total carbon (Table 8.2). These chemical and physical differences were summarized by the Multidimensional Scaling Analysis (MDS) performed (Figure 8.2).

Putting together results of the chemical, physical and biological analysis performed, appeared that the extension of the effects of aquaculture activities on the benthic ecosystem differed between Kimagro and Blue Island. Concerning Kimagro, the most disturbed stations were the ones located close to the cages and the perturbation seemed to follow a gradient in the direction of the principal currents. The situation appeared different for Blue Island where higher pressures were recorded for B1 and for B3, indicating that the sedimentation process probably act in a different way respect Kimagro. In fact, for Kimagro, due to the lower depth of the sea bottom (14 m), the main part of the wastes probably tended to settle close to the emission point and so the principal effects were recorded up to

200 m from the cages. Concerning Blue Island, where the depth of the sea bottom is higher, only the big size particles are settling close to the cages, while the most fine particles are transported by the currents, settling more diluited and at higher distance. Thus, the wastes "footprint" observed in the present study appeared more extended than the one found in other studies (Karakassis *et al*., 2000; Cancemi *et al*., 2003), underlining the importance of site-specific considerations and the main role of currents in the dispersion of wastes.

8.5 Conclusions

The present study showed that AMBI, M-AMBI and BENTIX could be useful tools in detecting benthic impacts caused by fish farm activity in Akrotiri bay. Even if both the farms resulted in a limited impact, the comparison among these calculations led to similar results, even if AMBI showed higher sensitivity than BENTIX. The application of M-AMBI allowed a better discrimination among the stations, and the worst conditions were found for Kimagro. The reference conditions used, derived from historical data, resulted adequate for the calculation and these values could be taken into account for further studies in the same area.

CASE STUDY III: ITALY, TUSCANY

9 Comparison between AMBI and BENTIX application to investigate the effects of two land-based fish farms in Coastal Transitional Ecosystems (CTEs): two cases study in Tuscany Region (Italy).

The objective of this study are (i) to assess the impacts of aquaculture on benthic assemblages in coastal transitional areas using AMBI and BENTIX as descriptors of their "quality *status*"; (ii) to compare the results derived from these two indices in order to establish the most suitable one to use in such areas.

9.1 Study area

The study was carried out in two land-based fish farms located in Tuscany region (Central Italy): "*La Rosa S.r.l.*" and "*Il Padule"* (Figure 9.1). Both the fish farms were located in marine transitional environments, a marine lagoon (Orbetello lagoon) and a salt marsh (Daccia-Botrona), for "*La Rosa S.r.l.*" and for "*Il Padule"* respectively. Concerning "*La Rosa S.r.l."*, at the time of samplings about 140 tons of Sea Bass (*Dicentrarchus labrax*) and 50 tons of Sea Bream (*Sparus aurata*) were reared in 34 ponds (volume = 1,053 m^3) and fed with commercial extruded pellet (Skretting®; 43-47% dry matter (d.m.) protein, 20% d.m. crude fat, 6.6-6.8% d.m. ash, 3% d.m. crude fiber, 0.8-0.9% d.m. phosphorous). The mean feed daily ratio was about 2.1 tons day^{-1}. The *"Il Padule*" fish farm produce about 400 tons $year^{-}1$ of *Dicentrarchus labrax* in brackish water, obtained by mixing marine waters with waters coming from the surrounding marsh and inflow rivers. The farm occupied a total surface of about 65 ha and comprised two head lagoon ponds receiving the water from three pumps with a maximum total flow of 3 $m^3\ s^{-1}$, 15 fish ponds and 11 final discharge lagoon ponds to depure water before release. The volume of fish ponds ranged from 4,500 m^3 to 27,500 m^3. Fishes were fed with a commercial pellets diet (Skretting®; 43-47% d.m. protein, 18% d.m. crude fat, 8-9.3% ash, 1.6-1.8% d.m. crude fibre, 1.05-1.25% d.m. phosphorous) and the mean daily ratio was about 4.5 tons day^{-1}.

9.2 Sampling strategy

The sampling activities were performed during the months of June and July 2010 and for each fish farm 3 stations were sampled (R and P stations for “La Rosa S.r.l.” and for “Il Padule”, respectively): one located after the ponds exit (R1, P1), another one located at the end of the final discharge lagoon ponds (R2, P2) and the last one located outside the fish farm (R3, P3), as reference site (Figure 9.1).

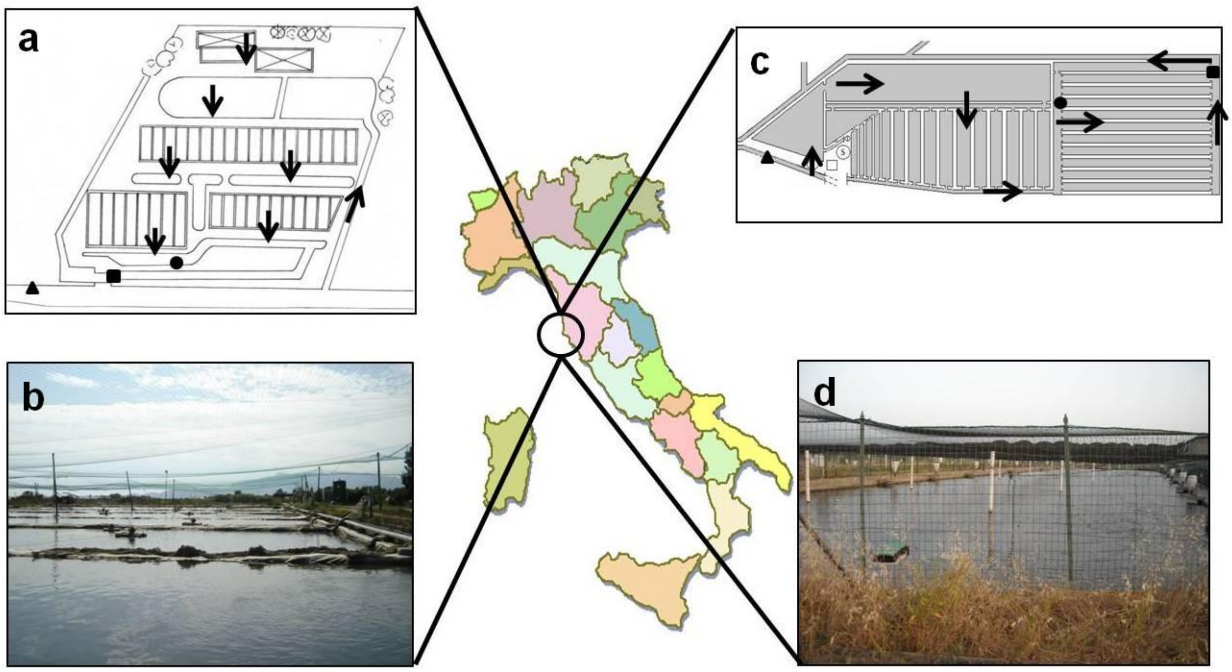

Figure 9. 1 Location of the study area (Tuscany, Italy); fish farm plan of “La Rosa S.r.l.” (a, b) and of “Il Padule” (c, d). Key:→ water flux direction; • , ■, ▲ : sampled stations; • : pond exit (R1, P1); ■: fish farm exit (R2, P2); ▲: reference site (R3, P3).

9.3 Results

9.3.1 Abiotic parameters

The mean values of the abiotic parameters measures are reported in Table 9.1. Concerning oxygen and temperature, no significant differences were found among the stations. Owing to the aquaculture activities, for both the fish farms, the highest oxygen concentration values were recorded at station 1 and 2 with maximum mean values of 7.35±0.52 mg l^{-1} and 6.40±0.53 mg l^{-1} for “*La Rosa S.r.l.*” (R2) and “*Il Padule*” (P1), while the reference sites showed lowest mean values (7.20±0.42 mg l^{-1} and 5.04±2.47 mg l^{-1} for “*La Rosa S.r.l.*” and for “*Il Padule*”, respectively). For “*La Rosa S.r.l.*” salinity was significantly lower in R3 (34.75± 2.5

‰) than in R1 (39.00±1.15‰) and R2 (39.00±1.15‰), while for "*Il Padule*" the measured values were constant among all the stations. For both the fish farms, the sampled sediments showed no homogeneity among the stations concerning physical and chemical characteristics. For "*La Rosa S.r.l.*" SWC% resulted significantly lower in R1 (28.18± 0.42) than R2 (33.47± 2.86) and R3 (35.58±0.52). Significant differences were found among the stations for OM% and TC% (Table 9.1). Total nitrogen percentage (TN%) resulted not detectable while the lower mean value for TH% was recorded for R2 (0.41± 0.22) and this station resulted significantly different from R3 that showed the higher mean value (0.79± 0.03) (Table 9.1). Heterogeneity among the stations was found also for the sediment grain size composition. Gravel percentage was significantly different among the station and R3 appeared characterized by the lowest relative percentage of gravel (2.09± 0.15) and by the highest percentage of mud (13.96± 0.70) (Table 9.1). For "*Il Padule*", significant differences were found among the stations for SWC% and OM%, while P3 showed the highest mean value of TN% (0.57±0.19) resulting significantly different from the other two stations. No significant differences were found among the stations for TH%, even if the mean values showed an increasing gradient from P1 (0.53± 0.02) to P2 (1.06± 0.03) and P3 (1.67±0.85). The three sampled stations appeared characterized by an high heterogeneity concerning the sediment grain size composition and P3 showed the higher gravel percentage (40.23±0.82) and the lowest sand percentage (54.57±0.037) (Table 9.1).

Table 9.1 Mean values and standard deviation (s.d.) measured for each station for water column and sediment parameters.

	Stations		O_2 (mg l^{-1})	O_2%	T (°C)	Salinity (‰)	SWC %	OM %	TC %	TN %	TH %	Gravel (%)	Sand (%)	Mud (%)
La Rosa S.r.l.	R1	Mean	7.28	86.75	25.75	39.00	28.18*	4.52^{A}	4.15^{D}	n.d.	0.63	9.63^{I}	87.22^{*}	3.15
		s.d.	0.59	7.27	0.37	1.15	0.42	0.36	0.21	n.d.	0.10	0.64	1.13	0.50
	R2	Mean	7.35	85.00	25.60	39.00	33.47	7.26^{B}	0.91^{E}	n.d.	0.41^{G}	13.37^{L}	82.82	3.81
		s.d.	0.52	4.69	0.22	1.15	2.86	1.48	0.38	n.d.	0.22	0.76	0.82	0.37
	R3	Mean	7.20	85.00	25.28	34.75^{*}	35.58	10.53^{C}	1.60^{F}	n.d.	0.79^{H}	2.09^{M}	83.96	13.96^{*}
		s.d.	0.42	4.97	0.33	2.50	0.52	0.99	0.15	n.d.	0.03	0.15	0.84	0.70
Il padule	P1	Mean	6.40	77.83	26.83	30.17	32.29^{AC}	5.47^{D}	3.86	n.d.	0.53	16.87^{G}	80.73^{L}	2.41^{*}
		s.d.	0.53	6.85	2.17	3.49	2.64	0.52	0.65	n.d.	0.02	0.36	0.84	1.20
	P2	Mean	5.73	69.17	26.57	30.17	67.96^{B}	12.06^{E}	1.78	0.16	1.06	6.78^{H}	87.42^{M}	5.80
		s.d.	0.81	7.88	1.91	3.49	4.72	0.18	0.46	0.14	0.03	0.39	1.08	0.70
	P3	Mean	5.04	71.67	26.05	30.17	17.66^{C}	18.04^{F}	6.64	0.57^{*}	1.67	40.23^{I}	54.57^{N}	5.20
		s.d.	2.47	9.85	2.02	3.49	11.07	3.27	3.45	0.19	0.85	0.82	0.37	0.99

Key: O_2: water oxygen concentration; O_2%: water oxygen saturation percentage; T: water temperature; Sal: water salinity; SWC%: sediment water content percentage; OM%: sediment organic matter percentage; TC%: sediment total carbon percentage; TN: sediment total nitrogen percentage; TH%: sediment total hydrogen percentage; n.d.: not detectable; *: significant differences ($p<0.05$). Capital letter and lower case letters: significant differences ($p<0.05$) between stations.

9.3.2 Biotic parameters

A total number of 487 individuals belonging to 18 different *taxa* was found for "*La Rosa S.r.l.*" and these data were similar for "*Il Padule*" where the total number of individuals was 467 and the number of *taxa* was 17. Concerning the sampled fauna composition, it appeared not homogenous among the stations. The cluster analysis and the applied SIMPROF test (Figure 9.2) showed that in "*La Rosa S.r.l.*" R3 clustered separately from R1 and R2 (Figure 9.2A), while in "*Il Padule*" the heterogeneity seemed higher and each station clustered separately (Figure 9.2B). For both the fish farms, no significant differences were recorded in number of *taxa* (S) and individuals (N) among the stations (Table 9.2). The Shannon index calculation showed its lowest values in P3 for "*Il Padule*" and in R3 for "*La Rosa S.r.l.*" and this latter, with a mean value of 1.20±0.30, resulted significantly different from all the other stations (Table 9.2). Even if biodiversity seemed lower outside the farms than inside them, R3 and P3 showed a benthic community more equally balanced in terms of *taxa* and specimens, and this fact was pointed out by the values of Simpson index (D) calculated for these sites that were lower than the ones found in the other stations (Table 9.2). For "*La Rosa S.r.l.*" the mean D value calculated for R3 (0.63±0.10) was significantly different from all the other stations (0.79±0.04 for R1 and 0.80±0.03 for R2) while for "*Il Padule*", P3 resulted significantly different from P1 with mean D values of 0.39±0.05 and 0.79±0.05 respectively. Concerning Pielou index calculation (J') the lowest mean values were found outside the fish farms, but any significant difference was not noticed among the stations (Table 9.2). The AMBI calculation underline the different situation of the two fish farms. For "*La Rosa S.r.l.*" the highest mean AMBI value was calculated for R3 (4.31±0.45) while R1 and R2 showed lower values (3.66±0.82 and 3.39±0.96 respectively) indicating that the "pressure" on the benthic community seemed higher outside the fish farm than inside it. For "*Il Padule*" the situation is different and the AMBI calculation showed significant differences between P1 and P2, with mean values of 2.08±0.74 and 3.32±0.25 respectively. Due to its higher mean AMBI value, P2 represented the site in which the benthic community health *status* seemed to suffer more (Table 9.2). The BENTIX calculation is reported in Table 9.2. For "*La Rosa S.r.l.*" this index showed similar results if compared to the AMBI, with the lowest mean value found for the R3 (2.14±0.19) and the highest one calculated for R2 (2.69±0.58). For "*Il Padule*", BENTIX showed a clear gradient of mean values underlining the better *status* of P3 (2.38±0.38) respect P1 (2.15±0.20) and P2 (2.17±0.09). Even if with some light differences, the trends of AMBI and BENTIX seemed quite similar (Figure 9.3), and these two calculations led to similar results.

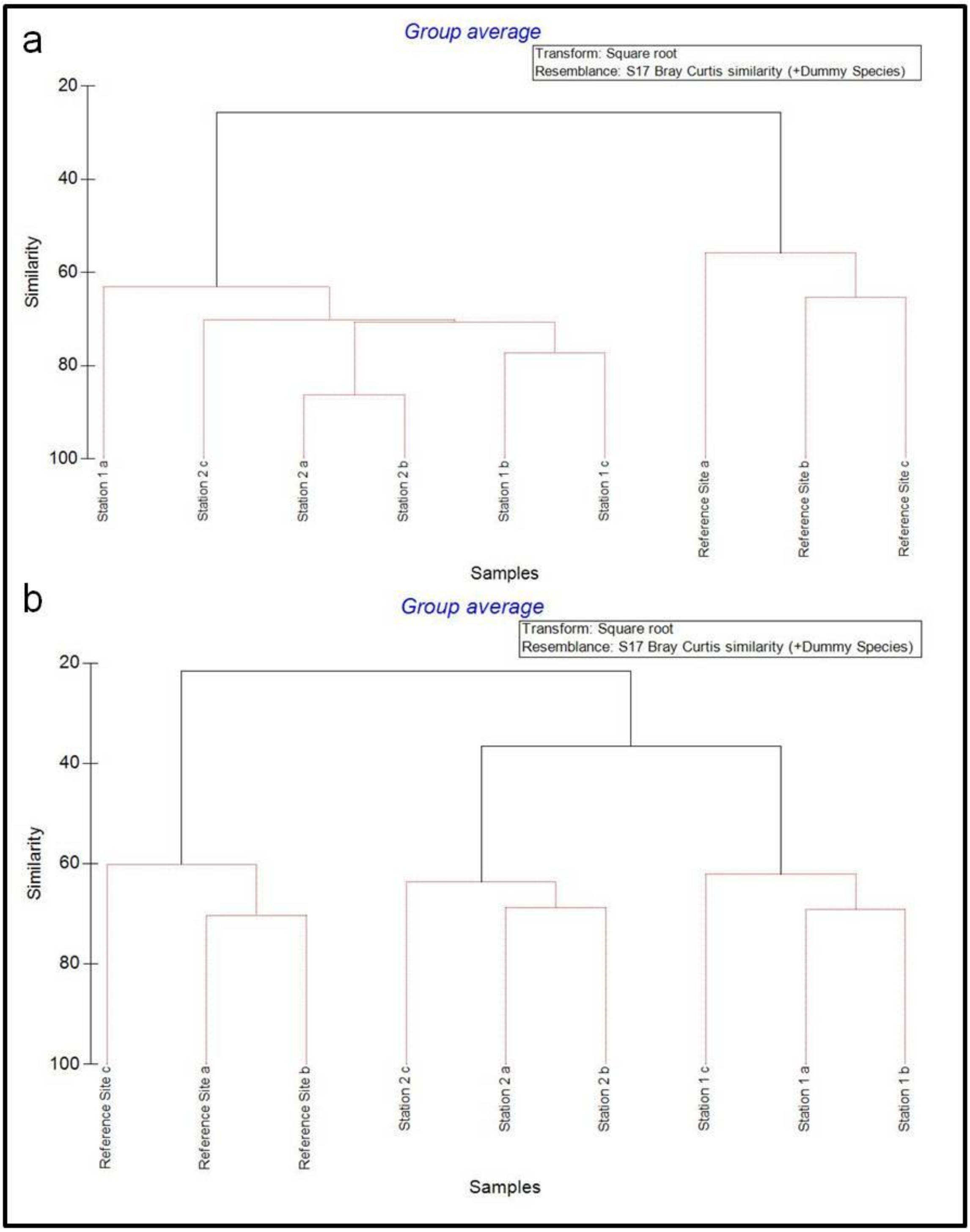

Figure 9. 2 . Cluster analysis performed on the fauna samples collected for "La Rosa S.r.l." (a) and for "Il Padule" (b). The Bray Curtis Similarity and the SIMPROF test were applied.

Table 9.2 Mean values and standard deviation (s.d.) calculated for each station for number of taxa (S), number of individuals (N), Shannon index (H'), Simpson index (D), Pielou index (J'), Azti's Marine Biotic Index (AMBI) and BENTIX. *: significant differences (p<0.05); capital letters: significant differences between the stations (p<0.05).

	Stations		S	N	H'	D	J'	AMBI	BENTIX
La Rosa S.r.l	R1	mean	7.33	42.33	1.65	0.79	0.83	3.66	2.36
		s.d.	1.15	6.66	0.13	0.04	0.08	0.82	0.23
	R2	mean	7.00	59.67	1.67	0.80	0.86	3.39	2.69
		s.d.	0.00	5.51	0.09	0.03	0.05	0.96	0.58
	R3	mean	6.33	60.33	1.20*	0.63*	0.72	4.31	2.14
		s.d.	3.06	58.16	0.30	0.10	0.18	0.45	0.19
Il Padule	P1	mean	8.33	58.00	1.70*	0.79^{A}	0.81	2.08^{C}	2.15
		s.d.	2.52	6.24	0.25	0.05	0.01	0.74	0.20
	P2	mean	4.00	48.00	0.91	0.52	0.66	3.32^{D}	2.17
		s.d.	0.00	38.97	0.33	0.23	0.24	0.25	0.09
	P3	mean	4.67	49.67	0.76	0.39^{B}	0.53	2.96	2.38
		s.d.	2.08	12.74	0.14	0.05	0.10	0.06	0.38

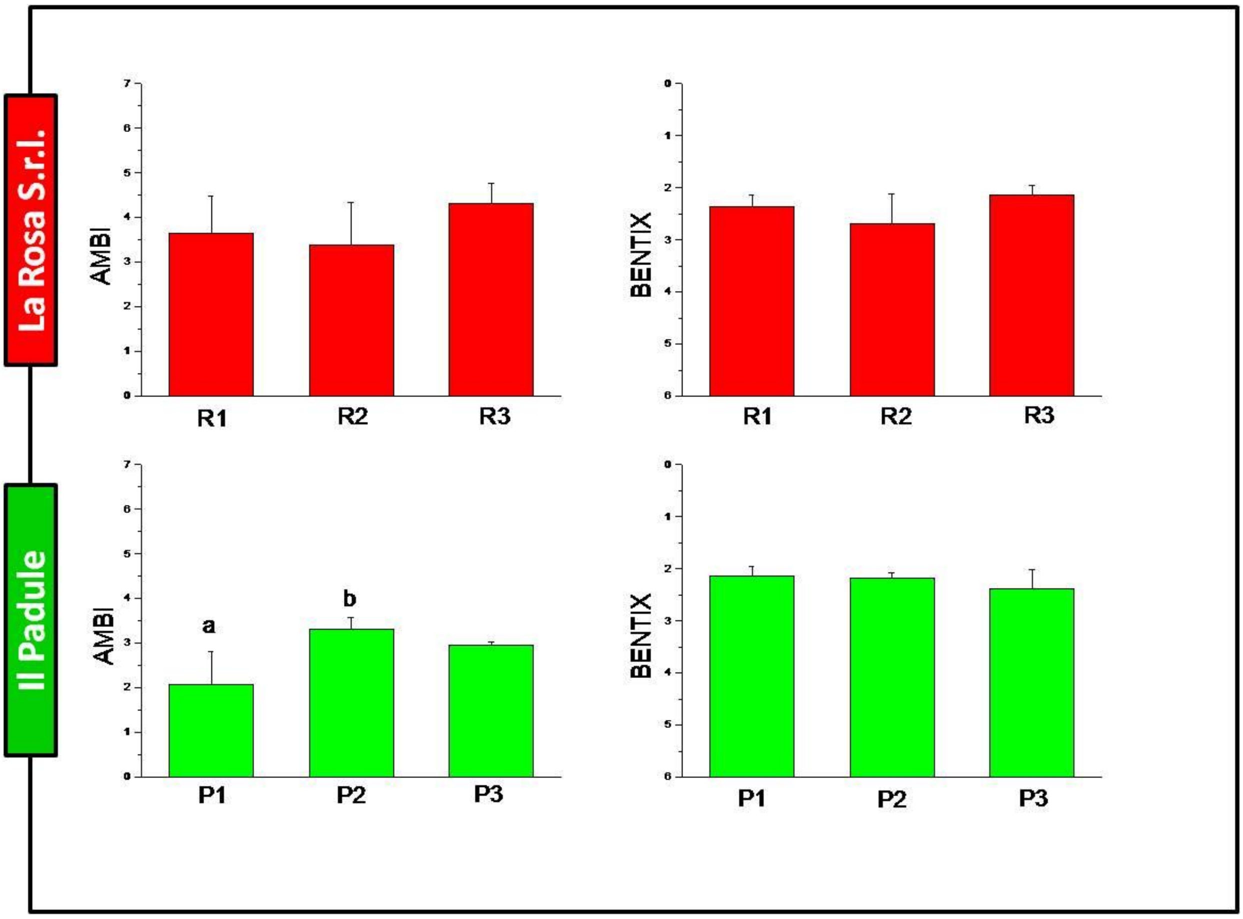

Figure 9. 3 AMBI and BENTIX trends found for "*La Rosa S.r.l.*" and for "*Il Padule*".

9.4 Discussion

Many studies have investigated the role of macrobenthic communities as descriptors of marine soft bottom health *status* analyzing changes in their community structure related to man-induced perturbation phenomena (Johannessen *et al.*, 1994; Karakassis *et al.*, 1999; Edgar *et al.*, 2005; Aguado-Gimènez *et al.*, 2007). The use of indices, such AMBI and BENTIX, to investigate these "changes" in Italian marine coastal areas already led to successful results but their application in transitional ecosystems was proved more complicated (Simonini *et al.*, 2009; Munari and Mistri, 2010). Thus, CTEs are naturally organic enriched environments and in these systems featured by low diversity and richness it is extremely difficult to distinguish between natural and anthropogenic stresses (Munari and Mistri, 2008). This concept is well known in literature and summarized in the concept of the "paradox of transitional water". This definition, firstly used by Dauvin (2007) was more widely explored by Elliott and Quintino (2007) which defined the paradox as follows: ”*the dominant faunal and floral community is adapted to and reflects the high spatial and temporal variability of highly naturally-stressed areas. However, this community has features very similar to those found in anthropogenically-stressed areas thus making it difficult to detect anthropogenically-induced stress. Furthermore, as transitional areas are organically rich the biota thus is similar to anthropogenically-organic rich areas. Becouse of this, there is a danger that any indices based on these features and used to plan environmental improvement will be flawed*”. The present study confirmed this paradox and for both the fish farms, the comparison among the stations sampled inside the farms and the ones locates outside, showed interesting results. Reference sites (R3 and P3) were characterized by lower values of diversity indices underlining a decrement of biodiversity (Table 2). For “*La Rosa S.r.l.*” this decrement was reflected in a decrement of the “quality” of the station, with calculated AMBI and BENTIX values respectively higher and lower respect all the other stations (Table 2). Thus, from the obtained results, seemed that “La Rosa S.r.l.” acted as “depurator” of the Orbetello lagoon. The situation was different for “*Il Padule*” where the decrement of biodiversity was not associated with worse values of AMBI and BENTIX (Table 9.2). The chemical and physical analyses performed underlined the peculiarity of R3 and P3 as well, and these stations showed higher percentages of organic matter (OM%), total carbon (TC%), total nitrogen (TN%) and total hydrogen (TH%). Moreover, these reference sites were also characterized by different sediment grain size composition that appeared more fine in R3 and more coarse in P3 (Table 9.1).

The oxygen supply derived by aquaculture activities could be an important factor influencing benthic communities and it could explain the differences found among the stations. In CTEs water oxygen level is subjected to fluctuation and the nutrient enrichment concomitantly with the extreme climatic conditions could lead to occasion and sometimes dramatic anoxia crises (Leonardi *et al.*, 2009). Even if in our samplings no significant differences were found among the stations concerning dissolved oxygen concentration and percentage of saturation, we could infer that the oxygenation practices operating by the fish farms limited oxygen fluctuations and avoid anoxia crises in the stations located inside the fish farms, while for the external sites conditions could be more critical. In order to confirm this hypothesis these parameters should be monitored for a period longer than the one in which these samplings have been performed but some evidences come from literature. Thus, several authors studied oxygen dynamics in these areas and in particular, for the Orbetello lagoon important oxygen fluctuations are well documented (Martelli and Nocciolini, 2006; Giusti and Marsili-Libelli, 2009).

The comparison between AMBI and BENTIX calculation revealed that the application of these two indices led to similar results for “La Rosa S.r.l.”, while for “Il Padule” their trends appeared slightly different (Figure 4). The percentage of *taxa* that were not attributed to an E.G. was very low for both AMBI and BENTIX methods. AMBI appeared to be more sensitive than BENTIX individuating significant difference among the stations (Table 2). Discrepancies in the results derived by the AMBI and BENTIX calculation could be due to the small number of individuals and *taxa* found in the sampled stations. Unlike AMBI, the BENTIX calculation is dependent by the sample size (Simboura and Zenetos, 2002), and this could represent a problem for the application of this index in CTEs. Whatever the case, the application of this kind of index is recommended as a part of a set of measures in order to minimize misclassification problems (Borja and Muxica, 2004) in the contest of the European Framework Directive (FWD), and their exact role will depend on the objectives of the study. These biotic index may be very useful for the FWD implementation, since the provided information is easy to learn and interpret, but as underlined by Aguado-Giménez *et al.* (2007) their application must be integrated with the multivariate analysis of physicochemical and macrobenthic parameters that represent a more accurate and statistically validated technique. Taking into account that the taxonomic classification effort needed for macrobenthic fauna is the same for calculating biotic indices as for multivariate analysis, possible sources of error could be reduced by using the latter because it is not necessary to group the specimen into tolerance groups, which is not always possible or accurate according to the information available on their autoecological features and even less based on experimental evidence (Gray, 1979; Majeed, 1987; Ponti and Abbiati, 2004). However, the application of the multivariate analysis alone could lead to partial understanding of the complex benthic ecosystem response to stress deriving from aquaculture activities. This resulted evident also in the present study and for this reason, the integration between these different approaches in order to get more complete information, is needed, especially for those complex environments represented by the CTEs,.

9.5 Conclusions

The present study showed that AMBI and BENTIX could be useful tools in detecting benthic impacts from fish farm activities in coastal transitional ecosystems areas (CTEs). Both the indices lead to similar results and these were confirmed by the chemical and physical analysis performed and by the multivariate approach. The independence of AMBI from sample size and consequently the higher sensitivity found comparing this index with BENTIX, seems to suggest that AMBI calculation could be preferred for the CTEs.

EPILOGUE

10. Epilogue: general considerations

Approaching the study of the impacts of aquaculture activities on the marine benthic ecosystems the scientist could set out on two different paths: focusing the attention on each single piece of the complex “puzzle” represented by this ecosystem or looking for a tool able to synthetize this complessity. The first approach, probably will lead the scientist to a more precise measurement of the parameters that characterize the studied environment, but the high number of analyses and therefore the high economic costs often represent an impediment to this kind of studies. Moreover, other problems will raise when the scientist will have the necessity to put together the obtained results and trying to derive from them general conclusions. From this point of view, the synthesis capability of biotic indices could represent an important argument that shall dispose the scientist to consider the use of these tools. If syshtesis is the strength point of a biotic index, this characteristic sometimes could mean weak capacity of discrimination between causes and effects and could lead the scientist to partial comprehension of the complex dynamics of benthic ecosystem. For these reasons, this study tried to combine the use of traditional abiotic and biotic measurements and calculations with the application of biotic indices as instrument of synthesis. By assessing the balance between a range of indices and their relative magnitudes, it could possible to ascertain the “biodiversity quality” of a site. Using numerical values to represent the pattern of biodiversity quality, it becomes possible to compare sites statistically over time or spatially (Feest *et al*., 2010). Thus, in comparison it is possible to prioritise sites, for an individual statistic (e.g. species richness or biomass), or for biodiversity quality, based on a suite of statistics (Feest 2009). This latter approach is the one adopted in this study, and it shares the base idea stated by Gaston and Spicer and reported by Feest *et al*. (2010): biodiversity cannot be encapsulated by a single number. A range of indices representing the various qualities of the biodiversity being studied is much more informative and open to interpretation (Feest et al., 2010).

In each case study, this combination of indices and analyses led to similar results, validating the indices application. Thus, from an operative point of view, this study seems to suggest that the use of biotic indices to assess impacts deriving from aquaculture activities could represent the first approach to the problem. If these calculations will results in the classification of the study area in the higher class provided by the index, the scientist could assume that this information is correct. On the contrary, if the area will be classified in a lower class, the scientist could investigate single pieces of the "puzzle", focusing the attention on each parameters.

However, the choice of the most suitable biotic index to use remain a crucial task. If each biotic index work very well in a limited geographical area, its application in a wider range of different scenarios still present several problems. In the present study, the application of AMBI in different Mediterranean areas underlined the importance of the software species database. Working on the enlargement of this database is of primary importance in order to expand the applicability of this index. At the beginning of this study, the AMBI database counted hundreds of species, but prevalently belonging to European Atlantic regions. This was due to the fact that AMBI was developed by Borja and collegues affiliated to the AZTI-Tecnalia Marine Research Division, operating in the Basque Country and so, since the last years, this index was tested and successfully applied to detect impacts along Atlantic coasts. So, to apply AMBI in Sardinia, Cyprus and Tuscany the costant development of the software database was necessary. At the end, 123 Mediterranean species were added to the database (see APPENDIX) and this allowed a more precise calculation. In some cases (e.g. Sardinia) without the assignation of new species to the AMBI Ecological Groups, the application of this index could not be possible. However, due to the lack of information present in literature, for 35 Mediterranean species found in the samplings, was not possible to assign any ecological group and at the end, these species remained not assigned (see APPENDIX). In the present study I tryed to validate the obtained AMBI results comparing them both with others abiotic and biotic analyses and with the BENTIX. The choice of this latter, as term of paragon for AMBI was principally due to three different reasons:

(i) BENTIX, was and still remain, the most widely used biotic index for the Mediterranean region, especially for Eastern areas (e.g. Aegean Sea);

(ii) even if AMBI and BENTIX derived both from a common theoretic base, they differ in structure and this could lead to differences in their sensitivity and discriminant capability;

(iii) in the scientific community, the comparison between these indices constituted a debated argument in these last years. Several authors tried to applied AMBI and BENTIX to detect impacts in different scenarios, exalting from time to time the higher sensitivity of one of them. This debate, fighted with papers strokes, create two main factions: the first one, referred to Borja, that supports AMBI, the second one, headed by Simboura, that supports BENTIX.

From this point of view, the present study wanted to be a contribute to this discussion and without the limit of belonging to one or to the other faction I tried to put in evidence differences between these two calculations. For both Cyprus and Tuscany cases study, the both the indices showed similar results, putting in evidence their suitability to detect impacts deriving from aquaculture activities. The upgrade of AMBI done with the present work led to the development of an index that, in the investigated cases study, revealed higher sensitivity than BENTIX.

However, a further consideration is needed. If on the one hand this study underlined the suitability of AMBI to detect impacts deriving from aquaculture activities, on the other hand some problems were also underlined. Problems principally concern the Ecological Quality *Status* (EcoQ) assessment of the studied areas, and particularly, the classification of the stations in the categories provided by the index. Taking into account the AMBI site disturbance classification and the relative boundary values (see chapter 4.2, Table 4.1), the classification of the stations sampled in this study is reported in the Table 10.1.

Table 10. 1 Mean AMBI values and relative site disturbance classification of the stations sampled in Sardinia, Cyprus and Tuscany.

	Stations	Mean AMBI value	Site disturbance classification
Sardinia	I1	1.64	Slightly disturbed
	I2	3.19	Slightly disturbed
	I3	3.40	Moderately disturbed
	I4	2.23	Slightly disturbed
	O1	1.36	Slightly disturbed
	O2	1.47	Slightly disturbed
	O3	1.61	Slightly disturbed
	O4	0.84	Undisturbed
	T0	3.82	Moderately disturbed
	T1	3.85	Moderately disturbed
	T2	2.83	Slightly disturbed
	T3	0.94	Undisturbed
Cyprus	K1	3.05	Slightly disturbed
	K2	3.00	Slightly disturbed
	K3	2.29	Slightly disturbed
	B1	2.50	Slightly disturbed
	B2	1.84	Slightly disturbed
	B3	2.47	Slightly disturbed
Tuscany	R1	3.66	Moderately disturbed
	R2	3.39	Moderately disturbed
	R3	4.31	Moderately disturbed
	P1	2.08	Slightly disturbed
	P2	3.32	Moderately disturbed
	P3	2.96	Slightly disturbed

A total number of 24 stations were sampled across the Mediterranean area, and according to the AMBI disturbance classification, 2 stations appeared "*Undisturbed*", 15 "*Slightly disturbed*" and 7 "*Moderately disturbed*". Moreover, it is significative to notice that 4 of the 7 stations classified as "*Moderately disturbed*" were located in Tuscany CTEs, where environmental conditions are particularly complex (see chapter 9.4). Thus, it appears evident the tendency of AMBI to classify sites in the lower categories of the disturbance scale, even if, from the chemical, physical and biological data obtained, the pressure on the benthic ecosystem appeared clear. The problem of an EcoQ overestimation was previously reported for AMBI by several authors (Simboura *et al*., 2005; Simboura and Reizopoulou, 2007, 2008).

More than the structure of the index, this overestimation derives from the boundaries values of the disturbance classification categories, and their ranges are probably too much wide for aquaculture. From a general point of view, these wide ranges adopted by AMBI are principally due to two main reasons. The first one is the necessity to simplify the final output of the index: few categories, higher clearness. The second reason is linked to the purpose of developing an index that could be used to assess impacts deriving from various natural and anthropic sources. Thus, among the human activities that could produce impacts on the benthic ecosystems, aquaculture is not the most damaging, and the application of AMBI to detect impacts deriving from more polluting activities (e.g. industrial discharges) did not showed problems of EcoQ overestimation (Costa-Dias *et al*., 2010). So, even if the effects of aquaculture on the benthic ecosystem are consistent there is a real risk to underestimate them if the disturbance classification will be considered as the final result of the analysis. For this reason in the present study this classification was not considered, and the attention was focused only on AMBI values. The only way to solve this problem could be the development of specific disturbance classification for aquaculture, reducing the range of the values of each quality category.

However, even if some aspect could be more improved, the upgraded AMBI resulted an excellent tool to apply in an aquaculture context. In fact, this study showed the high flexibility and resolution capability of this index in different scenarios. Even in Coastal Transitional Ecosystems (CTEs) context, where biotic indices calculations frequently fail, the AMBI showed good discriminant power (see chapter 9). Martinez-Crego *et al*. (2010) stated a list of six crucial aspects that often represent weak points for biotic indices; these aspects are:

1. Relevance to ecological integrity
2. Broad scale applicability
3. Early detection capacity
4. Feasibility of implementation
5. Definition of reference conditions

6. Link with causative stressors

At the beginning of this study, the first attempt to apply the old version of AMBI in the Mediterranean area underlined several weak points of this index, principally related to the broad scale applicability, the feasibility of implementation and the definition of reference conditions. On the contrary, the AMBI upgrade allowed to improve these aspects perfecting the previous index. In particular, the enlargement of the species database increased the feasibility of implementation of the index. The implementation of an higher number of species means higher early detection capability and higher broad scale applicability. The application of M-AMBI required the correct definition of reference conditions and this calculation increases the AMBI relevance to ecological integrity. Concerning the last aspect underlined by Martinez-Crego *et al.* (2010), the link with causative stressors, it remains a complex argument. In fact, if on the one hand there are numerous factors shaping benthic community (see chapter 3), on the other hand the impact deriving from aquaculture activities principally regards the organic enrichment of sediments and this often represent the main causative stressor. With regard to the organic enrichment, this study underlined the circumscribed extension of the aquaculture footprint. Thus, the area impacted by aquaculture activities appeared principally limited up to 200 m from the farms. Moreover, each case study showed the strong relationship between the sedimentation process and the physical characteristics of the area. In particular, currents, with their speed and direction, shape aquaculture footprint.

In conlusion, even if many issues still remain unsolved and rivers of ink will continue to flow about marine biotic indices and their application for the assessment of benthic ecological quality *status*, this work will remain at least a significative structural contribution to the development of AMBI. Moreover, the obtained results and the reference conditions used for M-AMBI application could be considered as guide lines for further studies in Mediterranean regions. The AMBI is ready, now it is the time to go on with its application.

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APPENDIX

List of *taxa* sampled in Sardinia. For each *taxa* the respective AMBI Ecological Group assigned for the AMBI calculation is reported. The new assigned species are underlined in yellow.

Taxa	**AMBI Ecological Group (E.G.)**
Abra alba	3
Abra prismatica	3
Achelia echinata	1
Acmira catherinae	2
Acmira cerrutii	2
Ampelisca diadema	2
Ampelisca gibba	1
Ampelisca ledoyeri	1
Ampelisca multispinosa	1
Ampharete acutifrons	2
Amphilochus picadurus	2
Amphilochus planierensis	2
Amphilocoides sp.	2
Anapagurus laevis	3
Anchialina agilis	2
Aphelochaeta marioni	4
Apherusa ruffoi	1
Apseudes latreillii	3
Aricidea capensis bansei	1
Armandia cirrhosa	1
Ascidacea sp.	3
Astrea rugosa	1
Atylus massiliensis	1
Axinulus croulinensis	1
Axonolaimus sp.	not assigned
Bathyporeia phaiophthalma	1
Bittum reticulatum	1
Bodotria scorpioides	2
Branchiomma lucullana	1
Branchiostoma lanceolatum	1
Calyptraea chinensis	1
Capitella capitata	5

Capitellida sp.	5
Capitomastus minimus	5
Caprella equilibra	2
Caprella grandimana	2
Cardioidea sp.	not assigned
Caulleriella bioculata	4
Caulleriella caputesocis	4
Cerithioidea sp.	2
Cheirocratus assimilis	1
Chiton sp.	2
Chone duneri	2
Chone filicaudata	2
Cirratulus cirratus	4
Corbula gibba	4
Corophium sp.	3
Coxicerberus remaneri	2
Cressa mediterranea	not assigned
Cumella limicola	2
Daptonema setosum	3
Diplodonta sp.	2
Donacidae sp.	1
Dorvillea rudolphii	4
Dosinia lupinus	1
Echinocyamus pusillus	1
Enoploides sp.	2
Enoplus meridionalis	2
Epacanthion durapelle	not assigned
Epsilonema cygnoides	2
Ericthonius sp.	1
Erinaceusyllis cryptica	not assigned
Eteone picta	3
Euchone rubrocincta	2
Euchromadora sp.	3
Eunice vittata	2
Eupolymnia nebulosa	3
Eurystomina sp.	not assigned
Eurysyllis tubercolata	2
Exogone dispar	2
Exogone gemmifera	2
Exogone naidina	2
Exogone rostrata	2
Exogone sp.	2
Exogone verugera	2

Fustiaria rubescens	1
Gammaridea	1
Gammarus aequicauda	1
Glycera alba	2
Glycera lapidum	2
Glycera sp.	2
Golfingia sp.	1
Goniada eremita	2
Gouldia minima	1
Gyptis mediterranea	2
Halichoanolaimus sp.	not assigned
Harmothoë sp.	2
Hesionuria elongata	2
Hesiospina aurantiaca	2
Hippomedon massiliensis	1
Idotea balthica	2
Idunella nana	2
Keferstenia cirrata	2
Kellia sp.	1
Lepidepecreum longicornis	not assigned
Leptochelia dubia	3
Leucon mediterraneus	2
Leucothoe incisa	1
Leucothoe oboa	1
Leucothoe spinicarpa	1
Levicardium crassum	1
Lichenopora radiata	not assigned
Limatula subauricolata	1
Linhomoeus sp.	not assigned
Lucinella divaricata	1
Lumbrinereis gracilis	2
Lumbrineridae	2
Lumbrineriopsis paradoxa	2
Lumbrineris latreilli	2
Lunatia guillemini	2
Lysianassa longicornis	1
Macroclymene santanderensis	1
Magelona filiformis	1
Magelona johnstoni	1
Malacoceros fuliginosus	5
Mediomastus capensis	4
Metaphoxus gruneri	1
Minuspio cirrifera	4

Minuspio multibranchiata	4
Monticellina dorsobranchialis	4
Muricidae	not assigned
Musculus marmoratus	1
Mysella bidentata	3
Mysidiacea	2
Natatolana borealis	not assigned
Nematoda	3
Nematonereis unicornis	2
Nephtys hystricis	2
Nepthydae	2
Nereis rava	3
Notomastus latericeus	3
Nuculana pella	1
Oncholaimidae	not assigned
Onuphis sp.	2
Ophiodromus pallidus	2
Ophiura albida	2
Orchomene similis	2
Orchomene sp.	2
Paradoneis ilvana	3
Paramysis helleri	2
Parapionosyllis brevicirra	2
Parapionosyllis elegans	2
Parapionosyllis labronica	2
Parapionosyllis minuta	2
Parvicardium ovale	1
Pectinariidae	4
Pectinoidea	4
Perioculodes aequimanus	2
Perioculodes longimanus longimanus	2
Pettiboneia urciensis	2
Pharidae	1
Phascolosoma granulatum	2
Phascolosoma sp.	1
Pholoë minuta	2
Photis longipes	1
Phtisica marina	1
Phyllodoce sp.	2
Pionosyllis sp.	2
Pisione remota	1
Pista cretacea	1
Plagiocardium papillosum	1

Pododesmus squamula	1
Polinices nitida	2
Polydora flava	4
Pontocrates arenarius	2
Pontonema parapapillifera	3
Prionospio fallax	4
Prionospio sp.	4
Prosphaerosyllis brevicirra	2
Prosphaerosyllis adele	2
Prosphaerosyllis xarifae	2
Protodorvillea kefersteini	2
Protopecten glaber	1
Psammobia costulata	1
Pseudoleiocapitella fauveli	5
Pseudomystides limbata	2
Pseudopotamilla reniformis	2
Pyramidellidae	1
Rhabdodemania mediterranea	not assigned
Ringicula auriculata	1
Rissoa sp.	1
Rissostomia lineolata	1
Scoletoma tetraura	2
Selachinematidae	not assigned
Serpulidae	1
Setosabatieria hilarula	4
Sphaerodorum flavum	2
Sphaeroma serratum	3
Sphaerosyllis hystrix	2
Sphaerosyllis prolifera	2
Sphaerosyllis taylori	2
Sphaerosyllis thomasi	2
Spisula subtruncata	1
Streptosyllis websteri	2
Syllides convolutus	2
Syllides edentatus	2
Syllis garciai	2
Syllis gerlachi	2
Syllis prolifera	2
Syllis variegata	2
Symplocostoma sp.	not assigned
Synchelidium haplocheles	1
Tellina distorta	1
Tellina donacina	1

Tellina pulchella	1
Tellina pygmaea	1
Tellinidae	1
Tellinoidea	1
Terebellidae	2
Tritaeta gibbosa	not assigned
Trochidae	not assigned
Trypanosyllis coeliaca	1
Tryphosella minima	1
Turritella biplicata	1
Turritella sp.	1
Veneroidea	1
Venus ovata	1
Viscosia sp.	3
Xenosyllis scabra	2

List of *taxa* sampled in Cyprus. For each *taxa* the respective AMBI Ecological Group assigned for the AMBI calculation is reported. The new assigned species are underlined in yellow.

Taxa	**AMBI Ecological Group (E.G.)**
Abra sp.	3
Alpheidae	2
Ampharetidae	2
Amphictene auricoma	1
Amphipholis squamata	not assigned
Amphitrite sp.	1
Amphitrite cirrata	1
Amphitrite Edwardsii	not assigned
Amphitrite johnstoni	1
Amphiura chiajei	2
Amphiura filiformis	2
Anapagurus laevis	3
Anchialina gracilis	2
Aphroditae	1
Apseudes latreilli	3
Arca noae	1
Arenicolidae	1
Aricia latreilli	1
Ariciidae	1
Asychis biceps	2
Athanas nitescens	1
Automate branchialis	not assigned
Callianassa tyrrhena	3
Capitella capitata	5
Capitellidae	5
Carditaoidea	3
Cheatozone corona	4
Cirratulidae	4
Cirratulus cirratus	4
Cirratulus filiformis	4
Clymene lumbricoides	1
Clymene Oerstendii	1
Corbula gibba	4
Cylichna cylindracea	2
Drilonereis filum	2
Eone nordmanni	not assigned
Eteone foliosa	3
Eteone picta	3

Ethusa mascarone	not assigned
Euchone rosea	2
Eunice Oerstedii	2
Eunice pennata	2
Eunice vittata	2
Eunicidae	2
Exogone gemmifera	2
Exogone sp.	2
Exogone verugera	2
Fauvelia martinensis	not assigned
Flabelligeridae	2
Gammaridea	1
Glycera convoluta	2
Glycera emerita	not assigned
Glycera sp.	2
Glyceridae	2
Goniada eremita	2
Gouldia minima	1
Haploscoloplos robustus	not assigned
Hermodice carunculata	2
Hesionidae	2
Heterocirrus sp.	4
Heteromastus filiformis	4
Hyale schmidti	1
Hyalinoecia bilineata	2
Hyalinoecia brementi	2
Jujubinus exasperatus	1
Lanice conchilega	2
Leptomysis burgii	not assigned
Leucothoe spinicarpa	1
Liocarcinus navigator	1
Llia nucleus	not assigned
Lophogaster typicus	1
Loripes lacteus	1
Loripinus fragilis	1
Lumbriconereis gracilis	2
Lumbriconereis impatiens	2
Lumbriconereis latreilli	2
Lumbrinereis fragilis	2
Lumbrinereis sp.	2
Macropipus arcuatus	1
Macropipus depurator	1
Maldanidae	1

Marphysa bellii	2
Marphysa sanguinea	2
Melinna palmata	3
Minuspio cirrifera	4
Muricidae	not assigned
Mysidacea	2
Nematoda	3
Nematonereis unicornis	2
Nereidae	3
Nereis diversicolor	3
Nereis irrorata	3
Nereis sp.	3
Nereis zonata	3
Nicolea venustula	2
Nicolea zostericola	2
Notomastus latericeus	3
Notomastus profundus	3
Notomastus sp.	3
Nucula sulcata	1
Nuculana pella	1
Onuphis eremita	2
Ophelia bicornis	1
Ophelia neglecta	1
Opheliidae	1
Paguridea	2
Paramysis helleri	2
Paraonis fulgens	3
Paraonis paucibranchiata	3
Parvicardium exiguum	1
Pectinaria	1
Penaeidea	not assigned
Phalacrophorus uniformis	not assigned
Pharidae	1
Pherusa plumosa	3
Phyllodoce maculata	2
Phyllodocidae	2
Pisa tetraodon	1
Pisionidae	1
Pista cristata	1
Plagiocardium papillosus	1
Sabellidae	2
Sipunculidae	1
Siriella clausii	not assigned

Sphaeroma serratum	3
Staurocephalus Rudolphii	4
Sternapsis scutata	3
Syllidae	2
Synalpheus gambarelloides	not assigned
Tellina sp.	1
Terebellidae	2
Terebellides stroemi	2
Veneroidea	1

List of *taxa* sampled in Tuscany. For each *taxa* the respective AMBI Ecological Group assigned for the AMBI calculation is reported. The new assigned species are underlined in yellow.

Taxa	**AMBI Ecological Group (E.G.)**
Aricia faetida	1
Bonellia viridis	not assigned
Capitellidae	5
Ceratonereis costae	2
Corophium acherusicum	3
Gammaridea	1
Gibbula sp.	1
Leptochelia savignyi	3
Leucothoe spinicarpa	1
Lysianassa longicornis	1
Magelona Johnstoni	not assigned
Maldanidae	1
Minuspio cirrifera	4
Nematoda	3
Nereis caudata	3
Nereis sp.	3
Nereis succinea	3
Nereis zonata	3
Nerine sp.	not assigned
Notomastus latericius	3
Odostomia acuta	2
Odostomia conoidea	2
Perinereis cultrifera	3
Pista sp.	1
Sphaeroma serratum	3
Talitrus saltator	1
Tanais cavolinii	2