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EVALUATION OF TECHNICAL AND ENVIRONMENTAL PROBLEMS IN OFFSHORE CAGE CULTURE OF THE MEDITERRANEAN GILTHEAD SEA BREAM *SPARUS AURATA* LINNAEUS, 1758

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A Lisa, Marina e Mario

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Chapter 4

Abstract

Aim of this study, was firstly to assess the seabed deposition of a fish farming facility located in the Alghero Bay (Western Mediterranean) by using the MERAMOD® particulate waste dispersion model, and evaluate actual scenario and a forthcoming situation represented by an enlargement of the fish farming area. The impact seabed surfaces forecasted by the model increased from 5.6 ha in the actual scenario up to 7.3 ha in the supposed potential condition.

The second part of the study aimed to describe the settlement and development of biofouling organisms on cage nets at the above–mentioned farming facility. This was done by investigating different developmental phases of the biofouling communities on net panels inside cages containing big and small gilthead sea bream (*Sparus aurata*) specimens. The rapid increase of biofouling on all the experimental panels positioned inside fish cages suggests that caged mariculture activities have the potential to provided an enhanced food supply to epibiotic communities.

Lastly, the objective of the third part study was to improve the knowledge of the ethological traits of different–sized gilthead sea bream specimens reared in floating cages. With this aim, the most common behavioural patterns of this species were observed during different times of the day and in the presence or absence of food. The results acquired show that the behaviour of fish reared offshore in the Alghero Bay is dramatically affected by the feeding rhythms in captivity.

Chapter 1

GENERAL INTRODUCTION ON CAGE CULTURE

1.1 Origin and evolution of fish culture

Fish farming is a very ancient activity. It is believed to have been practiced in China as early as 2000 B.C., and a classical account of the culture of common carp was written by Fan Lei in 475 B.C. (Villaluz, 1953). The Romans built fish ponds during the first century A.D. and later, during the Middle Ages, fish ponds for carp farming were built by religious men throughout Eastern Europe, where this activity was popular in the 12th and 13th centuries (Lovell *et al.*, 1978). In Southeast Asia, fish ponds were believed to have evolved naturally along with salt–making in the coastal areas, where the salt beds were utilized to grow milkfish during the rainy season. Early interest in fish culture in the United States was carried over from England before 1800 and was concentrated on propagation and culture of trout and salmon. By early in the 20th century, several forms of fish culture were fairly well established, such as milkfish farming in Southeast Asia, carp polyculture in China, carp monoculture in Europe, tilapia culture in tropical Africa, culture of indigenous finfish and crustaceans in estuarine impoundments in Asian and Southeast Asian coastal areas, and hatchery rearing of salmonids in North America and Western Europe (Beveridge, 1996). With the exception of salmonid culture, these forms of aquaculture were generally extensive, where the nutrient inputs into the system were limited to fertilizers and crude sources of foods, and yields were low (Lovell, 1998).

Although is the aquatic complement of agriculture, unlike this latter, which has been the most important way of obtaining food on land for several thousand years, aquaculture has until recent times contributed little in real terms to world fish or shellfish production (Bardach *et al.*, 1972). Rather than evolving in the direction of cultivation, hunter–gatherer methods of procuring food from the aquatic environment developed along a different path: by improvements in tracking methods and by increases in killing power.

There are several reasons because agriculture and aquaculture did not develop in the same way. First, why food resources in the seas and lakes were for long time, and until recently, abundant. The development of fisheries technology and increases in catch pressure were enough to meet growing demands and there was therefore little need to learn to farm. Moreover, the aquatic environment was unfavourable and something to be feared. It must have seemed impossible that a structure which could hold fish securely and withstand the forces of the tides and currents, waves and storms, could bee built in the sea. There were other technical problems, too, to overcome. Although the breeding and husbandry of animals and the harvesting and planting of seeds were

comparatively easy to realize on land, it was very difficult to feed several aquatic species, and to hatch the eggs and productively rear the offspring.

These problems in part stemmed from the fact that people working with organisms that were very different from themselves and into an environment about which they were for the most part uninformed. It was not until the rise of the biological sciences in the 19th century that the mysteries surrounding the physiology and reproduction of aquatic organisms and the role that the environment played in controlling these processes began to be solved.

Until recently, world fish demand was fulfilled by the expansion of capture fisheries. Development of this industry was most rapid during the 1960s, yields increasing by an average of 6% per year (Lawson, 1984). During the 1970s and 1980s, however, development was slow and irregular, production peaking at just over 70 million tonnes of fish in the first part of 1990s.

The main cause of the decline in catch fisheries production is the decreasing number of natural stocks that can sustain further increases in exploitation, and the situation has been accentuated by exorbitant increases in fuel oil prices, the development of Economic Exclusion Zones and over–capitalization of many of the world's fishing fleets (Beveridge *et al.*, 1997).

The agreement is that the next years capture fisheries landings might remain stable or decrease, providing that appropriate management of stocks and development of new fisheries can be achieved and that new fish products can be successfully marketed. Extrapolation of trend suggests that by the end of the first quarter of the 20th farmed fish production will have outstripped capture fisheries production and will be the most important means of providing fish for food. Nevertheless, this scenario may be over–simplistic, as it disregards likely shortages in some of the raw materials required for the intensive aquaculture and does not know growing limits on land and water availability (Beveridge *et al.*, 1994, 1997).

During the last fifty years, world aquaculture has grown from a production of less than a million tonnes in the early 1950s to 59.4 million tonnes by 2004 (Fig. 1.1), which represents more than half of the total catch on fisheries and one third of world production coming from the water (FAO, 2007). Of this production, 41.3 million tonnes, or 69.6%, was produced in China and 21.9% from the rest of Asia and the Pacific region (Fig. 1.2). The Western European region contributed for 3.5% with 2.1 million tonnes, while the Central and Eastern Europe region contributed 250,000

tonnes, or 0.4%. Latin America and the Caribbean and North America contributed 2.3% and 1.3%, respectively. Finally, production from the Near East and North Africa region and sub–Saharan Africa accounted for 0.9 and 0.2%, respectively (FAO, 2007).

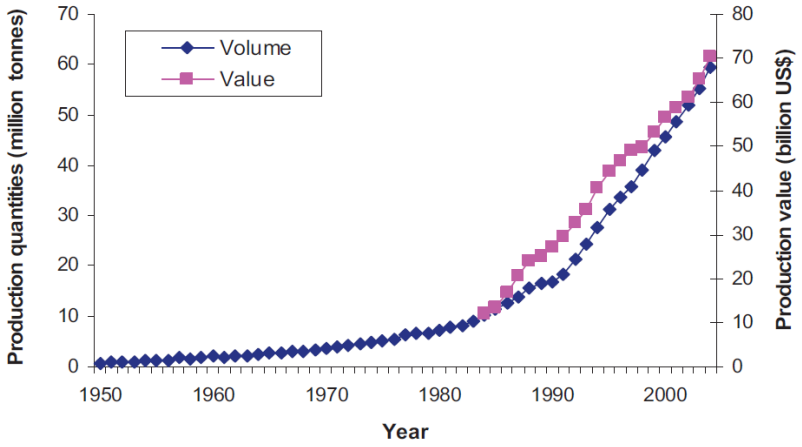

Fig. 1.1. Trend in total world aquaculture production and value (including plants) between 1950 and 2004 (from FAO, 2007).

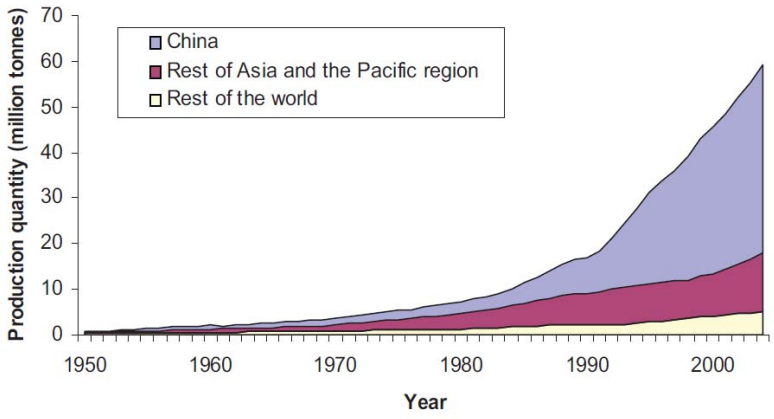

Fig. 1.2. World aquaculture production with China and rest of Asia and the Pacific region disaggregated from the rest of the world between 1950 and 2004 (from FAO, 2007).

Production within each region is diverse. In Asia and the Pacific region aquaculture production from South Asia, China and most of Southeast Asia consists of cyprinids, while that from the rest of East Asia consists of high–value marine fish. In global terms, 99.8% of cultured aquatic plants, 97.5% of cyprinids, 87.4% of penaeids and 93.4% of oysters come from Asia and the Pacific region. Meanwhile, 55.6% of the

world's farmed salmonids come from Western Europe, mainly from the northern region of the continent. Carps, however, dominate in the Central and Eastern Europe region.

In North America, channel catfish is the top aquaculture species in the United States of America, while Atlantic and Pacific salmon dominate in Canada. In the Latin America and Caribbean region, over the last decade salmonids have overtaken shrimp as the top aquaculture species group due to disease outbreaks in major shrimp producing areas and the rapid growth in salmon production in Chile.

The sub–Saharan Africa region continues to be a minor player in aquaculture despite its natural potentials.

High value marine species, such as sea breams, sea basses, turbot, and yellow tail tuna, are being cultured on a large commercial scale in Europe and Japan (Lovell, 1998). In the Mediterranean countries, for example, where the main represented farmed species are sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*), a significant increase in production has occurred in recent years (*i.e.* from 8,000 tonnes in 1991 to 125,000 tonnes at present).

Aquaculture will continue to grow and supply an progressive improvement of fisheries products consumed. This is assured because supply, price and quality of marine fish oscillate significantly because the marine environment is improperly managed and its yields is unpredictable. But when fish are cultured, like corn in a field, supply can be controlled more effectively. With the present technology and research base, yields and risks for a number of aquaculture enterprises are so predictable.

The origin of aquaculture in all probability is in the control of natural mortality through the capture and temporary keeps of organism which, in this period, increased in biomass. The simplest facilities to construct would have been earth ponds. These may have been little more than mud walls constructed to hold water temporarily and fish following the seasonal flooding of a river. Manipulation of growth through feeding with domestic scraps or agricultural wastes would have been a logical subsequently phase. However, control of spawning and recruitment is a relatively recent attainment as it is difficult to induce many species to rear in captivity. There are also many technical problems implicated in the hatching of eggs and the maintenance and feeding of larval and juvenile stages (Bardach *et al.*, 1972). Aquaculture has progressively evolved to obtain control of above mentioned phases.

Great advances have been made in the fields of nutrition, genetics, engineering, physiology and biochemistry, which have all contributed towards improved yields.

However, numerous types of aquaculture have opted for lower yields and significant savings in human resources and cost. For example, the release of juvenile into the wild involves increasing exploitation to the natural resources, but does not improves growth or reduces natural mortality (predation or disease). However, there are significant economic savings in terms of feeding and construction of facilities.

At the same time, interest is once more being turned towards fish culture that reduces expenditure on feeds. Feed costs can account for 60% of production costs or more at a farm (Chong, 1993) but can be clearly reduced if advantage is taken of naturally available foods. For example, in ponds, fertilization with inorganic or, better still, organic fertilizers, will stimulate the growth of organism at the base of the food web, while in lakes and rivers, cages or pens can be stocked with fish that will crop naturally available foods such as algae, zooplankton or suspended organic material (Beveridge, 1996).

In summary, aquaculture, or the farming of aquatic organisms, is achieved through the manipulation of an organism's life cycle and control of the environmental variables that influence it. Three main factors are involved: control of reproduction, control of growth, and elimination of natural mortality agents. Control of reproduction is an essential step, otherwise farmers must rely on naturally spawning stocks. The supply of fry from the wild may be restricted to a particular season and a particular area, and there may also be shortages due to over–exploitation of wild stocks. This step has yet to be realized in the culture of many, particularly marine, species. Growth can be increased through selection of broodstock and through feeding. Because rearing of carnivorous species is dependent upon the provide of high–protein fishmeal diets, there is significant scope for minimizing feed costs if the appropriate omnivorous–detrivorous–planktivorous species and systems are used (Beveridge, 1996).

At the conclusion, rearing systems are fundamental to all different aquaculture form. In fact, they are designed to hold organisms captive because they increase in biomass by reducing the predation losses and disease and by excluding competitors (Reay, 1979). Rearing systems should also facilitate management.

1.2 Rearing facilities

Rearing facilities for fish can either be land–based (*e.g.* ponds, raceways, tanks and silos) or water–based (*e.g.* enclosures, pens and cages). In the English language, the terms enclosure, pen and cage are used as synonymous and may be used

interchangeably. In aquaculture, however, this has given rise to a confusion, because the term “enclosure” is, the fact, used to illustrate something which could be both a cage or a pen while the word “pen” used in North America indicates a big sea cage. Beveridge (1996), for example, used these terms in a more restricted sense: “enclosure” was used to indicate an enclosed natural bay, where the shoreline forms all but one side, which is normally closed off by a solid or mesh barrier. In pen culture, all side of the cage, excluding the bottom, are man made, often being constructed from wooden poles and netting. The bottom of the pen, however, is formed by the sea bed. On the contrary, cages are enclosed on the bottom, and all side including the bottom are man made.

There are other differences among the aforementioned water–based rearing facilities. Pens and enclosures tend to be larger, ranging in size from around 0.1 ha to some which are well over 1,000 ha. Cages, however, typically have a surface area somewhere between 1 and 2,000 m^2. Moreover, because of their small size, cages are better suited to intensive culture methods than pens (Beveridge, 1996).

1.3 History of cage culture

In all probability, cages were first used by fishermen as a suitable holding facility for fish awaiting the sale. The most primitive types of holding cage may have been little more than custom–made fish traps or baskets, and such traditional types of holding facility have been in use in several parts of the world for generations (Beveridge, 1996).

The authentic cage culture, in which fish were held for rearing periods during which increased in weight, was until recently thought to be a comparatively modern development. According to Hu (1994), however, Zhou Mi described fry sales in the ancient Jiujjang River, in a book called Kuixinzhashi, written in 1243 during the Sung Dynasty (A.D. 960–1280).

In the Great Lake region of Cambodia floating cages have been used since the end of the last century (Lafont & Savoeun, 1951; Hickling, 1962; Ling, 1977; Pantulu, 1979). Species like snakeheads (*Channa* spp.), catfishes (*Pangasius* spp., *Clarias* spp.) and marble–headed gobies (*Oxyeleotris marmorata*) were held in wood or bamboo cages, fed on a mix of kitchen scraps and trash fish, and transported by river to the markets of Phnom Penh. Cages were either towed behind the boats or occasionally incorporated into the vessel to form a well–boat (Fig. 1.3). During the present century, this type of cage culture proliferates at the most part of the inferior Mekong delta and into Vietnam (Pantulu, 1979).

The floating bamboo cages have been in use since the early 1920s (Reksalegora, 1979) to rear *Leptobarbus hoeveni* fry captured in the Mungdung Lake (Jambi, Indonesia). A different form of cage culture appeared in Bandung (Indonesia) around 1940. Small bamboo and "bulian" wood cages were anchored to the bottom of organically polluted rivers and canals and stocked with common carp (*Cyprinus carpio*) which fed on wastes and invertebrates carried in the current (Vass & Sachlan, 1957; Costa-Pierce & Effendi, 1988).

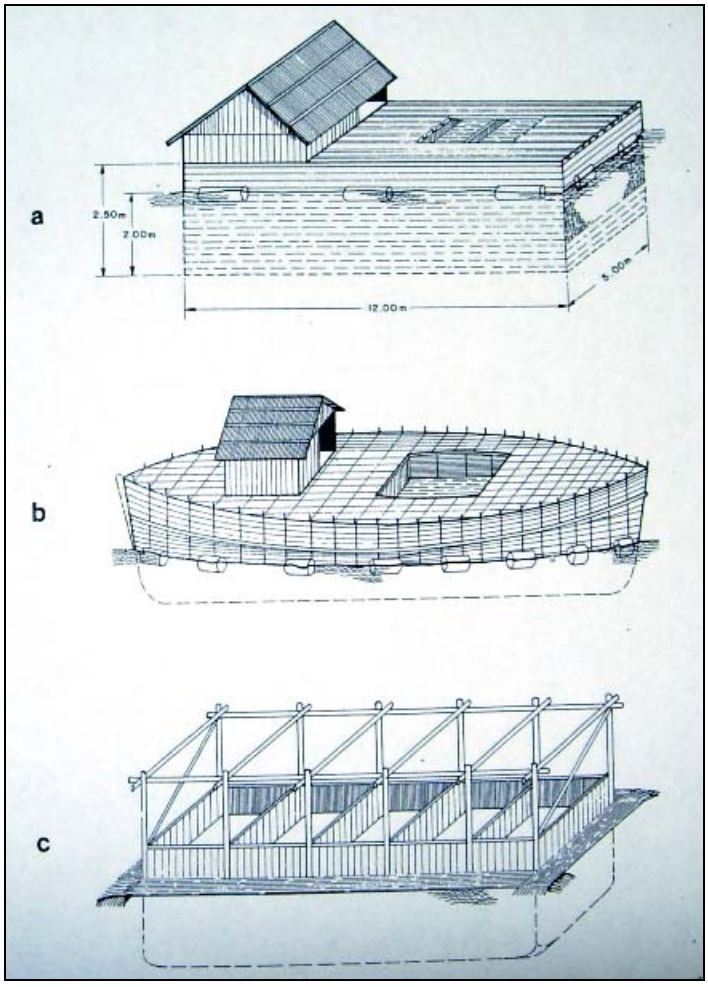

Fig. 1.3. Traditional fish cage designs, Indo–China: a=Southern Vietnam; b=boat–shaped cage from Cambodia; c=battery of small cages, Cambodia (from Pantulu, 1979).

Traditional cage culture, distinguished by its reliance on natural construction materials and natural or waste feeds, is still practised in many parts of Indonesia and Indo–China. However, although moderately successful, these methods of rearing fish had a largely localized influence and did not directly give rise to the current cage fish farming industry. Modern cages utilize synthetic mesh or netting materials and have

collars usually fabricated from synthetic polymers and metals although wood is still widely used in many designs. It is difficult to be exact about the origins of modern cage fish farming although Japan has undoubtedly been an central starting place. According to Milne (1974), Harada, Director of the Fisheries Laboratory at Kinki University, first started experimenting with cage fish culture in 1954, and commercial culture of yellowtail *Seriola quinqueradiata* followed three years later. In Norway, cages were being used to culture Atlantic salmon (*Salmo salar*) in the early 1960s, and in Scotland the White Fish Authority commenced salmon cage rearing trials around 1965. Surprisingly, tilapia (*Oreochromis* spp.) culture in cages is of even more recent origin and owes its beginnings to work carried out at Auburn University in the late 1960s (Schmittou, 1969).

For decades, cages were commonly used to maintain and transport bait fishes for tuna pole and line fishing (Ben Yami, 1978) although today they use has been largely superseded by live–bait holds in boats. In recent times, cages have been developed to hold fish, to check water quality of power station effluent, and to assess putative anthropogenic impacts (Yang, 1982; Little & Muir, 1978; Costa-Pierce & Effendi, 1988; Chang, 1989; Costa-Pierce, 1993; Beveridge & Muir, 1995).

1.4 Evolution of cage culture

Like other aquaculture activities, cage culture may be classified on the basis of feed inputs as:

- extensive (Fig. 1.4);
- semi–intensive (Fig. 1.5);
- intensive (Fig. 1.6).

In extensive culture, fish feed exclusively on available natural foods such as plankton, detritus and various organisms present in the aquatic environment.

Extensive cage culture is limited to fresh waters and may be practised in two types of environment: highly productive lakes and reservoirs and water bodies which receive sewage or domestic wastes. Primary production, which fuels all successive energy transactions in aquatic food webs other than in waste–fed systems and systems with high allochthonous inputs, is dependent upon the availability of essential nutrients (phosphorus and nitrogen compound), and light and temperature (Le Cren & Lowe Mc Connell, 1980; OECD, 1982).

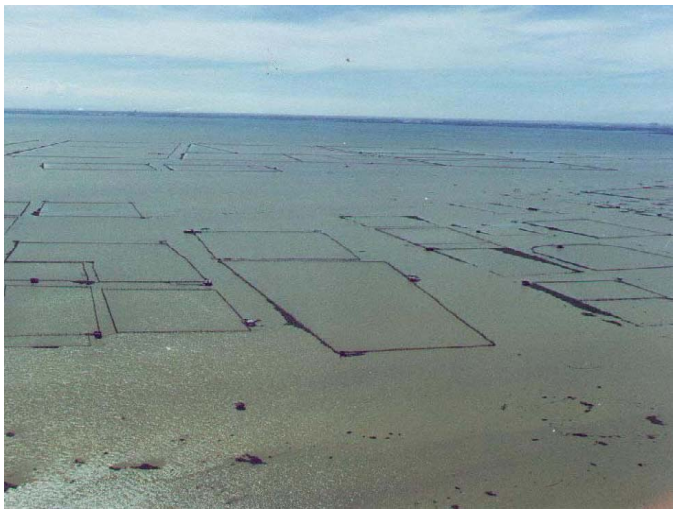

Fig. 1.4. Example of an extensive culture in the Philippines.

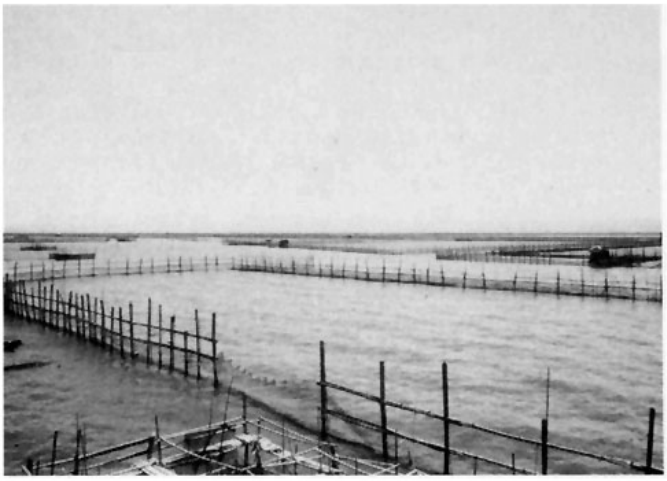

Fig. 1.5. Example of a semi–intensive culture in the Philippines.

Fig. 1.6. Example of an intensive fish culture in the Mediterranean.

Systems with high nutrient loadings are likely to be highly productive. However, productivity is also strongly correlated with latitude (Brylinsky, 1980), and between temperate and tropical zones there is a considerable increase in the range of annual primary production values. Extensive cage culture on any scale is at present only practised in the Philippines and China (Beveridge, 1984; Li, 1994). In Europe and in North America, extensive cage rearing of juvenile planktivorous stages of salmonids, coregonids and pike (*Esox lucius*) is carried out, often using lights to attract zooplankton (Bronisz, 1979; Uryn, 1979; Jager & Kiwus, 1980; Holm & Moller, 1984; Mamcarz & Novak, 1987).

Sewage–fed ponds and streams and rivers subject to high loadings of domestic waste have proved suitable for extensive cage culture (Gaigher & Toerien, 1985; Edwards, 1992), although there is concern about the public acceptability and health risks associated with fish grown in such systems (Buras, 1993). It is possible to depurate such fish (Buras, 1993), however, or incorporate them into diets for other species (Edwards, 1992).

Semi–intensive culture involves the use of low protein (<10%) feedstuffs, usually compounded from locally available products, to supplement the intake of natural food. In tropical fresh waters, semi–intensive rearing of fishes is the most common method of cage culture. Species that feed low in the food chain, such as *Oreochromis niloticus*, *O. mossambicus*, *O. aureus*, and bighead, silver (*Hypophthalmichthys molitrix*) and common carp, are fed on a variety of materials including rice bran, wheat middlings, brewery and domestic wastes (Pantulu, 1979; Dela Cruz, 1980; Coche, 1982; Beveridge & Phillips, 1988; Costa-Pierce & Soemarwoto, 1990; Beveridge & Muir, 1995). Semi–intensive cage fish rearing is also practised to a limited extent in eastern Europe (Muller & Varadi, 1980; Martyshev, 1983). However, apart from some experimental works with herbivorous species such as siganids and mullets (Pitt *et al.*, 1977; Tahil, 1978), semi–intensive cage culture is not practised in marine environments.

In intensive culture operations, fish depend exclusively on an external supply of high protein (>20%) food, usually based on fishmeal. Intensive cage culture is largely confined to the rearing of high value carnivorous species. In fresh water, salmonids and channel catfish (*Ictalurus punctatus*) are reared intensively, while in the marine environment Atlantic salmon, yellowtail, sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) are the principal intensively reared species.

Some intensive rearing of caged tilapias and carps omnivorous fish that typically

have a low market value is practised in parts of the world where they fetch high market prices (*e.g.* North America, Singapore, Taiwan). Limitations in the length of the growing season in these countries also encourage the use of intensive feeds. Trash fish is still the principal type of feed used in yellowtail and grouper culture. Formulated pelleted dry diets have been developed and are commonly available for salmonid, channel catfish, sea bream and sea bass culture.

Nonetheless the installation designs used to rear fish in offshore site can be complex, several cages are simple to assemble and can be constructed in a day. Cages are also easily managed. Observation of stock is facilitated and, unlike pens, fish may be supplied relatively easily using little scoop. Once installed land–based culture systems can be difficult or expensive to change. However, cage farms can be extended simply by adding a few more cages.

Fish grown in cages can succumb to fin and skin damage through abrasion (Moring, 1982), although disfigurations can be kept to a minimum if cages are sited and moored properly, if appropriate rearing densities are adhered to, and if fish are carefully handled (Boydstun & Hopelain, 1977). According to Li (1994), caged carps show less signs of physiological stress than free–swimming fish. Studies confirm that cage–reared fish can be superior to fish reared in other systems and even wild fish, in terms of condition factor, appearance and taste (NORDA, 1984; Li, 1994).

1.5 Selection of fish farm sites

Selection of site in any fish farming operation is of vital importance. It significantly influences cost–effective variability by determining capital expenditure and affecting conduct costs, production and mortality. Cage and pen based aquaculture systems suffer in comparison to land–based operations in that there is fewer room to make a mistake in site selection. Poor pond sites, for example, may be enhanced by using filters and sediment traps to eliminate suspended material.

The are some important guidelines for choice site of cage that must be followed. The first concerns the physicochemical conditions that determine whether a species can prosper in an environment (temperature, salinity, oxygen, currents, pollution, algal blooms, exchange); the second comprises those factors that must be considered in order to place a cage system effectively (weather, shelter, depth, substrate); and the third is the profitability and establishment of a facility (logistics, legal aspects, social and economic considerations).

Cage site must have good water quality. Sites should not only be uncontaminated by toxic industrial pollutants but also should assure the physicochemical conditions requirements of the species to be farmed.

For example, an increase of temperature augments metabolic rate and causes a parallel increase in oxygen utilization. Similarly, there is an increment of the production of both ammonia and carbon dioxide. Salinity is a measure of the dissolved solids present in water and is expressed in parts per thousand (‰) or Practical Salinity Units (PSU). Its importance to aquaculture concerns essentially the control of osmotic pressure, which can significantly affect the ionic balance of aquatic animals.

Moreover, sub–optimal environment conditions also contribute towards stress, leading to increased vulnerability to parasitosis and reduced resistance to disease (Alabaster & Lloyd, 1980; Pickering, 1981; Anderson, 1990; Schreck, 1990). Fast fluctuating temperatures and salinities are frequently more dangerous than seasonal changes, though some species are more tolerant than others (Stickney, 1979; Poxton, 1995).

In marine areas, temperature variations are complicated by salinity which varies between 32 and 40‰ and is affected by evaporation and precipitation. In open sea areas, where the stability of water conditions depends upon tidal turbulence and batimetry (Pingree *et al.*, 1978), stratification typically occurs in deep waters with low current velocities, while in coastal marine areas the freshwater runoff significantly influences the temperature regime.

For the reason that water density depends by salinity and by temperature, the mixing of seawater and freshwater runoff requires energy, and the mixing level depends principally on the volume of freshwater runoff and available mixing energy (wind and tidal).

A further factor that influences the choice of a site is the benthic community structure. In fact, when water column shows a stratification, benthic oxygen demand can cause a dramatic deoxygenation of the hypolimnion. Thus, at the end of stratification, the upwelling of deoxygenated, hypolimnetic water can result in fish kills (Zoran *et al.*, 1994). The high particulate waste flow associated with cage farm may greatly increase benthic oxygen demand at the site, which reduce the dissolved oxygen (DO) concentration below and around cages.

In summary, sites that are durably stratified and where algal blooms can cause cyclically reduced oxygen condition, should be avoided if possible. Marine sites which

have considerable bottom currents and which disperse sedimenting wastes are desirable.

One of the major problems of suspended solids is on rear fish since high levels cause gill damage. Suspended solids have also been implicated in diseases and in reduced fish growth. Reduced growth may partially be caused by the consequence of turbidity on visibility, disturbing food intake (Sigler *et al.*, 1984).

Good water exchange at a site on projected for intensive facility is essential in order to reduce the accumulation of wastes and all the associated problems. In marine areas, this means at sites where there are very good bottom and moderate or strong currents consequently the exchange period is in days rather than weeks (Beveridge, 1996).

The other problems in selecting a fish farm site is fouling, that decreases the specified mesh size of netting while increasing mesh surface area. The reduction in mesh size restricts the flow of water through cages, thus reducing the rate of DO supply and waste metabolite removal. Furthermore, the increased resistance to water flow can cause deformation of the bag and consequential decrease in cage volume, at the same time increasing stresses on the cage structures. The additional weight of fouling can lead to net failure and makes net changing difficult.

In conclusion, fouling is of most importance at marine site with low velocities of current and is significantly influenced by temperature and nutrient status (Beveridge, 1996).

1.6 Environmental impact of aquacultural activities

Aquaculture, like many other human activities, uses and transforms resources into commodities valued by society and, in so doing, produces wastes. So, environmental services are required to assimilate wastes. Impacts stem from these 3 processes: the consumption of resources, the transformation process itself and the production and assimilation of wastes, which not only impose costs on society at large but have implications for the sustainability of the aquaculture project (Beveridge *et al.*, 1994).

With the expansion of aquaculture during the latter part of the 20th century, criticisms began to appear. For example, with respect to salmon culture, the list of objections is everything from visual pollution to excessive noise and odours (Stickney, 1990). Major objections have been associated with degraded environmental quality, disease transmission, use of antibiotics, and interaction of escapees with wild population.

The main environmental problems associated with aquaculture activity are related with wastewater dissolved nutrients like phosphorus (limiting nutrient in continental waters), nitrogen (limiting nutrient in marine waters) and with organic particles (Enell & Ackefors, 1992).

The uneaten feed, fish excretion and the chemicals used in farming practices, also represent the primary sources of aquaculture wastes. Actually, an estimated percentage values of uneaten feed waste can range from 1 to 30% or more, often because of supercharged or because of an inefficient supply system, which reduces the ingestion rate. Even the feed dust can increase dispersed solid into the water.

Ingestion is dependent upon a sequence of events in which fish must first recognize that there is food present. They must then be able to arrive at food (strong currents, for example, may wash pellets out of the cage before they can be ingested) and be motivated (appetite, visual appearance) to capture it.

As ingested material passes through the gut it is attacked by enzymes, the products of digestion are absorbed into the bloodstream while the undigested fraction is released as faeces. Metabolic breakdown products such as CO_2, 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 which have either become dislodged or have been discarded as a result of *in situ* net cleaning, mortalities and blood from certain types of harvesting operation, may be released into the environment (Beveridge, 1996).

In addition, it has been calculated that, normally, about 25% of the nutrients given to fish, is incorporated in their flesh, whereas about 75% stays in the environment. The 75% of this nutrients is represented by 62% of N in dissolved form and 13% in solid form, contrary to the P that is 11% in its dissolved form and 66% in solid sedimentable substance.

With a conversion index of 1.3 and a content of P and N respectively of 1.62% and 8.45% on the dry weight, it has been estimated that the release in the environment is 10 kg of P and 90 kg of N per tonne of fish produced. These quantities can be calculated as follows: 2.2 kg of P in a dissolved form, 7.3 kg of P in particle form, 61 kg of N in dissolved form and 17 kg of N in particle form per tonne of fish produced. Other factors that can influence alteration of the natural system could be: the rearing system used (intensive or extensive), the reared species, the location of facilities and the production volumes.

The intensive rearing techniques employed for the supply of food in a granular form, have usually a greater negative effect on the environment compared to the extensive breeding ones, where the food is provided by the natural environment.

Nevertheless, the extensive system involves less handling if compared to the intensive one, where other economic activities such as fish monger's shops, transformation industry, transport, and distribution of the catch and transformation of the same in feed. All this chain of economic activities is advantageous only if the system is capable of producing high value and quality market products.

As far as the breeding in floating cages is concerned, in which the nutrients as well as the organic matter are directly discharged in the environment, it has been estimated that about 10% of P is present on dead fish during the breeding phase. Data reported in Tab. 1.1 show as an example the quantity of nitrogen and phosphorous released in the water environment by various human activities (included aquaculture) in Northern Europe countries facing the Baltic Sea in 1989 (the total production of salmons in floating cages the most important in those countries, was 189,300 tonnes in that year).

Tab. 1.1. Quantity of N and P released in the water environment by various human activities in Northern Europe countries in 1989 (modified by Ackefors & Enell, 1994).

Source	N (tonnes)	P (tonnes)
Agriculture	607,800	12,800
Woods and forests	87,600	3,600
Urban sewage	214,600	33,700
Industry	32,900	6,600
Aquaculture	14,200	2,400
Atmospheric deposit on the sea surface	448,000	6,700
Nitrogen fixation	134,000	–
Total	1,539,100	65,800

The release of N is dominated by the agricultural activities (the major source), followed by the atmospheric deposit on the marine surface and by the urban sewer system.

While, as far as the P is concerned, urban sewage and agricultural activities are the main sources for this nutrient. This fact shows that aquaculture has a minimum

impact if compared to other human activities.

On the other hand, the composition of feed is very important in relation to the discharge of aquaculture activities (Ackefors & Enell, 1994; Talbot & Hole, 1994; Ackefors, 1999). Clearly the total nutrients discharged in the environment have a mutual relation with the contents of the diets. Any strategy that aim to reduce the discharges have to formulate the right feed, containing the necessary (and available) quantity of this nutrients in order to guarantee an adequate growth together with a suitable management of the feeding by the breeders. Both N and P are essential nutrients in fish diet (Ketola, 1975; Lovell, 1978; Ogino & Takeda, 1978; Ogino *et al.*, 1979; Sakamoto & Yone, 1980; Watanabe *et al.*, 1980) and must be present in the feed in the right quantity to satisfy the needs which are different, according to the species (Wilson *et al.*, 1982; Cho *et al.*, 1985). The need of the protein and amino acids for fish have been studied by several authors (Halver & Tiews, 1979; Tacon & Cowey, 1985). Fish need a high content of proteins in their diet, between 36% and 55% (Tacon & Cowey, 1985), and can be variable according to the species and during the different phases of growth (Dabrowsky, 1977).

As a general rule, we can say that carnivorous fish need a greater amount of proteins compared to the herbivorous ones, and among the same species smaller fish have a greater need of proteins compared to the bigger ones. Furthermore, a diet lacking in proteins can reduce the growth and causes loss of weight (Wilson & Halver, 1986).

A reduction of the eliminated N can be obtained by increasing the proportion of lipids and/or reducing the contents of proteins in the diet. At the moment, this tendency in the formulation of commercial feed for fish is rather generalized. The reduction of eliminated P can be obtained using flours with low content of soluble P in the feeds (Alsted, 1991). To do this, an adequate knowledge both of the need of the species receiving the feed and the characteristics of the ingredients (profile of nutrients, digestibility, palatability, etc.) is required.

Since the 1980s, the development of new formulated feeds (thus improving production) has allowed the reduction of the conversion index, together with N and P contents in fish diets. At the same time, the total energetic content has been increased (Johnsen & Wandsvik, 1991; Ackefors, 1999) which allowed a further reduction of the contents of nutrients discharged in the environment. Furthermore, the increase of the use of extruded feeds improved the conversion index factor, which allowed a further reduction of discharging in the environment.

The main advantages of extruded feeds are: a better digestibility of carbohydrates, a better use of vegetable proteins, and greater buoyancy and stability of the granules (thus giving fish a larger probability of catching the food), besides a slower and more efficient digestion.

The improving of the conversion factors, further to minimize the effect of aquaculture in the environment, has allowed a better use of the feeds and a faster growth of fish, making aquacultural activities more convenient.

Research on and production of feeds for sea bream and sea bass followed the experience already made in rearing salmon. Despite all, information on nutritional needs of these species is still insufficient and must be constantly updated in order to both reduce the conversion rate and to minimize the environmental impact of aquacultural wastes. The published data on retention rate of N and P in fish, greatly differ even within the same species. The reasons for these changes are probably due to differences of the quality of the ingredients in the feed and erroneous assessments of the quantity of food ingested and mortality in the cages. The estimation of the quantity of nutrients released in the environment tends to vary depending on several factors: a) type of food supplied; b) size of reared animals; c) digestibility of the different components of the diet; d) farming method and feeding techniques used (Munday *et al.*, 1994). In intensive salmonids culture, for example, N and P release was found to be highly dependent from the conversion rate as well as from their total content in feeds (Ackefors & Enell, 1990). These nutrients have a proportional relationship: thus, when one or both of them decrease, the quantity released in the environment decreases, too. Type of food and feed management also should added to these factors. In fact, the increasing use of dry feed in salmonids has reduced the release nutrients quantity per tonne of fish product (Beveridge, 1996).

The management is also crucial in the production of waste. In fact, if charged as a result of improper assessment of biomass, it can be assumed a substantial increase in the uneaten food and the corresponding environmental consequences (Munday *et al.*, 1994). It is also necessary to take into account the differences between manual and automated fed. Thorpe *et al.* (1990) estimated that in manual feeding salmon, ingestion was of 67% and only 33% with automatic feeding.

1.7 Environmental effects of cage fish farming

Cage aquaculture has continued to attract largely unproven negative publicity as

an environmental polluter. Despite the output of nitrates and phosphates from cages is considered insignificant in terms of contributing to nutrient loading (the quantities being small compared with inputs from other anthropogenic sources), they may have local impacts on eutrophication and algal blooms (Folke & Kautsky, 1989; Folke *et al.*, 1994). Therefore, nutrient waste from intensive cage aquaculture can be important in both local and regional terms. In fact, although there is little direct evidence that organic waste released from fish farms can cause problems, it has been established that fish farm wastes stimulate dinoflagellate growth (Nishimura, 1982) and that biotin, found in fish farm wastes, activates toxin production of marine dinoflagellates (Roberts *et al.*, 1983; Graneli *et al.*, 1993).

Moreover, because of their effects on the environment, the release of chemotherapeutants and escapes of fish are considered particularly interesting to involve (Beveridge, 1996).

Cage fish farming does not always result in changes in sediment chemistry or in macrobenthic community structure (Johannessen *et al.*, 1994), the degree of nutrient enrichment depending upon species being farmed, food, management, currents and depth. For organic carbon, for example, Hargrave (1994) cites a five hundred–fold range in sedimentation rates under salmon cages. Effects of solids loadings, however, are apparent at many marine and freshwater sites. Faeces and waste food, especially from intensively managed operations, have much higher levels of carbon, nitrogen and phosphorus than sediments (Enell & Lof, 1985; Merican & Phillips, 1985). The result is that sediments below and in the immediate vicinity of cages have elevated levels of organic matter and nutrients (Gowen, 1990; Hall *et al.*, 1990; Kupka-Hansen *et al.*, 1991; Angel *et al.*, 1992; Kelly, 1992; Cornell & Whoriskey, 1993; Johnsen *et al.*, 1993; Wu *et al.*, 1994; Berg *et al.*, 1996).

Sedimented food and faeces stimulates microbial production, changing sediment chemistry, structure and function (Enell & Lof, 1985; Kaspar *et al.*, 1988; Hall *et al.*, 1990, 1992; Holby & Hall, 1991, 1994; Kupka-Hansen *et al.*, 1991; Kelly, 1992; Sowles *et al.*, 1994; Berg *et al.*, 1996). Changes are positively correlated with waste loadings and accumulation: oxygen demand increases and sediments become increasingly anaerobic and reduced.

The organic matter released into the environment in solid form is easily degraded in sediment under aerobic conditions. The oxygen used in this process is termed BOD (Biochemical Oxygen Demand), and its values range between 2.0 and 4.5 kg day^{-1} ton^{-1}

of fish product (115–120 grams of oxygen per kilogram of feed supply). This fact can reduce dissolved oxygen concentration in the sediment, causing chemical changes that promote further the phosphorus and nitrogen release from organic material present in the water column, which thus accelerate the eutrophication process. Lack of oxygen may adversely affect the health of reared fish and at certain times of the year (summer termocline) causes total deoxygenation of the water.

While in open marine environments this fact does not create serious problems, in case of low current velocities, the anaerobic activity of sulfate–reducing and methanogenic bacteria cause the production and release of carbon dioxide, hydrogen sulfide and methane in the sediment. These compounds are released under the cages and can induce, because of their toxicity, mortality in farmed fish.

This effect decreases rapidly with an increasing distance from cages. Nevertheless, the real extent of hypernutrification and the impact degree on benthic community depend on the size of the farm and the hydrography of the water body within which the farm is located.

As already said, the deposition of organic matter can influence benthic assemblages, but despite of structural changes on meiofauna communities (*e.g.* abundance of large nematodes), most of the studies has been focused on benthic macrofaunal species. These studies showed that the depletion of dissolved oxygen in organic enriched sediments causes the death (or disappearance) of not disrupted soft–sediments typical species, leading to a reduction in richness and diversity of species from 90 to 100% under the cages. Thus, there tends to be a positive correlation between sedimented material and biomass of macrobenthos and a negative correlation between organic matter and diversity: heavily impacted sediments are dominated by pollution–tolerant species, while less tolerant taxa disappear (Brown *et al.*, 1987; Nakao *et al.*, 1989; NCC, 1989; Ritz *et al.*, 1989; Gowen *et al.*, 1991; Tsutsumi *et al.*, 1991; Weston, 1991; Angel *et al.*, 1992; Hargrave, 1994; Johannessen *et al.*, 1994; Wu *et al.*, 1994; Beveridge, 1996).

The macrofaunal biomass does not show a linear relationship with enrichment degree. Indeed, while some authors have noted a reduction of this biomass, others do not. It is therefore very difficult to predict the variation of macrofauna biomass, because this parameter depends on the size and density of opportunistic species. When the organic matter flows in moderate amount, a biostimolation characterized by the enrichment of macrofaunal diversity and biomass may occur, despite different studies

are contradictory. The alteration speed of benthic community after the installation of breeding plant and speed recovery of these communities after disappearance depend on a number of physical and biological parameters (*i.e.* current, bathymetry and recruitment cycle). As a general rule, benthos alterations occur in a few months, while its recovery takes much longer time.

Excretory products are dispersed in the water column by currents while solids (*i.e.* uneaten food, faeces) fall towards the lake or sea bottom. During sedimentation, a quantity of the dispersed food is eaten by fish (Phillips *et al.*, 1985; Carss, 1990), whereas a quantity breaks down in form of minute particles. A portion of these are solubilized, the quantities released depending by faecal and uneaten food composition, physical properties, temperature, depth of water and turbulence (Phillips *et al.*, 1993). Nutrients are also released from sedimented solids (Enell & Lof, 1985; Hall *et al.*, 1990, 1992; Holby & Hall, 1991, 1994; Kelly, 1992) and it has been estimated that about 60% of total phosphorus and about 80% of total nitrogen wastes are dispersed in the water column (Pettersson, 1988; Holby & Hall, 1991; Wallin & Håkanson, 1991a, b; Hall *et al.*, 1992).

Nutrients release causes enrichment (fertilization) of the water surrounding, so that resulting in an increase in primary production of affected areas (eutrophication), altering locally specific algal composition. The increase in algal biomass, both microscopic and macroscopic that can reaches significant levels (seaweed blooms), resulting in an turbidity increase and a reduction of the water column dissolved oxygen by subsequent decomposition of this biomass. In extreme cases, these blooms can produce high concentrations of toxic algae (*e.g.* red tides).

High levels of phosphorus and nitrogen, which can produce eutrophication of water and phytoplankton blooms may also cause massive mortality among farmed species and between organisms of the proximate area. Nevertheless, there is no evidence that would say that aquaculture activities can produce these blooms (Iwama, 1991). In rearing cages in Scotland, for example, an increase of the ammonium concentration has not produced increases of plankton (Gowen & Bradbury, 1987).

Despite all, in many countries there are restrictions on the discharge of fish farming wastes into the environment. In some, however, cages are prohibited from certain types of water body (Van Houtte, 1993).

Hypernutrification is often evident in dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), turbidity and Secchi disk depth

values. At marine sites where dilution is very rapid, effects are often temporary and only apparent during slack tides, when momentary elevations in ammonia levels or decreases in dissolved oxygen level can be measured (Kadowaki *et al.*, 1978; Gowen *et al.*, 1989; Aure & Stigebrandt, 1990; Gowen, 1990; Weston, 1991). Nevertheless, Wallin & Håkanson (1991a, b), studying impacts of Swedish and Finnish coastal cage fish farming, found strong correlations between fish farm loadings and dissolved nutrient levels, especially between total nitrogen and total phosphorus loadings from farms and inorganic nitrogen and phosphorus concentrations in surface waters.

Eutrophication, as indicated by increases in plankton and fish standing crop or productivity, is readily apparent in many fresh waters used for cage aquaculture (Beveridge, 1984; NCC, 1990; Cornell & Whoriskey, 1993; Costa-Pierce, 1996).

The degree of eutrophication is dependent upon the characteristics of the water body and the size, nature and management of the cage operation.

Many studies have failed to find any influence on productivity in marine waters (NCC, 1989; Aure & Stigebrandt, 1990; Gowen, 1990; Weston, 1991), while others have found only weak relationships between nutrient loadings and chlorophyll *a* (Wallin & Håkanson, 1991a, b). This fact is not surprising, given the degree of water movement and flushing at most sites. Highly enclosed, poorly managed sites can show signs of eutrophication. Wu *et al.* (1994) found in Hong Kong a pronounced dissolved oxygen sag, apparent up to 1 km from the cages, although changes in suspended solids and chlorophyll *a* levels are insignificant. Studies conducted in the Baltic, a highly brackish area with limited currents and water exchange, show enhanced growth and production of macroalgae and changes in fish community structure and function in the vicinity of the cages (Koivisto & Blomqvist, 1988; Ruokolahti, 1988; Henriksson, 1991; Rönnberg *et al.*, 1992; Beveridge, 1996).

Nevertheless, if we compare an aquacultural activity with other sources of nutrients, we can reasonably affirm that it constitutes an almost negligible source of eutrophication, although when the size of a facility increases, there are more likely risks of an environmental impact.

1.8 Effects of cage fish farming on wild populations

The detrimental effects of a rearing facility depend on species reared, fish density, farming method, type and frequency of food supply, but also on climatic and hydrographic site conditions that can affect the dispersion of wastes together with water

replacement.

Fish farms attract pelagic and benthic species, as well as birds and marine mammals (Beveridge, 1984, 1988; Carss, 1993a, b, 1994; Pemberton & Shaughnessy, 1993; Beveridge *et al.*, 1994). Another effect that sea cages can cause on the surrounding environment is linked to their ability to operate as FADs (Fish Aggregating Devices). In fact, the presence of a large structure attracts many species of fish that tend to cluster around it. Moreover, the supply of food for rearing fish generate excess of food that spilled outside the cages can be partly ate by the species present in the surrounding of the facility, thus altering community trophic structure.

One of the major factors that contributes to this phenomenon is the excess of food around the cages, which causes an enrichment of seabed sediments. A soft or moderate enrichment may also support the increase in mean number of organisms under intensive cages fish farming (Iwama, 1991).

In freshwater environments, Loyacano & Smith (1975) described an increase in number and weight of wild fish around fish farms compared to catch in control areas. Studies on *Salmo salar* and *Oncorhynchus mykiss* freshwater fish farming, showed a significant increase in total number of individuals and biomass. This results 5 times higher in cages than the areas from which the cages were removed (Carss, 1990). On the other hand, the population densities of *Pollachius virens* in 3 separate areas around open sea cages farming were about 12 times higher than those observed in control zones far from any aquaculture facility (Carss, 1990).

Other studies reported increases in infauna and epifauna, such as crabs, nudibranch, anemones, sea urchins and sea cucumbers, as well as certain benthic species such as *Chironomus plumosus*, *C. anthracinus*, *Macoma baltica* and *Potamothix hammoniensis*, all connected with the degree of organic matter in sediment (Partanen, 1986; Dobrowolski, 1988).

On the contrary, large depositions of organic matter lead to a reduction in number of species and, in extreme cases of anaerobiosis, immediately under the cages at heavily impacted sites an azoic zone, devoid of oxygen and macrobenthos, may be apparent (Earll *et al.*, 1984; Iwama, 1991).

Outside cages this is an area of organic enrichment where exceptionally high numbers of opportunistic species such as small polychaetes *Capitella capitata* and *Scolelepis fuliginosa* with densities between 100 and 10,000 individuals m^{-2} occur (Mattsson & Linden, 1983). These organisms tend to vicariate the filter–feeding

macroinvertebrates, typical of sediments with a moderate organic enrichment and potential redox between 150 and 200 mV. These are usually in large number up to 20–50 meters from the cages, although in some sites, because of poor management or unusual hydrographic conditions, effects are evident at a distance of 100–150 meters (NCC, 1990; Kupka-Hansen *et al.*, 1991; Weston, 1991), where there is a recovery of typical organism of these areas (Brown *et al.*, 1987).

Also echinoderms are the group showing the largest decline in abundance. These are the first species to vanish with increasing organic sediments.

There are others macroscopic examples of enrichment of the sediments under fish or shellfish cages, which is represented by a white mantle on sediment formed by filamentary bacteria belonging to the *Beggiatoa* genus, moving into the space between the oxygenated and anoxic sediment layers where occurs the production of hydrogen sulfide (Jorgensen, 1977). In some cases the *Beggiatoa* layer cover up to 50% of the available space below the cages. In Scotland farms, this species create at a distance of 10–15 meters from the cages a ring joining starfish, sea cucumber and dead nudibranch. At a distance of 20–30 meters from the cages the sediment had a very low potential redox, with a greyish–brown colour (Earll *et al.*, 1984).

As regard the recovery time of the benthic community below the cages, this can recovers (in terms of abundance) in 1–1.5 years depending on the contribution of received organic substance (Johannessen *et al.*, 1994), although the total recovery of the ecosystem may be longer because of the large quantity of organic matter in sediments (Goldburg & Triplett, 1997).

1.9 Effects of cage fish farming on Posidonia oceanica meadows

Posidonia oceanica, the main and endemic seagrass species in the Mediterranean Sea, with its sparse sexual reproduction (Díaz-Almela *et al.*, 2006, 2007), is the slowest–growing seagrass species (Marbà & Duarte, 1998), requiring centuries to re–colonize coastal areas (Meinesz & Lefevre, 1984; Duarte, 1995; Marbà *et al.*, 2002; Kendrick *et al.*, 2005). Thus, since a number of injures of *P. oceanica* meadows can be irreversible, they are now protected by various international conventions, agreements and other legal documents (Holmer *et al.*, 2008). In fact, seagrasses play major ecological roles in the coastal zone (*e.g.* prevent coastal erosion, increase coastal biodiversity, oxygenate water and sediments).

Many environmental requirements for coastal fish farming (*e.g.* good water

quality and adequate water renewal) are, unhappily, similar to the habitat of *P. oceanica*. So, the weak enforcement of regulations of fish farm siting (and the irregular effectiveness in monitoring or impact assessment procedures among Mediterranean countries) has allowed that a significant number of fish farms are placed over or very near *P. oceanica* meadows (Pergent-Martini *et al.*, 2006). As a result, the forecasted multiply of fish farming may increase speed the decline of this important marine habitat. The negative effect of fish farming on *P. oceanica* meadows has been frequently demonstrated (Delgado *et al.*, 1997, 1999; Pergent *et al.*, 1999; Ruiz *et al.*, 2001; Pergent-Martini *et al.*, 2006; Holmer *et al.*, 2008).

Posidonia oceanica meadows are highly defenceless to marine fish facility as showed its losses in the vicinity of fish farms (*e.g.* Delgado *et al.*, 1997; Ruiz *et al.*, 2001; Cancemi *et al.*, 2003; Holmer *et al.*, 2003; Marbá *et al.*, 2006), which continue even after farming cessation and water quality recovery (Delgado *et al.*, 1999).

The turn down of *P. oceanica* near fish farms has been ascribed mainly to the decline of sediment quality (Holmer & Nielsen, 1997; Terrados *et al.*, 1999), most probably determined by organic matter sedimentation (Duarte, 2002; Holmer *et al.*, 2003).

Sedimentation in seagrass meadows has not yet been measured near fish farms, but it is likely that the presence of seagrass meadows increases the organic loading of the sediments due to enhanced sedimentation of waste products. Aquaculture waste products in sediments have been traced to considerable distances (*i.e.* greater than 300 m) around Mediterranean fish farms by application of sensitive tracers such as the stable isotopes $\delta^{13}C$ and $\delta^{15}N$ (Sarà *et al.*, 2004).

Before irreversible losses occur, therefore, it is essential to increase true predictors of fish cage culture impacts on *P. oceanica* meadows. In recent years, in an attempt to minimize the impact to the benthos, new fish farms, in particular in the Mediterranean area (with exceptions as in Greece where an extensive archipelago allows more sheltered locations), have been installed relatively far from the coasts (1–5 km) at deeper and more exposed sites (Holmer *et al.*, 2003).

1.10 Effects of introduced species in cage fish farming activities

The use of introduced species in aquaculture is not new. There is no documentation as to when common carp, native to China, came to Indonesia or Mozambique tilapia came to Indonesia. Similarly, rainbow trout had crossed the oceans

even during the steamer days. But with air transport and increased global commerce, the rate of introductions has increased in recent years (FAO, 2007).

The breeding of exotic species is increased particularly for several reasons like: a) advantage in terms of growth; b) more efficient feed conversion; c) better resistance to disease; d) hardiness to handling and environmental fluctuations; and e) better tolerance to crowding.

Alien species have been used successfully to generate increased income and social benefits in many parts of the world. They have, however, also been identified as a major threat to biodiversity and as a vector for pathogens (FAO, 2007).

Their transfer from typical environments, in addition to the physical transfer of new species, can produces their pathogens movement. On the other hand, it is known that reared specimens frequently escape from the cages during the daily handling or because of net–cage broken because of meteorites events or vandalism acts. These organisms may lead to environmental deterioration due to the habitat modification, competition, predation or because of the interbreed with native species (Cognetti *et al.*, 2006).

At present, several methods and techniques to avoid the undesirable effects of aquaculture on aquatic biodiversity are available (beginning with the choice of suitable sites, as well as improved management and nutrition). Another method largely used is the encouragement of closed farming systems.

Exotic species rearing activities also favour the proliferation of microorganisms, due to nutrients and organic matter released and to the existence of microorganisms in the digestive intestinal tract of reared fish. In general, micro–organisms increase with the addition of nutrients and organic matter, whereas decline due to a chemical treatment of fish diseases (Beveridge *et al.*, 1994). In past years, together with aquaculture development, a strong increase in the use of chemicals such as medicines, anaesthetics, vaccines and disinfectants, has occurred. Some of these are used as biocides to controlling bacteria, fungi and protozoa or as antifouling (Beveridge, 1986). The 1980s were the years of their maximum use, but after then there was a rapid decrease in their use up to 90% particularly correlated of damage on marine organisms and humans. (Beveridge *et al.*, 1994).

However, the problem of escaped exotic stock has not received as much attention until recent times. This attention came with the introduction of Pacific white shrimp, due to the opposition to its use by environmentalists frightened that it may carry exotic

diseases and modify local biodiversity by displacing a local species (FAO, 2006).

1.11 Zoning for cage fish farming development

Cages are usually sited in the full of activity coastal zone or in multi–purpose lakes and reservoirs. The area they occupy, although not large by comparison with ponds (cages are deeper and hold higher stocking densities), or in terms of a country's shoreline or freshwater resources, can nevertheless be important (Beveridge, 1984).

Space occupied by cages may otherwise serve other purposes. If the allocation of space has not been fair, or is perceived as such, social tensions can develop. This is apparent in several regions, where conflicts between people and cage fish farmers sometimes arise. But it is not just the space occupied by cages that must be considered.

An intensively sited and poorly managed cage farm can adversely affect landscape values. Indeed, visual impact is one of the most important causes of public concern about cage farm developments in several regions of the world (SWCL, 1988), and has been an important factor in the development of aquaculture and tourist facilities. Such problems should, of course, be identified and resolved at the planning stage.

1.12 Fishmeal supply to meet needs of cage fish farming

Many aquaculture species are piscivore, since in the wild they partially or totally eat fish. For this reason, feed for the early stages must contain a high content of fish flours and fish oils. However, the world's production of these ingredients, based on the South Pacific and the North Sea catches, has suffered since the 1980s a sharp drop caused by over–exploitation. In fact, this resource was used not only to produce feed for fish and shellfish but also for feeding chickens, pigs, cattle and to a lesser extent for the production of pharmaceutical products.

The main part of world fish culture production is of carps and tilapias grown in the tropics and sub–tropics. A small component of fish production, an estimated 10%, comes from intensive cage culture. Nevertheless, salmon farming in particular is becoming an important consumer of fishmeal. By the mid–1990s world production of Atlantic salmon was around 400,000 tonnes. If an average food conversion ratio of 1.3:1 is assumed, then 520,000 tonnes of salmon food were needed to sustain the industry. Since the fishmeal component of salmon diets is 50% and 5 tonnes of fish is required to produce 1 tonne of fishmeal, then 1.3 million tonnes of industrial fish were

being used to support the industry, equivalent to some 15% of global fishmeal supplies and around 5% of total capture fisheries production. A further 78,000 tonnes of fish oil is required (15% inclusion rate), equivalent to 5% of world supplies (Folke & Kautsky, 1992; Chamberlain, 1993; Tacon, 1994; Beveridge, 1996).

For this reason, and following several scientific research, there was a partial or in some cases a total replacement of amino acids and fat of fish meal and fish oil with those of vegetable origin. Nevertheless, it is important to underline how recent studies on human nutrition have highlighted a higher nutritional value of aquaculture products with respect to that of similar wild species.

Extensive cage culture of tilapias in Philippine lakes has caused overgrazing with the result that poorer farmers have been forced of production while those remaining have become increasingly reliant on supplemental food (Beveridge, 1984; Santiago, 1995). Thus, from one perspective, cage aquaculture is not a major drain on finite resources except, perhaps, with regard to fisheries products. Intensive cage aquaculture is an important consumer of fishmeal and fish oil, and must accept some responsibility for over–exploitation of fish stocks, with dramatic consequent effects on wildlife (Monaghan, 1992; Pauly & Christensen, 1995).

Impacts on resource use are more apparent when the ecological footprint (*i.e.* the area of land and water required to provide resources and services) or energy flow are considered. Berg *et al.* (1996) compared inputs of natural capital and energy flow for intensive cage culture of tilapias on Lake Kariba and semi–intensive pond culture carried out nearby. Although the former used less energy, the latter proved much more efficient in terms of energy transfer. In general, however, it is difficult to say whether cage aquaculture is more resource hungry than other forms of aquaculture as a comprehensive analysis of land–based and water–based aquaculture production has yet to be carried out (Beveridge, 1996).

Therefore, the development of sustainable and responsible aquaculture will allow the decrease of pressure by market demand on fisheries, promoting the recovery of natural populations. Aquaculture may also contribute to a further recovery of the fishing industry through restocking programs included in integrated management of natural resources (FAO, 2007).

1.13 Biouling problems in cage fish farming

Biofouling is defined as the settlement and attachment of aquatic plants and

animals onto hard substrates introduced into the aquatic environment by human activity. It occurs to some degree at all cage sites, although marine sites are worse than fresh water, especially if the environment is warm and eutrophic and if prevailing low currents.

Colonization begins with the development of a bacteria film and algae (Milne, 1970; Santhanam *et al.*, 1984). Nevertheless, the growth and biodiversity of the fouling community is correlated both the environmental conditions and the materials used, but is also significantly affected by temperature, productivity of the environment and by the cultured species (Chamberlain & Strawn, 1977; Cheah & Chua, 1979). Actually, culture of herbivores is not a problem because the action of the fish, which continually graze on the netting, thus reducing biofouling cages increase (Pantastico & Baldia, 1981).

Biouling increases vertical forces on cage structures and netting and reduce mesh size, thus limiting water exchange and increasing drag (Kuwa, 1983, 1984; Santhanam *et al.*, 1984; Greenland *et al.*, 1988; Aarsnes *et al.*, 1990; Lai *et al.*, 1993; Løland, 1993a, b). Milne (1970), for example, used immersion trials to determine changes in weight and in twine thickness of net panels. Increases in current forces can be as much as 12 fold, while increases in weight can be up to 200 fold affecting both routine operations and flotation. A number of net failure at commercial marine cage fish farms have been attributed to biofouling (Milne, 1976; Hugenin & Ansuini, 1978; Lovegrove, 1979).

There are some options if biofouling at a site is severe: a) management procedures can be modified to cope with the problem; b) biofouling can be tackled using chemical or biological control agents; c) a biofouling resistant or rotating design can be used.

Farmers may consider the use of fouling resistant materials in the construction of the cages. Some companies manufacture polyethylene netting inlaid with copper wire and, while this may be readily fabricated into conventional net bags design, it is much more expensive, costing much more than a nylon net bag of similar dimensions. Moreover, the copper gives limited protection and nets must either be replaced every year or two or treated with conventional antifouling compounds after the copper wire has corroded.

The majority of commercial fish farmers use nylon or polyethylene net cages, and either accept the increased labour involved in cleaning, or resort to the use of antifouling compounds. While the savings in manpower offered by rotating designs or copper alloy cages may not justify higher initial capital expenditure, they have a number

of other advantages over conventional net bag systems that should be carefully assessed by prospective farmers.

1.14 Some final issues

Various factors are driving the aquaculture sector to intensify. The main motivating force appears to be the limited availability of sites. As the availability of sites for aquaculture is becoming increasingly limited and the ability to utilize non–agricultural land is restricted, along with economic drivers, the aquaculture production systems are being progressively more intensified.

Intensification may sustain productivity of farming facility, but this comes at a cost in terms of water quality and fish health. In fact, not all farmers are able to intensify and, as production costs increase, part of the sector may reduce intensity to lower costs or reduce vulnerability to health or environmental problems.

The farmers constantly look for innovative ways to use land and water environments for production. The exploration of new systems not only requires identification of suitable areas, but also needs to use tools such as surveys, studies of carrying capacity, water quality monitoring and Geographical Information Systems (GIS), remote sensing and mapping (FAO, 2006).

The rapid development of aquaculture can only be achieved through an aquaculture model that links ecological and technical aspects of applying ecological design principles and environmentally friendly aquaculture. This fact, linked with a rational strategy would boost its social and economic perception. As a result, aquaculture possibly can be transformed into a eco–sustainable and socially responsible practice, developing as an integral part of the management of natural resources and thereby making possible the restoration and maintenance of natural ecosystems affected by these activities. Cages suffer by comparison with other aquaculture systems in being particularly vulnerable to both environmental variables and anthropogenic hazards. The site selection process is concerned with choosing the best environment so that the mortalities are minimized, growth and production are maximized and the venture is as profitable as possible. Unfortunately it is not often possible to choose the ideal site.

Neither is it always possible to predict the problems that might occur or to examine risks as thoroughly as one might like. Damage caused by drifting objects and pollution owe much to chance and it is usually very difficult to investigate factors such as fouling or wastes effect that affect fish welfare (Beveridge, 1996).

1.15. References

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Chapter 2

APPLICATION OF THE MERAMOD® MODEL

2.1 Introduction

In the last decade, cage rearing of sea bass and gilthead sea bream has experienced a period of exponential growth in the Mediterranean region. However, little detailed information is yet available on the real environmental impacts of this farming system (Karakassis *et al.*, 1998, 2000; Apostolaki *et al.*, 2007; Sarà, 2007a, b; Holmer *et al.*, 2008).

Many fish farming have been investigated about the interactions of aquaculture operations and sediment chemistry processes (Gowen & Bradbury, 1987; Weston, 1990; Silvert, 1992; Davies *et al.*, 1996; Findlay & Walting, 1997; Kempf *et al.*, 2002), oxygen availability (Enell & Löf, 1983; Hall *et al.*, 1990; Findlay & Walting, 1997), and changes to the benthic assemblages structure (Brown *et al.*, 1987; Henderson & Ross, 1995; Kempf *et al.*, 2002), but generally, it has been assumed that impacts will, at least qualitatively, follow the pattern established in northern latitudes (Black *et al.*, 2001).

As sea–cage aquaculture continues expanding rapidly along the Mediterranean coast both in the number of farms and production, it is necessary to be more accurate when predicting potential impacts of this practice. Consequently, much research has focused on modelling the dispersal of sea–cage farm wastes to infer the impact of aquaculture more precisely and thus improve management actions and find the most suitable places to locate farms (Cromey & Black, 2005).

After food supply and as a consequence of fish metabolic activity, the outflow waters from intensive aquaculture systems contain a variety of constituents that can have detrimental effects on the health of benthic organisms and the surrounding environment (Yokoyama *et al.*, 2006), which, in turn, can produce a negative feedback on the aquaculture system (Brambilla *et al.*, 2007a).

Key constituents include deoxygenating and eutrophicating matter from uneaten feed and excreta. To fulfil environmental protection requirements, great improvements in feed and feeding technologies have been made in the past few years to enhance the food quality by increasing nutrient retention. Nowadays, nitrogen and phosphorous retention ranges between 10 and 49%, and 17 and 40%, respectively. Similarly, nitrogen and phosphorus release in faeces ranges between 3.6 and 35%, and 15 and 70%, respectively, while dissolved nitrogen and phosphorus excretions range between 37 and 72%, and 1 and 62%, respectively (Piedrahita, 2003).

A general review of modelling approaches to fish farm impacts has been

undertaken by Silvert & Cromey (2001). Models developed on fjordic ecosystem dynamics (Ross *et al.*, 1993a, b, 1994) did not provide information at high resolution. Gowen *et al.* (1989) described a simple model for predicting carbon deposition rates from marine fish farms based on a current meter record and the production of a site. Although this type of model is a useful starting point, it only provides limited information regarding the deposition of carbon (see Panchang & Richardson, 1992 for a review). It does not include any of the physical and biological parameters which determine the fate of organic material once it has reached the seabed, nor does it include fish husbandry factors affecting variation of input over time.

Other approaches include description of an algorithm which calculates particle distributions backwards from the seabed to surface (Gowen *et al.*, 1994; Silvert & Sowles, 1996), while Hevia *et al.* (1996) used a graphical programming approach to map deposition on the seabed. The use of Geographical Information Systems (GIS) is an increasingly popular method for management of fish farm impacts (Ali *et al.*, 1991; Ross *et al.*, 1993a, b; Aguilar-Manjarrez & Ross, 1995; Nath *et al.*, 2000).

More sophisticated fish farm models (*e.g.* AWATS), which include complex hydrodynamics of tidal/wind–driven current and waves and associated resuspension processes, are less common (Panchang *et al.*, 1997). Validation of current fish farm models rarely include a resuspension component, and the AWATS package represented a significant advance in this field (Dudley *et al.*, 2000).

There is a continuous debate as to the magnitude of critical thresholds for resuspension. Low thresholds have been used in resuspension models of freshly deposited material, but these do not include fish farm wastes (Sanford *et al.*, 1991; Cromey *et al.*, 1998).

Over recent years, the modelling of waste deposition and benthic impacts from fish culture operations have increasingly been recognized as an important component of the management process (Henderson *et al.*, 2001; Silvert & Cromey, 2001; Pérez *et al.*, 2002). Therefore, a number of fish culture waste sedimentation models have been developed (Panchang & Richardson, 1992; Cromey *et al.*, 2002a, b; Pérez *et al.*, 2002; Corner *et al.*, 2006) which provide predictions of the increased flux and, in some cases, also predictions of the nature and scale of effect, from proposed and existing farm sites on the proximal benthic environment. In practice, these models must adequately represent and characterize all of the important processes that act on and define the waste material settling through the water column and depositing on the seabed.

Integration of the processes acting on the waste material post–deposition (*e.g.* resuspension, degradation) is also an important factor to be considered in such models (Chamberlain & Stucchi, 2007).

A combination of factors including production levels, feed characteristics (*e.g.* ingredient composition, digestibility and physical structure), and feeding efficiency influence the quantity and quality of material exiting a farm structure in the form of fish fecal material and uneaten feed (Chamberlain & Stucchi, 2007). The spatial fate and the extent to which the seabed is affected depends both on the type and quantity of this material on benthic conditions will be site specific and influenced by local physical condition (*e.g.* hydrodynamics), the characteristics of the receiving environment (*e.g.* bathymetry, seabed typology, benthic oxygen supply), and the sensitivity of resident organisms (Brambilla *et al.*, 2007a). The above factors all require consideration during model development and parameterization.

Over time, as aquaculture waste models have developed from simplistic linear equation based systems (*e.g.* Gowen *et al.*, 1989) through incorporation of spatially varying flow fields, detailed bathymetric grids and use of Lagrangian particle tracking algorithms (Panchang & Richardson, 1992; Cromey *et al.*, 2002a, b; Pérez *et al.*, 2002; Doglioli *et al.*, 2004; Corner *et al.*, 2006), key processes have been incorporated into simulations, increasing predictive skill. However, data on the quantity and physical and chemical properties of the waste material produced from the farm are equally critical in predicting the nature and scale of effect to the receiving environment and uncertainty in these values will result in a corresponding uncertainty in model outputs.

The overall accuracy of model predictions will be determined by the suitability of the model to the test environment, the processes the model simulates, how the model is configured and parameterized, and the quality of input data used. For utility in management decision frameworks, where the objectives are generally the assessment of "effect/impact" relative to natural and/or background conditions, it is necessary that model outputs, which are in the form of a predicted waste flux, are correlated with a measure of "actual" or "change to" benthic status (Chamberlain & Stucchi, 2007).

Some of the above models have been compared with measured flux (using sediment trap techniques) with varying levels of accuracy [*e.g.* Cromey *et al.* (2002a) ±20% and ±13%; Stucchi *et al.* (2005) overestimated by ~500%; Corner *et al.* (2006) ±58.1%] which itself is an important step in model development, but the majority make no interpretation of the consequent effect on benthic condition. It should be noted that

the level of confidence placed on interpretations of model accuracy calculations has to be weighed carefully against the observed highly variable efficacy of sediment traps deployed in shallow near–shore locations around finfish farms (Stucchi *et al.*, 2005).

A small number of semi–empirical models have been developed that correlate the effect of increased sedimentation from fish culture operations with benthic ecosystem processes such as the Benthic Enrichment Index (BEI) in sediments (Hargrave, 1994), indices of benthic diversity (Cromey *et al.*, 2002a), and benthic oxygen demand (Findlay & Walting, 1997; Stigebrandt *et al.*, 2004). When such relationships between predicted flux and benthic status can be demonstrated to be significant, model predictions of the degree and spatial extent of effect may be made at other locations having similar substrates, bathymetry and hydrographic conditions (Chamberlain & Stucchi, 2007).

This paper presents the application of the aquaculture waste model MERAMOD® (Cromey *et al.*, 2002a, b) at a finfish farm site in the Alghero Bay (Sardinia). We examine the effect of uncertainty in 3 parameters (percent waste feed, carbon concentration of feed and fecal material) applied within the model and one process (resuspension) on predictions of carbon flux to the seabed. These parameters and processes align somewhat with the sensitivity analyses carried out by Brooker (2002), who identified the factors of bathymetry, settling velocity settings, percent waste feed rate and FCR (Feed Conversion Ratio defined as the ratio of feed used to production) as having the greatest effect on predicted deposition. We then assess the relative contribution of fecal material and waste feed components, integrating the associated uncertainty, on the overall predicted flux.

Finally, model simulations are then examined in conjunction with field measurements of benthic status to explore relationships between predicted flux and alterations to seabed conditions and assess the predictive skill within the modelled envelope of uncertainty. Information on farm configuration (cage dimensions, layout and positions) and feed input data (to specific cages) are also important and necessary data requirements.

The fish culture waste model MERAMOD® consists of 4 process–based modules that are applied consecutively: a grid generation model; a particle tracking/dispersion model; a resuspension model; a benthic impact model (Brambilla *et al.*, 2007b).

In general, particulate waste dispersion models can provide a cost–effective approach to evaluate wastes releases in site selection and biomass limits in terms of

local environmental capacity. They can also be helpful in supporting decision–making for environmental regulation and management by testing several pre–production scenarios for given environmental situations (Corner *et al.*, 2006). Across Europe, several modelling strategies have been designed to enable predictions for managing environmental impacts of marine fish culture (Henderson *et al.*, 2001). In Scotland, the DEPOMOD® model (Cromey *et al.*, 2002a), from which MERAMOD® originates, is now widely used for the Environmental Impact Assessment (EIA) and to estimate the likely seabed deposition (SEPA, 2003).

The aim of this study, therefore, was to assess the likely seabed deposition of fish farm wastes by using the MERAMOD® model in order to evaluate the actual scenario and the forthcoming situation represented by an enlargement of the farming area (about 8 ha), with the addition of 4 new submersible fish cages having a volume of about 2,500 m^3 and hypothetical mean daily amount of feed of 50 kg $cage^{-1}$.

2.2 Materials and methods

2.2.1 Study area

This part of the study was carried out between June 2006 and December 2007 at the fish farming facilities of "La Maricoltura Alghero s.r.l." located in the Alghero Bay (North–Western Sardinia, Latitude 40°33'43.9''N, Longitude 8°16'09.0''E; Fig. 2.1). During this period this fish farm occupied a surface area of about 2.5 ha, on a 38 m water depth average where only gilthead sea bream specimens (*Sparus aurata* Linnaeus, 1758) were reared in 5 round "tension–legs" cages (REFA®) of 800 m^3 and 4 round "tension–legs" cages (REFA®) of 2,500 m^3. Fish density ranged from 0.4 to 20 kg m^{-3} and the provided daily feed ratio was estimated to be 40÷190 kg $cage^{-1}$ with a total daily average of 98 kg $cage^{-1}$ (Brambilla *et al*., 2007b).

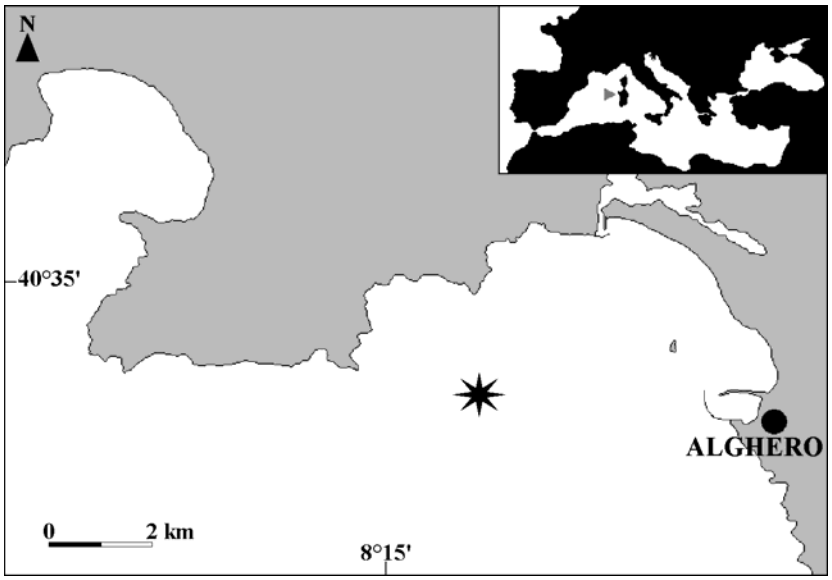

Fig. 2.1. Study area (asterisk indicates fish farm position in the Alghero Bay).

The solids deposition on seabed arising from the fish farm and associates changes in the benthic community were estimated by the application of the MERAMOD® model, Version 1.4 (see Cromey, 2004 for details).

2.2.2 Description of the MERAMOD® model

A general review of modelling approaches to fish farm impacts was undertaken by Silvert & Cromey (2001). A number of sedimentation models have been developed which predict the magnitude and spatial extent of the deposition of particulate matter from finfish farms. These models typically attempt to predict the trajectory of particles

of waste (waste feed pellets and/or faecal material) as they fall through the water column and are deposited on the seabed (Hevia *et al.*, 1996).

The fundamental forcing parameters used in these models were initially reported by Gowen *et al.* (1989) as the hydrographic regime, depth of water column, and the settling velocity of the waste material (Fig. 2.2). Over time, increasingly complex models have been developed, improving the use of these fundamentals with the incorporation of spatially varying flow fields and detailed bathymetric grids. The use of Lagrangian particle tracking algorithms to describe the trajectory of individual particles from a defined point in the water column to their intersection with the seabed (*e.g.* Panchang & Richardson, 1992; Cromey *et al.*, 2002 a, b) was another significant advance in techniques.

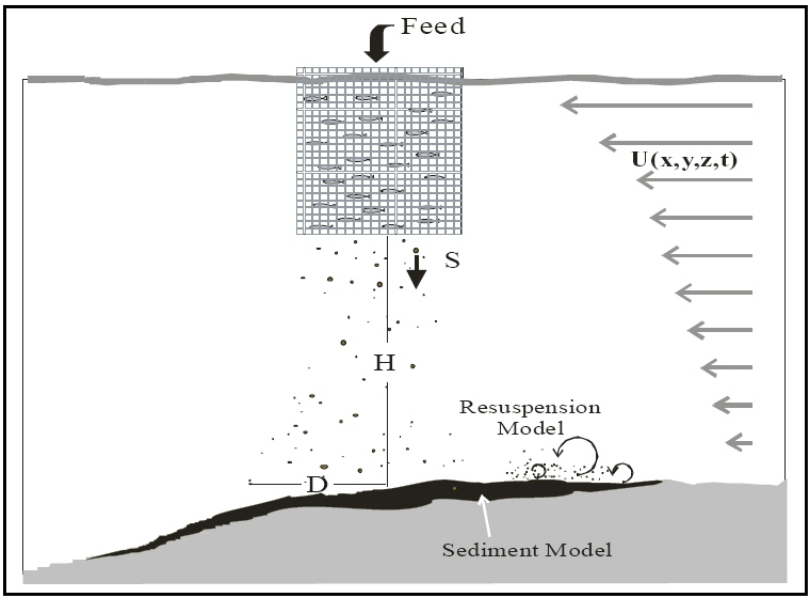

Fig. 2.2. Scheme of settling particle waste under a fish rearing cage.

Information on farm configuration (net pen dimensions, layout and positions), fish production (species, biomass, size) and feed input to the site are important necessary data requirements. Data on quantity and quality of waste material produced from the farm are critical in determining the nature and scale of effect on the benthos. Once wastes settle onto the bottom, the currents, if sufficiently strong, may transport wastes through resuspension and saltation processes. Physical removal and transport of material away from a point source through resuspension processes depend on a number of key factors (Clarke & Elliot, 1998; Cromey *et al.*, 1998) and often result in a reduction of material available to the benthic community proximal to the farm.

The final component or step in the modelling process is to predict some measure of change in the benthic community and/or sediment quality as a result of increased flux or accumulation of waste material. A number of semi–empirical models have been developed that predict measures of benthic impact such as the benthic enrichment index in sediments (Hargrave, 1994) or indices of benthic diversity (Cromey *et al.*, 2002a). When such relationships can be demonstrated to be significant, model predictions of the degree and spatial extent of benthic impact may be made at other locations having similar substrates, oceanographic and hydrodynamic conditions.

In Fig. 2.3 there is a schematic representation of how the individual modules are integrated in the DEPOMOD model (very similar to those used in MERAMOD®). Although the carbon degradation G–model (Westrich & Berner, 1984) is implemented in the model, it was not used in model validation.

The grid generation module generates an array used by subsequent modules containing bathymetry, cage and sampling station positions. Fine grid cell resolution is desirable (*e.g.* 10 m), where the limits of the predicted deposition footprint are expected to be less than 100 m away from the cages and spacing of sampling stations is small. For a larger deposition footprint, cell resolution of 25 m is more appropriate.

The particle tracking model describes transport of particles from the surface to the seabed. Large numbers of particles are used to represent the waste material which are assigned appropriate settling characteristics, although beyond a threshold further increases in particle numbers results in an insignificant change in bed particle distributions. Information on feed input and food to waste conversions (mass time^{-1} cage^{-1}) allow definition of the solids loading arising from the farm. Particles are subject to settlement through the water column while being advected in two dimensions by hydrodynamic data. These data are implemented into the model as a number of layers, where each data set represents a layer with different current amplitude and direction.

Typically, three data sets are implemented to represent shear in the water column, and this layered method is more accurate at describing the water column than fitting a logarithmic profile to one data set. Such profiles are unsuitable for use in areas around fish farms where shear and stratification in the water column are often significant. For short time scale modelling studies (*e.g.* 24 h), the use of 10–min current observations has been found to be appropriate, whereas for longer term steady–state solids accumulation predictions, hourly averaged data is desirable. Particles are subject to random walk in 3 dimensions as a representation of turbulence and, as particles intersect

the bed, information is stored for use in further modules. For a particular level of solids accumulation, the benthic response model gives a prediction for 2 benthic indices [*i.e.* Infaunal Total Index (ITI) and Total Abundance (TA)]. These relationships were validated using data from some Mediterranean marine farms.

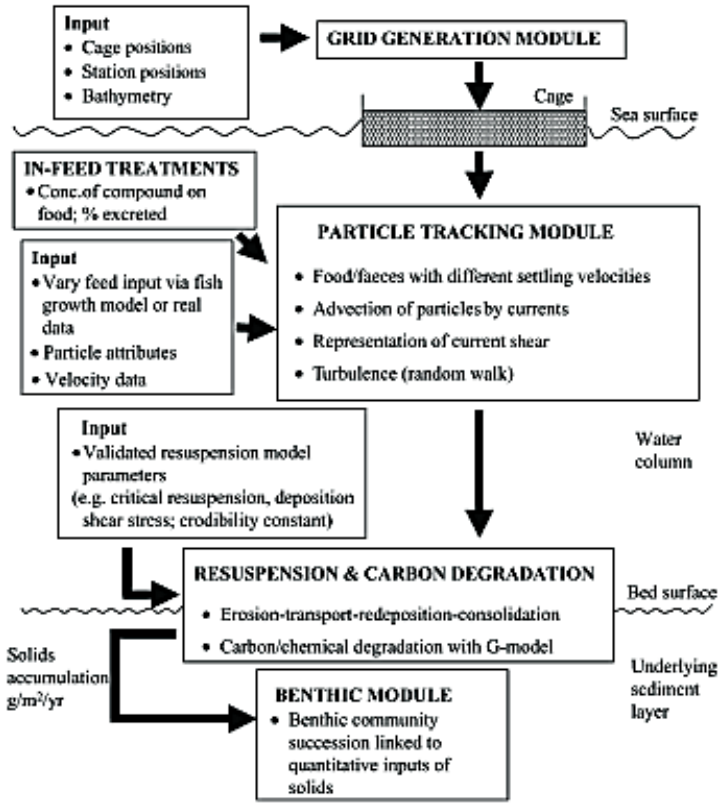

Fig. 2.3. Integration of the DEPOMOD modules and associated input data used for modelling benthic impacts (from Cromey *et al.*, 2002a).

In the following paragraphs, the main parts of the MERAMOD® model protocol are explained in detail (Cromey, 2004). All the sampling procedures used to carry out this part of the study are also described.

2.2.2.1 Validation of the MERAMOD® model

The validation is divided into 3 stages:

- validation of the particle tracking model using solids flux (AFDW – ash free dry weight) in 57 sediment traps at MD8 was undertaken. This study was 13 days in length and sampled across a range of flux values (65 – 7535 g AFDW m^{-2} y^{-1}) from under cage to intermediate field (50 m);

- validation of the particle tracking model using solids deposition (TDS – total dry solids) in a series of six 24 hours sediment trap studies from the spring and autumn 2002 cruises [MD1 (3 experiments), MD5 (2 experiments), MD3 (1 experiment)]. These studies concentrated on the high flux zone underneath the cages and included different depths of sediment traps within the same experiment, including traps directly attached to the cage (net) bottom;
- validation of the benthic response model using benthic community data from six sites.

This established relationships between modelled flux and numerous descriptors (species (S), abundance (A), biomass, A/S, Shannon Weiner, Simpson, Eh (4 cm) allowing the model to be used for planning and monitoring scenarios. Useful relationships were also found between modelled flux and relative abundance of indicator species and families.

2.2.2.2 Model capability

The model validation resulted in acceptable agreement between observed and modelled variables. This resulted in MERAMOD® being satisfactorily validated for predicting flux and benthic response for Eastern Mediterranean fish culture operations. Any reasonable predictive capability in an environment where both sediment trap data and benthic community descriptors vary over such short spatial and temporal scales is acceptable. In addition, a number of tests undertaken in the validation studies showed model performance to increase when using the wild fish module, species–specific faecal settling rates and highly detailed husbandry data. Importantly, the quality of input data used in the model directly effects its capability and this is particularly true of hydrographic and husbandry data.

2.2.2.3 Model limitations

The model should be used to predict flux and benthic response with special regard to the model accuracy specified, determined during model validation exercises. The level of accuracy expected also varies on the level of flux predicted. In addition, use of the benthic response module requires care as the reliability differs between the relationships established for each descriptor. Although primarily a data input issue, the detail of husbandry data used in the model effects predictions significantly. Use of monthly summarised husbandry data can be limiting due to the range of fish size and

species being farmed within a cage group. This model has not been tested in hard substrate, underwater cliff areas nor does it include a validated resuspension component. It does not include flocculation or disgregation behaviour of particles.

2.2.2.4 Model use

The user should take care to use appropriate settling velocities for the species being modelled. There are important differences between salmon, sea bass and bream faecal settling rates and so the most up to date information in the literature should be sought. The user should also seek detailed husbandry data for the site being modelled. Accurate hydrographic data are also required for this environment. The model is set up with depth, cage layouts and sampling station locations. Hydrography, settling characteristics, feed input and wild fish module settings are then input to the model. A flux/deposition model then summarises the flux at the sea bed. Degradation of particulate material can also be undertaken with the G–model (Westrich & Berner, 1984). This model does not predict resuspension effects.

2.2.2.5 Model validation detail

To validate a deposition model for marine fish farms in the Eastern Mediterranean, flux predictions of ash free dry weight (AFDW) of waste material arising from the farm were compared with observations of sediment trap data (*i.e.* g m^{-2} y^{-1}). Model input data were more detailed than usually used in such models, with cage specific data used for food and faecal settling velocities according to feed type and species respectively, as well as feed input. Detailed hydrodynamic data obtained at three depths were also used in the modelling as well as the effect of wild fish feeding on the fate of discharged farm waste. Diver deployed sediment traps on 8 transects at distances 5, 10, 15, 25, 35 and 50 m from the experimental cage were deployed for a period of 13 days and then analysed for AFDW.

Comparisons between observed and predicted AFDW resulted in a satisfactory regression line when appropriate adjustments were made to observations to account for natural background sedimentation (observed deposition = 1.04 predicted deposition +82 g m^{-2} y^{-1}, R^2=0.61, n=57).

Accuracy of predictions of AFDW were dependent on the level of deposition with the best accuracy achieved in the mid–range of deposition 501÷2,500 g m^{-2} y^{-1} (±29%) and reduced model performance at low (0÷500 g m^{-2} y^{-1}) and high (2,500+)

depositional zones (±111% and ±35% respectively). The model performance represents a significant improvement on current models validated for this type of environment and species.

The model was validated across a range of observed deposition values (65÷7535 g AFDW m^{-2} y^{-1}) which is uncommon for models of this type. The study also showed that model performance was improved when species–specific faecal settling data were used.

2.2.2.6 Data for setting up the MERAMOD® model

- current velocity data for an area close to the fish farm site (include information on heights of instruments above bed, total depth of water column at mooring, position of mooring, time (*e.g.* GMT) and direction formats (e.g. degrees true or magnetic);
- some knowledge of the vertical structure of the water column; shear in the water column can be represented in MERAMOD® by setting up layers in the model represented by different current velocity records;
- horizontal and vertical dispersion coefficients for the area;
- bathymetry of the area of interest either from a site survey or from an Admiralty chart of the area;
- number and dimensions (length, width and depth) of cages and the proposed/existing positions of these cages;
- feed input data (kg food d^{-1} for the farm) and mean fish size for the intended scenarios; information on the proportion of different fish species being farmed is also required as faecal settling rates vary between species (its required feed input data for the whole release period (kg food per unit time), general data requirements for modelling of total deposition (g m^{-2}) or sediment concentration (g kg^{-1}) of a component adhered to the waste material;
- information on water content and digestibility of the food to be used at the stage of the growing cycle to be modelled; some assessment of feed wasted according to husbandry practice is required (MERAMOD® defaults available);
- food and faecal settling velocity for the fish species being modelled (data from measurements undertaken in the MERAMED® project and literature values are available for sea bass and sea bream);

- background information on wild fish populations and their behaviour around the farm.

The following data would be useful for assessment of site characteristics and interpretation of model results:

- benthic macroinvertebrates present at the study site and the value of some benthic indices (*e.g.* species, total abundance, biomass, Shannon–Weiner index, Evennes, etc.) for sampling stations at the site;
- sediment type and characteristics for the proposed/existing site general data requirements for modelling of total deposition from a single release of waste material (g m^{-2}).

It is furthermore necessary to have additional information about:

- concentration of component on feed (g component kg^{-1} food);
- excretion of component by fish (*e.g.* 90% excreted);
- mass and total period of time for component in feed is being used (*e.g.* 100 kg $cage^{-1}$ over 7 days).

2.2.2.7 Model output

Predictions are given in an ASCII text data file showing predictions for the grid and for sampling stations. Predictions are also given in a x, y, z ASCII file which can be used in a contouring package for visual display.

2.2.2.8 Model data input in this study

Bathymetry data of the study site was provided with an echo sounder interfaced with a GPS positioning system. These data were fixed in latitude and longitude in relation to the cages.

Conversion of latitude and longitude to UTM datum was effected as linear scale and the model used a linear scale for grid generation. All of these data were entered into a contouring package (*e.g.* Surfer for Windows TM, Golden software), contoured and then exported as a grid with equal spacing between nodes. MERAMOD® can import these grids via ASCII files in DSAA format. Resolution of the data will depend on the survey method, but production of a grid with resolution not more than 25 m is suitable. In this study, a mesh of 25 m was used.

2.2.2.9 Cage layout and positioning

The cage layouts were obtained during an on–site survey carried out in June 2006 (Fig. 2.4). information about diameter, depth and rearing volume of each net cage were also collected from the farmers. Positioning of the cages in relation to the bathymetry was also recorded.

Fig. 2.4. Aerial view of "La Maricoltura Alghero" fish farming facility.

Tab. 2.1. Characteristics of the cages at "La Maricoltura Alghero" fish farming facility.

Measures	Pre-growing cages	Growing cages
Diameter (m)	8	16
Depth (m)	11	12
Volume (m^3)	800	2,500

2.2.2.10 Sampling station positioning

Positional data of the sampling stations were recorded and converted to the same datum as the positional data collected for cages and bathymetry (Fig. 2.5).

This method requires a reasonable degree of accuracy as the transect may be located along a high deposition gradient. Any error in station position in the model grid may result in a difference in predicted flux (g solids deposited m^{-2} bed yr^{-1}) of an order of magnitude.

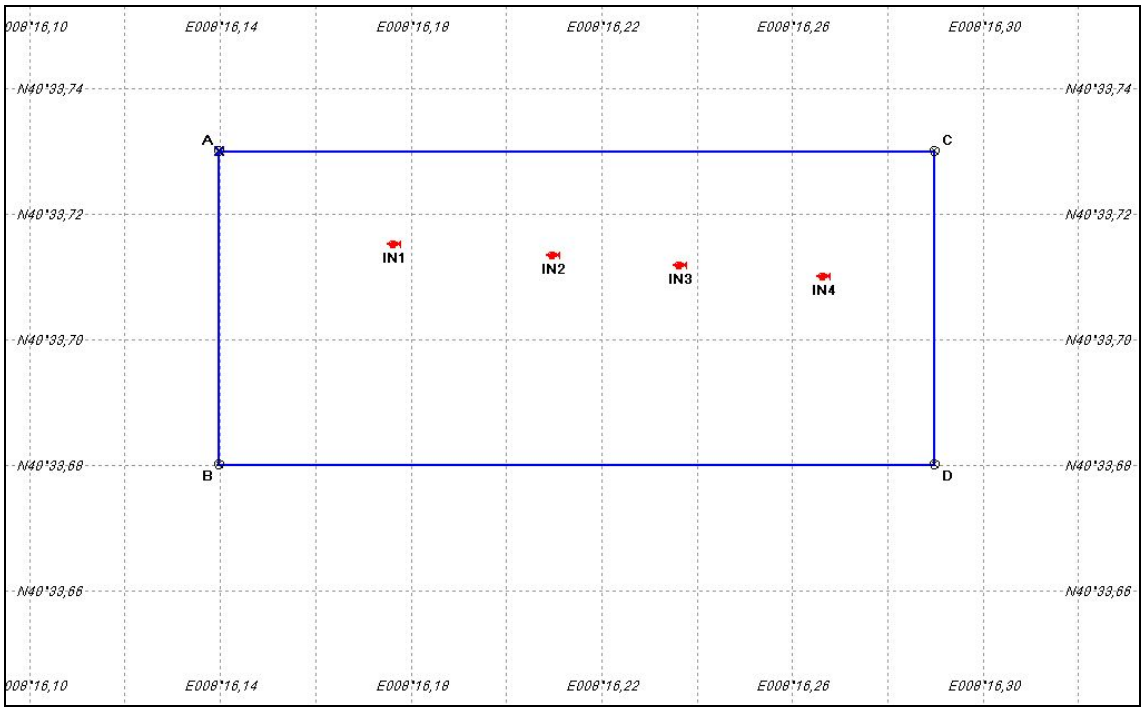

Fig. 2.5. Grid positioning of the fish farming area and sampling stations therein.

2.2.2.11 Husbandry data

Detailed information on husbandry were also collected from the farmers. In particular, sea breams was fed with an extruded feed pellet produced by the Aller Aqua Company characterized by 42÷56% protein, 18÷21% of fats, 7.5÷12% ash, 0.5÷2.5% fiber and 1.1÷1.4% phosphorus content. During the sampling period, the daily ration varied from 40 to 190 kg cage^{-1} of feed, with a daily average of 98 kg cage^{-1}. Feed conversion index was estimated to be 2.2:1.

Information on feed input (*i.e.* kg cage^{-1} d^{-1}) is required to run the model, but also information on fish species, number, mean weight, total biomass and pellet diameter should be obtained. These data will be required either for the farm, individual cage groups or individual cages depending on the study. The time interval of these data will also depend on the study, but is normally on a month by month basis. Feeding and defecation events can be set up in the model, so information on the number of feeding events daily should be obtained as well as the feeding method (*i.e.* hand or automatic).

Very little information exists in the literature on the evacuation of faecal material by farmed fish in relation to feeding times, so the user may wish to create defecation events over the course of the whole day rather than specific times, particularly for longer term studies.

2.2.2.12 Feed pellet considerations

The value of uneaten feed as a percentage of feed input is difficult to quantify and few studies exist in the scientific literature. Wastage depends on husbandry feeding method and the level of care taken to prevent overfeeding. Modelling studies currently use between 1 and 5% of feed input lost as uneaten feed pellets.

Feed digestibility and water content can usually be obtained from manufacturers specification sheets and default data are available in the model. Digestibility may well vary with feed pellet type, temperature and fish size. The 3 variables in this section cannot be varied over time within the period modelled nor between cages in the model. However, different model scenarios can be used to test the effect of varying these parameters.

2.2.2.13 Hydrodynamic data

Assessment of the quality of hydrographic data is essential and care should be taken in all aspects of data implementation as these sensitive data can affect model predictions considerably. The model requires current speed (in cm s^{-1}) and direction (degrees magnetic or true). In addition, the total depth at the location of the current meters and the depth and height of the current meters above the bed are required for input data.

In this study 3 data sets were implemented into the model and each set represented a layer with different current amplitude and direction. Hydrodynamic data were collected from July to December 2006 by using a Sensor Data Current Meter, model SD 2000 (Fig. 2.6) at 3 different depths levels (*i.e.* –5 m, –15 m, and –25 m from the surface; or 33 m, 23 m, and 13 m from the sea bottom, respectively). The sampling sites corresponded to the 4 vertices of the fish farming area (Fig. 2.6, Tab. 2.2).

Tab. 2.2. Coordinates of sampling points of hydrodynamic data collection.

Sampling points	Latitude	Longitude
A	40°33,7320'N	8°16,1496'E
B	40°33,6547'N	8°16,1711'E
C	40°33,7284'N	8°16,3452'E
D	40°33,6711'N	8°16,4491'E

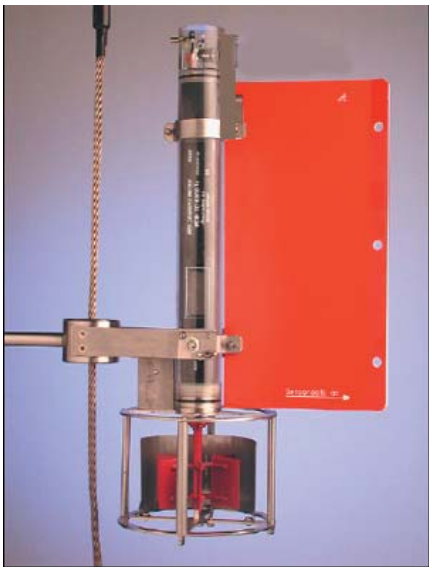

Fig. 2.6. Sensor Data Current Meter (model SD 2000).

Although the model does not use meteorological data, these are important for the interpretation of hydrographic measurements and for an assessment of general flow patterns in the study area.

2.2.2.14 Settling rates – faeces

Faecal settling rates of the farmed species are required by the model. The MERAMED® project measured settling rates of over 2000 sea bass and sea bream faecal particles and these are available as default data. If another farmed species is being modelled, faecal settling data for this species should be used, if available (Cromey, 2004).

Combined data for both bass and bream are also provided by the model, so these may be used to approximate the faecal settling rates of another species. In addition, where species (*i.e.* sea bass and sea bream) are not specified on a cage by cage basis in the model, these combined data best represent both species.

2.2.2.15 Settling rates – food

A relationship measured between feed pellet diameter and settling velocity is provided with the model so that an appropriate settling velocity can be set. The data for different pellet sizes and types (*i.e.* pelletized and extruded) is also available in addition to the general relationship. Where pellet diameter data are unknown, the average and standard deviation settling velocity of the whole data set should be used. Feed pellet

diameter is constant through the modelled period.

Information on feed pellets is more commonly available in the literature, particularly for salmonid feeds. The most likely reason is that feed pellets are easier to obtain for experimentation than faeces (Chen *et al.*, 1999; Holmer & Kristensen, 1994; Stewart & Grant, 2002).

2.2.2.16 Wild fish populations

The user can specify the percentage removal of uneaten feed pellets by wild fish in the water column and on the sea bed. In addition, removal of faecal material in the water column and sea bed can be modelled. Background information on wild fish populations at the study site in conjunction with the findings of the MERAMED® wild fish workpackage will assist in setting appropriate values in this module. In addition, some of the experimental work detailed in the quality assurance field handbook can be used to measure directly the effect of wild fish populations at the site on the fate of uneaten feed pellets. However, it is recommended that if modelling is being undertaken with a number of sites wild fish module settings should be constant across all sites during initial comparisons. As settings of this module directly effect predicted flux at the sea bed and benthic effect, adequate justification of settings used is required (Cromey, 2004).

2.2.2.17 Dispersion coefficient data

Drifting buoy or dye studies to assess the dispersion characteristics of a water body are not common for areas around fish farms. These studies are more commonly associated with long seas outfalls of domestic sewage, industrial discharges or marine dumping grounds. The MERAMED® project undertook a number of drifter surveys using six DGPS drifting buoys at Mediterranean fish farms (fix interval 30 s; accuracy 57% ± 1 m, 99% ± 4 m; sock depth 6 m; see Cromey, 2004 for details).

The main limitation of such studies is that only a snap shot of conditions are obtained during the survey period. In the absence of site specific data, examination of the range of values measured in the MERAMED® project may assist in setting an appropriate value. In Scotland, regulatory models apply a standardised horizontal dispersion coefficient (kx, ky) of 0.1 m^2 s^{-1} unless site specific data are provided (SEPA, 2003). Kx is resolved for the model x–axis (090° – 270° axis) and ky for the model y axis (000° – 180° true axis). It is recommended a value of 0.001 m^2 s^{-1} is used

for the vertical dispersion coefficient (kz) in MERAMOD® (Cromey, 2004).

2.2.2.18 Standardisation of data

MERAMOD® model input data generally fall into one of three categories comprising of site specific survey data, site specific data obtained from the farmer and standardised (default) data.

- Input data category 1 – Site specific data measured by survey hydrographic data (current speed and direction), bathymetry and cage and sampling station layout are necessary for modelling a site and should be given priority during survey planning. Occasionally dispersion coefficients are available from a specifically designed survey. For validation of the model predictions, benthic data and/or sediment trap data from the site can be used to test the model predictions if these are available. The wild fish population and the effects on the fate of wastes are also site specific.
- Input data category 2 – Site specific data obtained from the farmer husbandry data normally fall into this category and are required for accurate modelling. Occasionally information on cage layout is obtained from the farmer if not obtained during site survey.
- Input data category 3 – Standardisation of data (default data) – Percentage of feed input wasted as uneaten pellets, feed digestibility, feed water content, feed and faecal settling velocities are commonly standardised and assigned as default data across sites. This assists comparisons of different scenarios of the same site and between sites. Standardising these data means that differences in predicted flux and benthic effect will be primarily a result of the differences in the model input data of feed input, hydrography and bathymetry.
- Complexity of scenarios – It is good modelling practice to decide on objectives of a modelling study prior to the site survey so that the appropriate data can be collected during the survey. During the modelling exercise it is essential to begin with a simple scenario and increase the level of complexity in stages, with appropriate checks on model output. In addition to building confidence in the model, the effect on model predictions of increasing scenario complexity can be assessed. Increasing the complexity of the modelling scenarios can make little difference to model predictions, depending on the site characteristics and the

detail being added. A simple robust model that performs reliably is more desirable than a model requiring extensive data input. Despite this, a simple model should still use good quality input data and where the reliability of these data are uncertain, sensitivity should be tested.

2.2.3 Description of the sampling phases

In order to validate the results of the MERAMOD® model, sampling of sediment and macrozoobenthic fauna were carried out during 2 different phases. The first one was performed in June 2007 (hereafter T0) at 4 sampling stations positioned below the fish rearing cages (hereafter indicated as I1, I2, I3, and I4) at a distance of approximately 30 m from each other, and at 4 control sampling stations outside the fish farming area (hereafter indicated as O1, O2, O3, and O4) oriented toward the cardinal points and located at a distance of approximately 500 m from the centre of the fish farming area (Fig. 2.7).

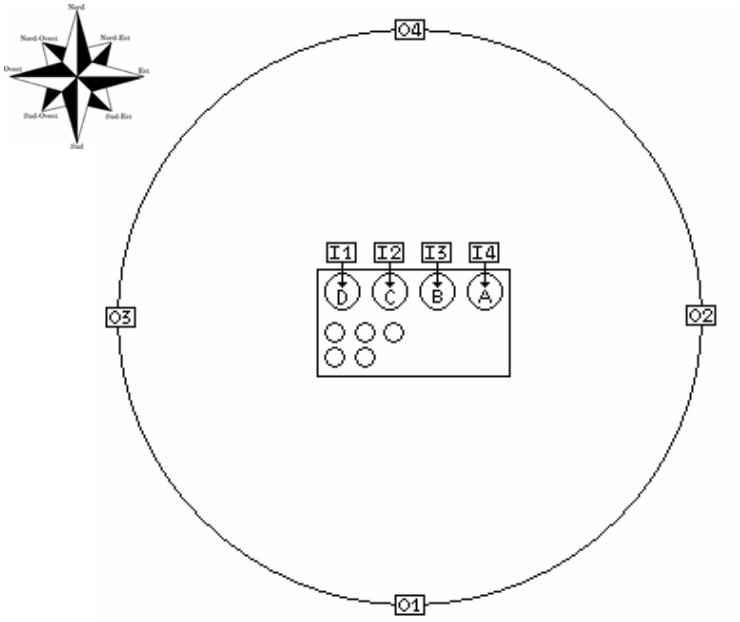

Fig. 2.7. Position of the stations during the first phase of the sampling.

Macrozoobenthic assemblages were sampled using a 0.132 m^2 Van Veen grab with 12 l volume (Fig. 2.8). During this phase of the study, 3 replicates were performed at each sampling station for a total of 24 samples of macrofaunal assemblages collected inside and outside the fish farming area.

Fig. 2.8. Sampling phase of macrofaunal assemblages with the Van Veen grab.

The second sampling phase was carried out in 3 distinct periods: July 2007 (hereafter T1), September 2007 (hereafter T2), and December 2007 (hereafter T3), respectively. Within the fish farming area, sampling of sediment and macrozoobenthic fauna were carried out at the same 4 stations (*i.e.* I1, I2, I3, and I4), while outside the perimeter of the facility samples were collected at 4 stations (*i.e.* O1, O2, O3, and O4) along a transect oriented towards West–East (Fig. 2.9).

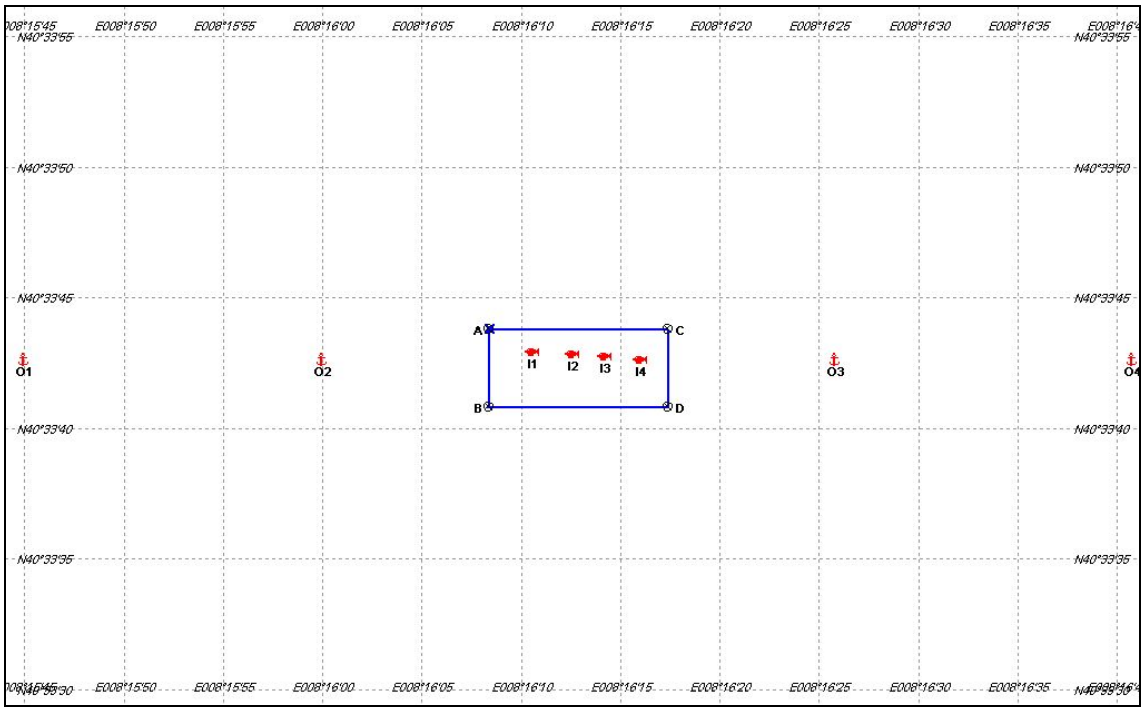

Fig. 2.9. Position of the stations during the second phase of the sampling.

In particular, 2 of these stations (the most outer, *i.e.* O1 and O4) were the same already investigated during the first sampling phase, while the others (the most inner, *i.e.* O2 and O3) were positioned at about half the distance (*i.e.* 250 m) far from the centre of the fish farming area (Fig. 2.9). Macrozoobenthic assemblages and sediment samples were collected using the same Van Veen grab used during the first sampling phase (i.e. T0).

During both the above mentioned–sampling phases, samples were placed in individual plastic bags and transferred to the laboratory for subsequent analyses within 2 h after field collection.

2.2.4 Sorting and identification of macrozoobenthos

In the laboratory, the samples of macrofaunal assemblages collected during both the 2 sampling phases were first sieved through a 500 μm mesh, and then preserved in 4% buffered formaldehyde. Rose Bengal solution was used as a staining agent to facilitate the sorting. All the collected specimens were identified at the lowest possible taxonomic level.

2.2.5 Statistical analyses

The number of taxa and abundance of individuals were counted for each sample. One–way analysis of variance (ANOVA) was then used to detect differences in mean number of taxa and individuals of the macrofaunal assemblages collected during both sampling phases inside and outside the fish farming area.

The homogeneity of variance was always tested using Cochran's test and data were appropriately transformed where necessary. If transformations did not produce homogeneous variances, ANOVA was used nevertheless on untransformed data after setting α=0.01 to compensate for the increased likelihood of type I error (Underwood, 1997). ANOVAs were always performed using the STATISTICA software package.

Macrofaunal assemblage structure was also analyzed by multivariate statistical techniques using the PRIMER software package (Plymouth Marine Laboratory; Clarke & Warwick, 2001). Similarity of macrofaunal assemblages between sampling stations was calculated using the Bray–Curtis coefficient (Bray & Curtis, 1957). The data of macrozoobenthos abundance for each monitoring period were pooled and graphically represented in two–dimensional ordination plots by non–metric multi dimensional scaling (nMDS) and cluster analyses.

A one–way analysis of similarities (ANOSIM) was then used to examine differences among macrofaunal assemblages collected inside (IN) and outside (OUT) the fish farming area. Finally, the similarity percentage (SIMPER) procedure (Clarke & Warwick, 2001) was employed to identify the major taxa contributing to dissimilarities between IN and OUT macrozoobenthic assemblages, with 2.0% being arbitrarily selected as the threshold value.

2.3 Results

2.3.1 MERAMOD® model

In the Figs. 2.10 and 2.11 are illustrated, respectively, the results obtained from the application of the MERAMOD® model to the actual scenario and the forthcoming enlargement of "La Maricoltura Alghero" fish farm.

Current velocity recorded in the sampling period ranged between 0.1 and 30.4 cm sec^{-1} at 5 m depth, between 0.1 and 7.6 at 15 m, and between 0.1 and 5.1 at 25 m from the water surface, respectively. Mean current direction ranged between 264 and 316 magnetic degree within the water column, and the maximum level of total solid flux deposition forecasted by the model was about 3,800 g m^{-2} bed $year^{-1}$ for both scenarios (Brambilla *et al.*, 2007b).

The impacted seabed surface was mainly located just under the fish farming facilities and increased from an area of about 5.6 ha in the actual scenarios (Fig. 2.10) to 7.3 ha in the hypothetical future situation (Fig. 2.11). The degradable fractions of total deposition were 76 and 78%, respectively. The maximum level of total carbon flux deposition predicted was equal to 1,350 g m^{-2} bed $year^{-1}$ for both scenarios, while the percentages of degradable carbon fraction amounted to 80 and 82% for each scenarios, respectively.

The installation of 4 new fish cages with a hypothetical mean daily amount of feed of 50 kg $cage^{-1}$ will increase the impact seabed surface to about 1.7 ha, with a total solid and carbon flux deposition levels of approximately 0–400 g m^{-2} bed $year^{-1}$ and 0–150 g m^{-2} bed $year^{-1}$, respectively, under the new supposed fish cages location (Fig. 2.11).

2.3.2 Macrozoobenthic assemblages

As far as macrofaunal assemblages is concerned, during the first phase of the study [*i.e.* June 2007, hereafter T0 (Fig. 2.8)] 216 taxa were globally identified, mainly composed by Polychaetes (87 species), Crustaceans (64 species), Nematodes (36 species) and Molluscs (22 species).

The histogram illustrated in Fig. 2.12 shows a clear dominance in mean number of macrozoobenthic taxa recorded at the stations positioned far from the cages (hereafter OUT) compared to those investigated near them (hereafter IN). Indeed, the mean number of taxa at the IN stations ranged between 17 (station I3) and 33 (station I1), whereas at the OUT stations ranged between 25 (station O4) and 63 (station O1),

respectively.

The mean number of individuals of macrofaunal taxa recorded at each station was also considerably lower in the IN stations (Fig. 2.13). In detail, the mean number of individuals at the IN stations varied from 71 (station I4) to 137 (station I1), whereas at the OUT stations varied between 154 (station O4) and 235 (station O1), respectively.

One–way ANOVA performed on both the mean number of macrozoobenthic taxa (Fig. 2.12) and mean number of individuals of (Fig. 2.13) showed significant differences for the factor "Position" (*i.e.* the distance from fish rearing cages) in both cases (Tab. 2.3).

As far as multivariate analysis is concerned, both the 2–dimensional nMDS ordination plot and the cluster dendrogram illustrated in Fig. 2.14 indicates significant differences in macrofaunal species composition at the 2 investigated areas (*i.e.* IN and OUT). In fact, while the samples collected near the fish rearing cages (IN) formed an evident cluster, the samples taken far the cages (OUT) tended to form 2 separated clusters: a bigger one containing the stations O1, O2 and O3 and a smaller one containing only station O4. Furthermore, one–way ANOSIM test indicated that there were significant differences (global $R=0.734$; $p<0.001$) between the macrozoobenthic assemblages found near and far from the fish rearing cages.

SIMPER analysis (which results are illustrated in Tab. 2.4) revealed that 9 species individually contributed by more than 2% to the dissimilarity between IN and OUT areas. In particular, species like *Pisione remota* (8.74%) *Photis longipes* (7.42%), *Capitella capitata* (7.09%) and *Polydora flava* (4.07%) were found to be responsible for this dissimilarity.

During the second phase of the study, the sampling of the macrozoobenthic assemblages (at the stations illustrated in Fig. 2.7) was carried out during 3 different periods, and specifically: July 2007 (hereafter T1), September 2007 (hereafter T2) and December 2007 (hereafter T3), respectively.

As regards the results for T1, 202 taxa were globally found, mainly Polychaetes (84 species), Crustaceans (55 species), Nematodes (33 species), and Molluscs (25 species). In the samplings of T2, a total of 192 taxa was collected, largely represented by Polychaetes (72 species), Nematodes (55 species), Crustaceans (35 species), and Molluscs (25 species). Finally, during the last period of this phase of the study (T3), 153 taxa mainly belonging to Polychaetes (59 species), Nematodes (44 species), Crustaceans (22 species), and Molluscs (20 species), were overall identified.

One–way ANOVA performed on mean number of taxa (Fig. 2.15) and mean number of individuals (Fig. 2.16) of the macrozoobenthic assemblages collected during T1 showed significant differences for the factor "Position" (*i.e.* the distance from fish rearing cages) in both the cases (Tabs. 2.5 and 2.6). As far as T2 is concerned, significant differences were found only for macrofaunal taxa diversity (Fig. 2.17, Tab. 2.5), but not for the number of individuals (Fig. 2.18, Tab. 2.6). The same results were found for the samples of macrozoobenthos collected during T3 (Figs. 2.19 and 2.20) (Tabs. 2.5 and 2.6).

The 2–dimensional nMDS ordination plot relative to the first sampling of the second phase of the study (*i.e.* T1) reported in Fig. 2.21 shows a clear–cut separation among 3 clusters of macrofaunal assemblages. In particular, besides noting a first separated cluster formed by the OUT stations farer from the cages (*i.e.* O1 and O4), and a second one composed by all the IN stations, a third minor cluster formed by the OUT stations at an intermediate distance from the cages (*i.e.* O2 and O3) can be observed. This latter is very close to that formed by the IN stations. However, the 2 principal clusters are divided by less than 10% similarity in the dendrogram reported in Fig. 2.21.

The nMDS ordination plot and the cluster dendrogram for the sampling carried out in September 2007 (T2, Fig. 2.22) illustrates a fairly similar trend, except for the samples collected in the station O4, that clustered apart from the others. These results were also confirmed by nMDS ordination plot and the cluster dendrogram for the sampling carried out in December 2007 (T3) reported in Fig. 2.23.One–way ANOSIM test revealed that there were significant differences between the 2 groups (IN and OUT) for all 3 sampling periods, with values of global $R=0.463$ ($p<0.001$) for T1, global $R=0.565$ ($p<0.001$) for T2, and global $R=0.603$ ($p<0.001$) for T3, respectively.

SIMPER analysis (which results are illustrated in Tabs. 2.7, 2.8 and 2.9) revealed that less than 10 taxa individually contributed by more than 2% to the dissimilarity between the macrofaunal assemblages collected at each sampling time. In particular, for T1 (Tab. 2.7) *Photis longipes* (16.92%), *Aricidea capensis bansei* (8.5%), *Capitella capitata* (6.77%), and *Pisione remota* (5%) were primarily responsible for the dissimilarities, as well as *Photis longipes* (19.73%), *Linhomoeus* sp. (12.62%), *Abludomelita aculeata* (5.85%) and *Chone duneri* (5.49%) for T2 (Tab. 2.8). During T3 (Tab. 2.9) the principal contribution to the dissimilarities was provided by *Linhomoeus* sp. (19.01%), *Photis longipes* (13.47%), *Sebateria sp*. (10.47%) and *Chone duneri* (5.32%).

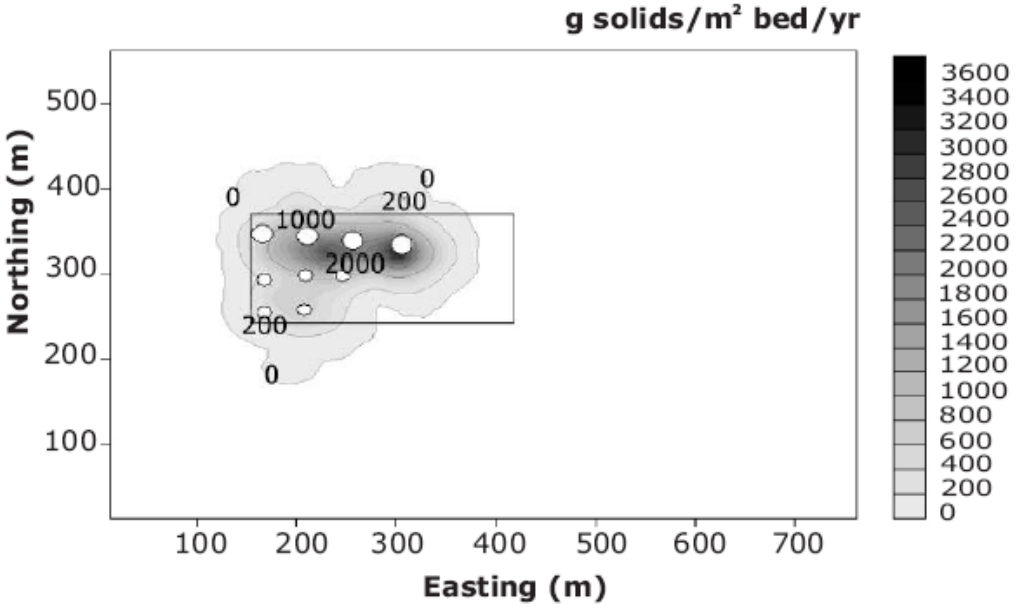

Fig. 2.10. Total solid flux deposition (g solids m^{-2} bed $year^{-1}$) forecasted by the MERAMOD® model for the actually scenario of the fish farm studied.

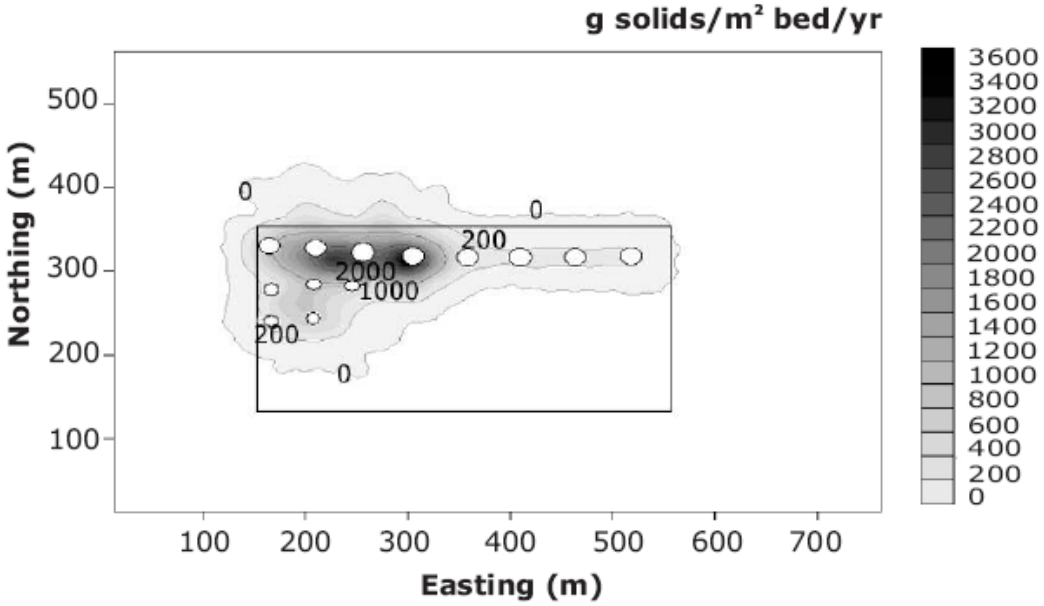

Fig. 2.11. Total solid flux deposition (g solids m^{-2} bed $year^{-1}$) forecasted by the MERAMOD® model for the future enlargement of the fish farm studied.

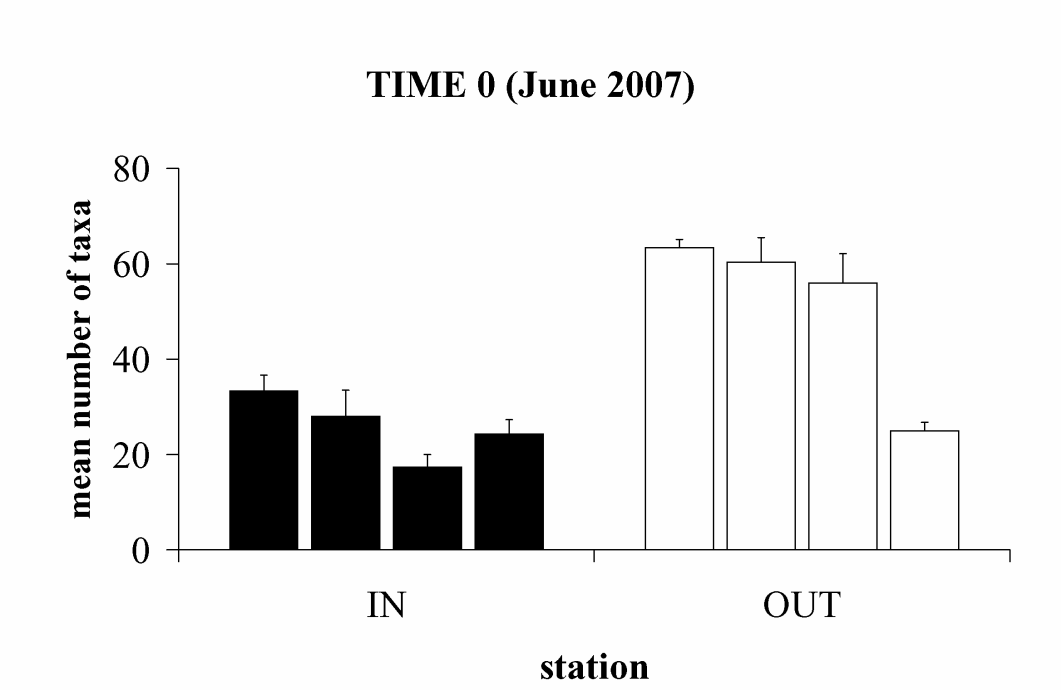

Fig. 2.12. Number of macrozoobenthic taxa collected at each station during the first phase of the study.

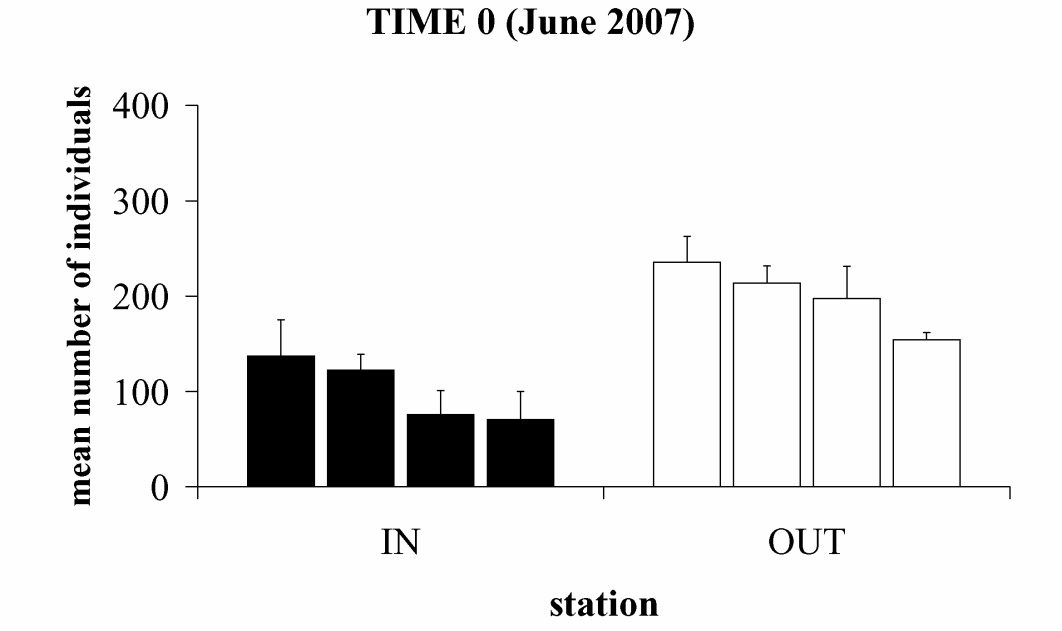

Fig. 2.13. Number of individuals of macrofaunal taxa collected at each station during the first phase of the study.

TIME 0 (June 2007)

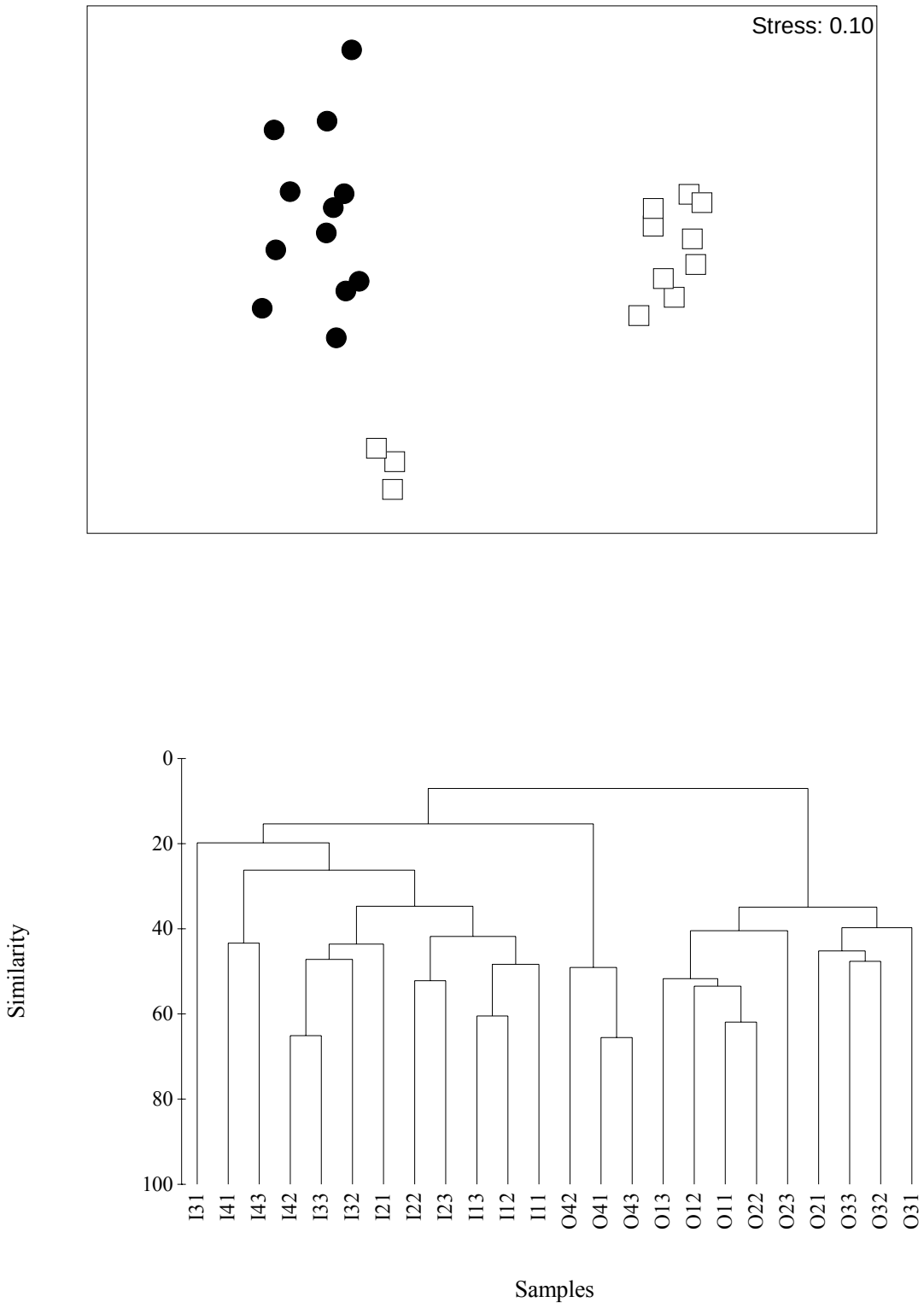

Fig. 2.14. Multidimensional scaling and cluster analysis performed on the Bray–Curtis similarity matrix to test the differences among macrofaunal assemblages inside and outside the fish farm area during the first phase of the study (I=IN; O=OUT).

Tab. 2.3. Results of ANOVAs for the effect of distance from cages on the mean number of macrofaunal taxa and individuals detected in sampling stations during the first phase of the study (significant differences are marked in bold).

		Taxa			Individuals		
Source of variation	df	MS	F	*p*	MS	F	*p*
Position	1	2.53	18.78	**0.000**	58410.67	23.89	**0.000**
Residuals	22	0.14			2444.96		
Cochran's test			0.602	ns		0.547	ns
Transformation				ln(*x*+1)			none

Tab. 2.4. Results of SIMPER analysis showing macrozoobenthic taxa contributing most (in order of decreasing percentage) to dissimilarity between IN and OUT areas and their average abundance during the first phase of the study.

Species	Dissimilarity contribution	IN (avg. abundance)	OUT (avg. abundance)
Pisione remota	8.74	0.00	25.08
Photis longipes	7.42	4.25	15.08
Capitella capitata	7.09	20.00	0.08
Polydora flava	4.07	11.67	0.00
Protodorvillea kefersteini	3.82	0.08	11.25
Aricidea capensis bansei	3.76	11.25	2.67
Sphaerosyllis hystrix	2.63	0.00	7.50
Lumbrineris latreilli	2.13	0.17	6.00
Apseudes latreillii	2.08	0.25	4.67

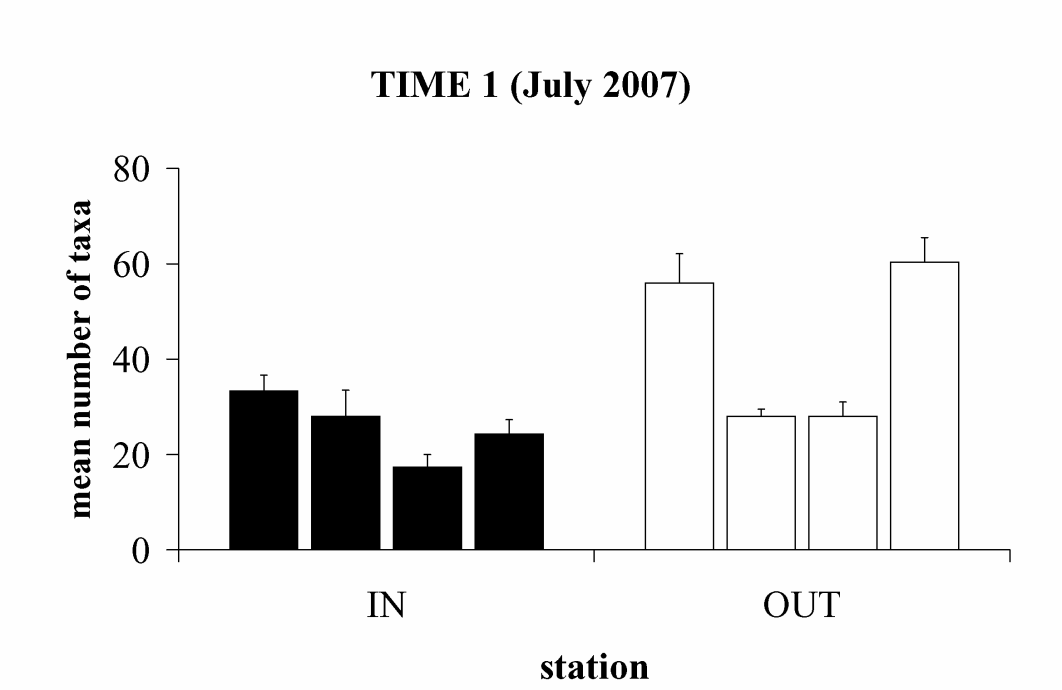

Fig. 2.15. Number of macrozoobenthic taxa recorded at each station during the first sampling of the second phase of the study.

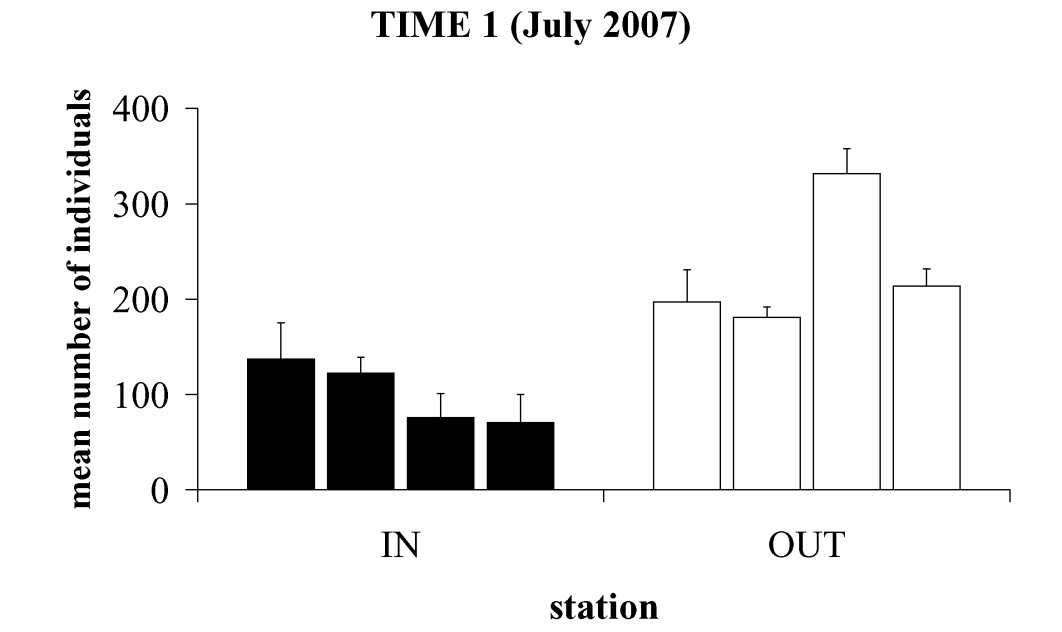

Fig. 2.16. Number of individuals of macrofaunal taxa collected at each station during the first sampling of the second phase of the study

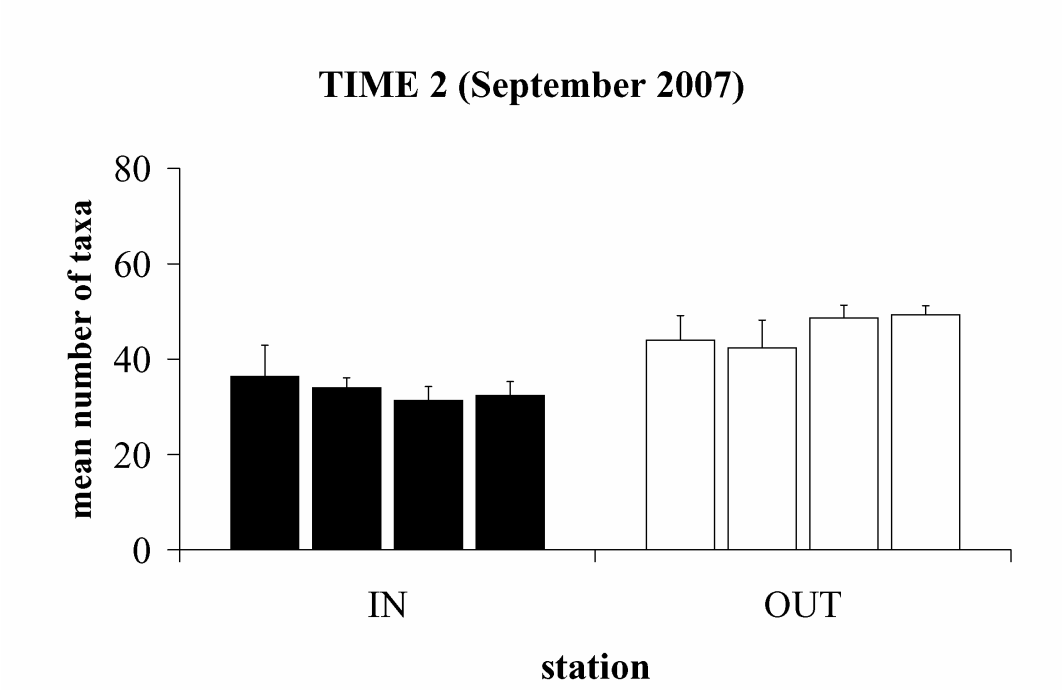

Fig. 2.17. Number of macrozoobenthic taxa recorded at each station during the second sampling of the second phase of the study.

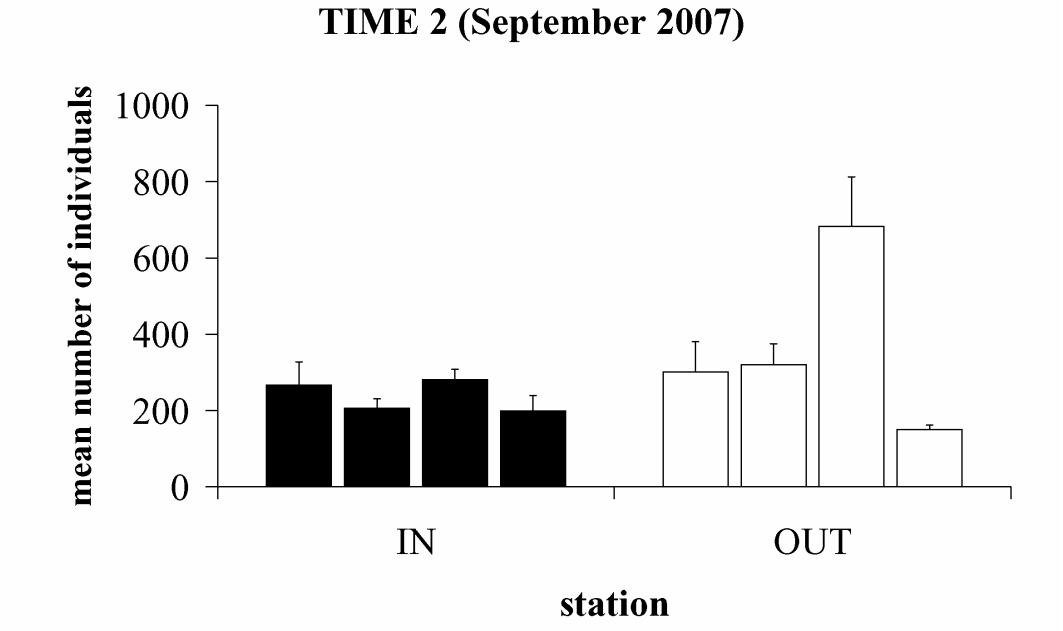

Fig. 2.18. Number of individuals of macrofaunal taxa collected at each station during the second sampling of the second phase of the study.

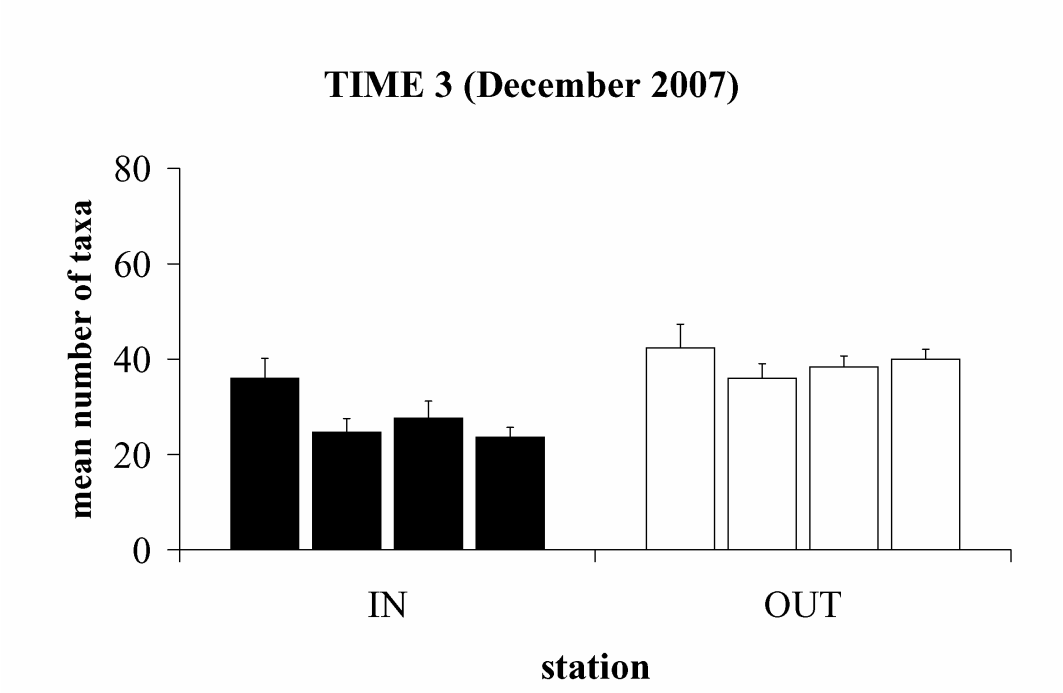

Fig. 2.19. Number of macrozoobenthic taxa recorded at each station during the third sampling of the second phase of the study.

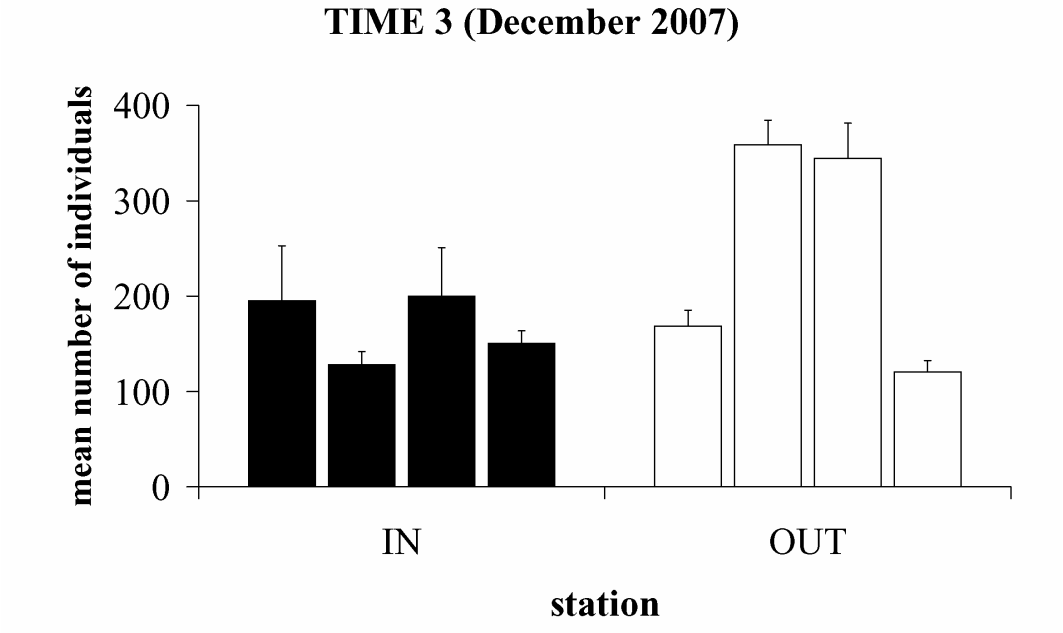

Fig. 2.20. Number of individuals of macrofaunal taxa collected at each station during the third sampling of the second phase of the study

Tab. 2.5. Results of ANOVAs for the effect of distance from cages on the mean number of macrozoobenthic taxa detected in sampling stations (significant differences are marked in bold).

		T_1			T_2			T_3		
Source of variation	df	MS	F	*p*	MS	F	*p*	MS	F	*p*
Position	1	1.36	10.36	**0.004**	950.04	21.68	**0.000**	748.17	19.06	**0.000**
Residuals	22	0.13			43.81			39.26		
Cochran's test			0.591	ns		0.553	ns		0.621	ns
Transformation				ln(*x*+1)			none			none

Tab. 2.6. Results of ANOVAs for the effect of distance from cages on the mean number of individuals of macrofaunal detected in sampling stations (significant differences are marked in bold).

		T_1			T_2			T_3		
Source of variation	df	MS	F	*p*	MS	F	*p*	MS	F	*p*
Position	1	100104.17	25.77	**0.000**	94000.17	3.08	0.093	38001.04	4.27	0.0507
Residuals	22	3884.33			30546.42			8890.63		
Cochran's test			0.655	ns		0.917	<0.01		0.752	ns
Transformation				none			none			none

TIME 1 (July 2007)

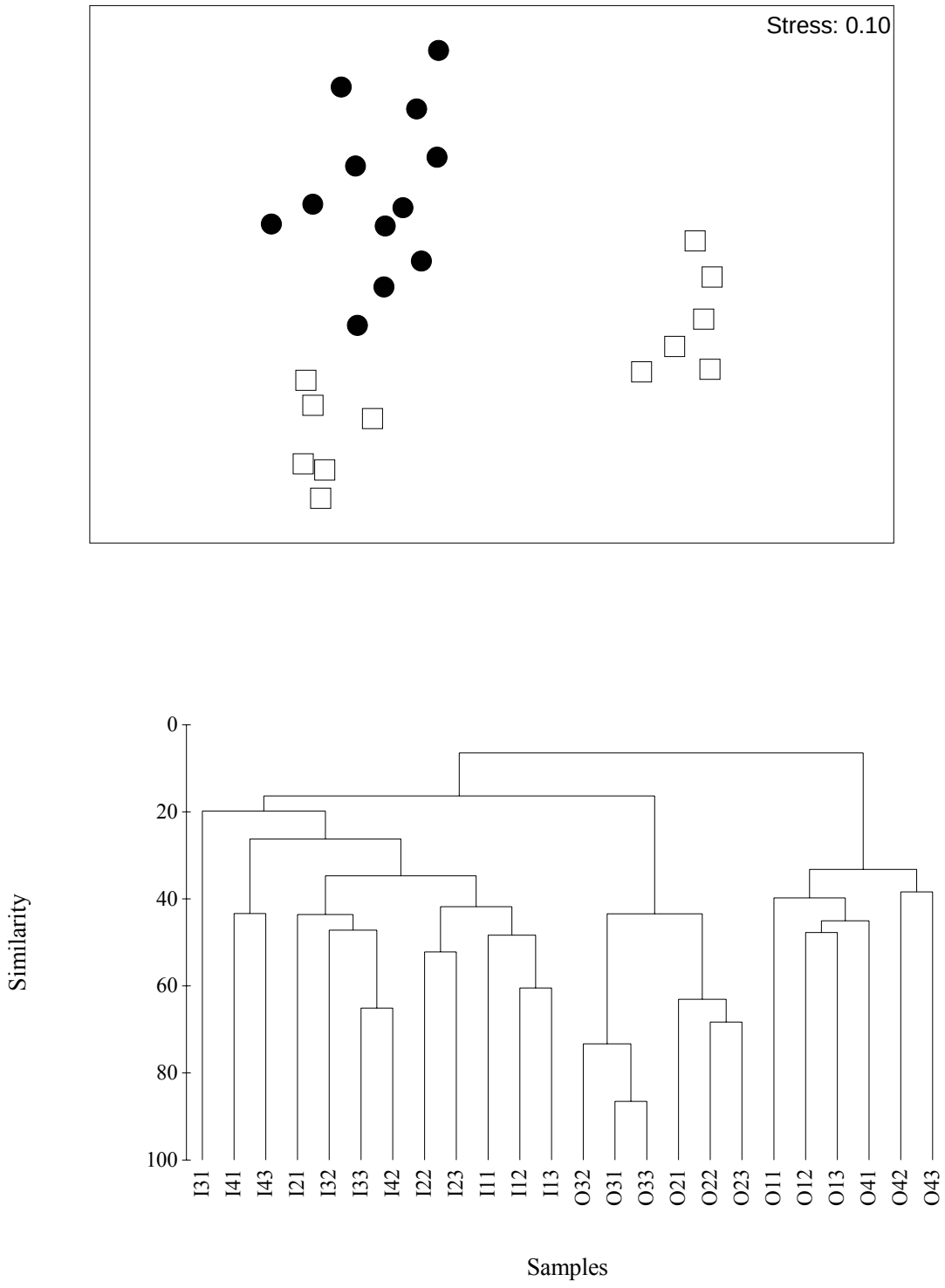

Fig. 2.21. Multidimensional scaling and cluster analysis performed on the Bray–Curtis similarity matrix to test the differences among macrofaunal assemblages inside and outside the fish farm area during the first sampling of the second phase of the study (I=IN; O=OUT).

TIME 2 (September 2007)

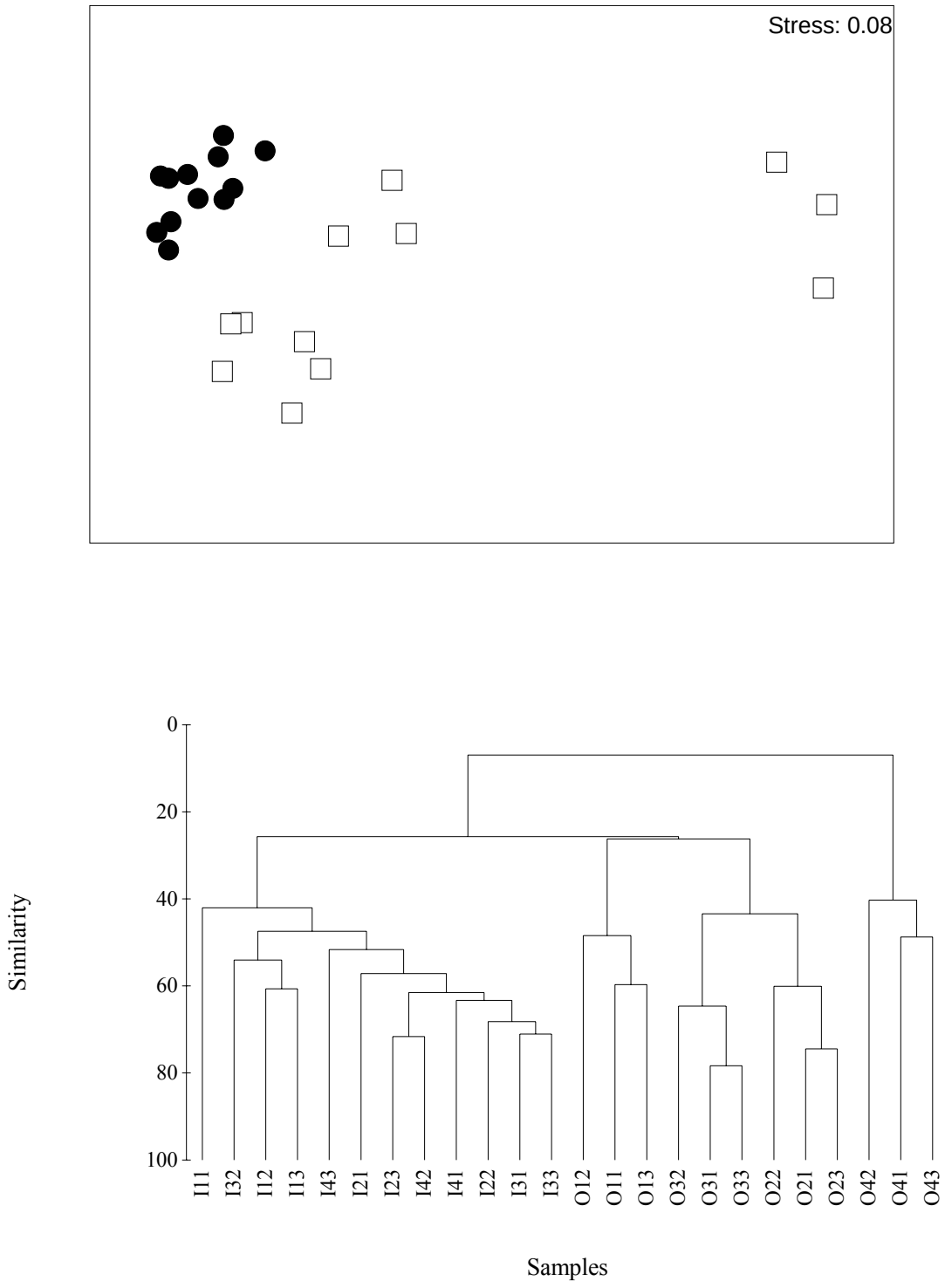

Fig. 2.22. Multidimensional scaling and cluster analysis performed on the Bray–Curtis similarity matrix to test the differences among macrofaunal assemblages inside and outside the fish farm area during the second sampling of the second phase of the study (I=IN; O=OUT).

TIME 3 (December 2007)

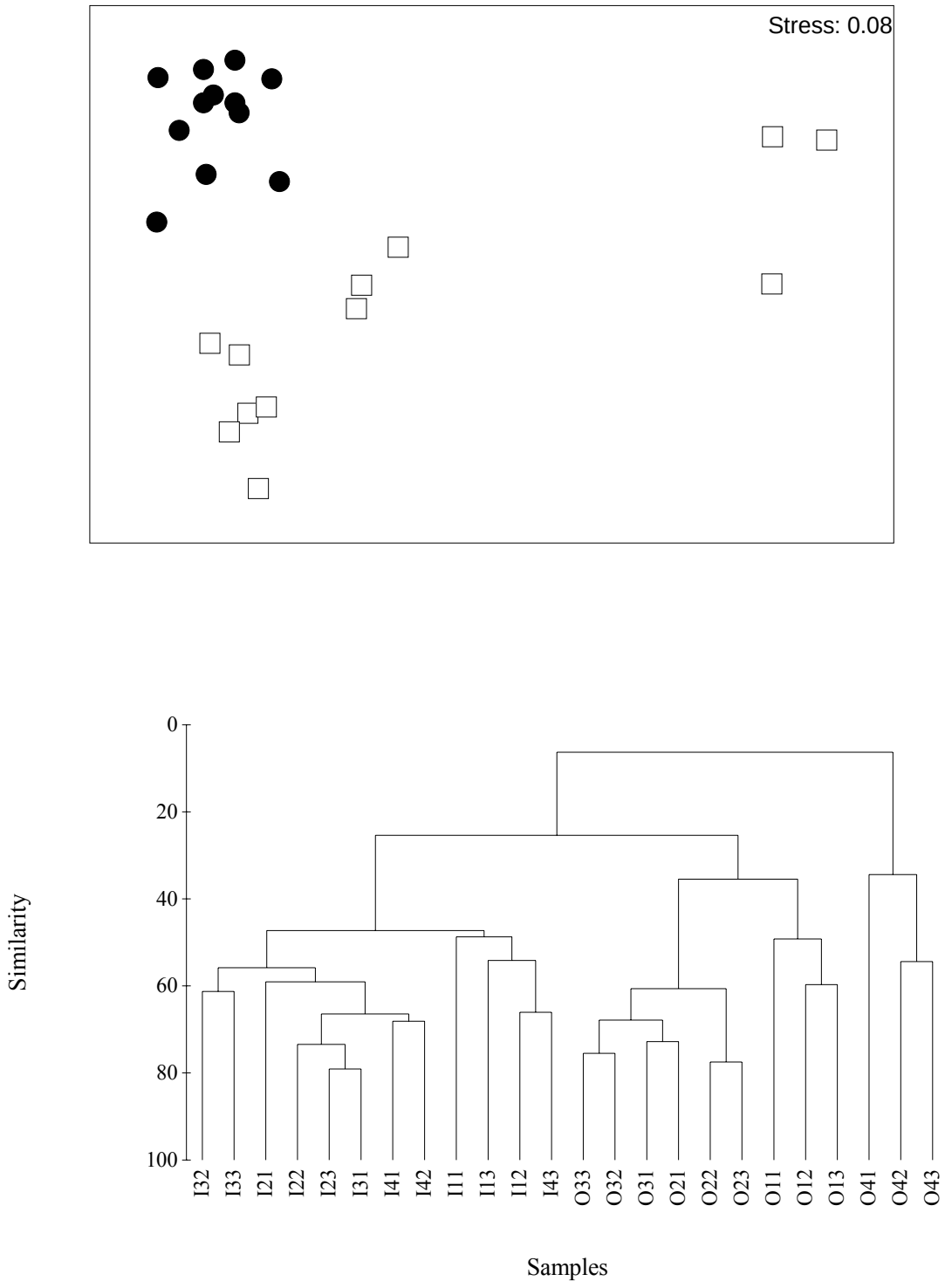

Fig. 2.23. Multidimensional scaling and cluster analysis performed on the Bray–Curtis similarity matrix to test the differences among macrofaunal assemblages inside and outside the fish farm area during the third sampling of the second phase of the study (I=IN; O=OUT).

Tab. 2.7. Results of SIMPER analysis showing macrozoobenthic taxa contributing most (in order of decreasing percentage) to dissimilarity between IN and OUT areas and their average abundance during the first sampling of the second phase of the study (July 2007).

Species	Dissimilarity contribution	IN (avg. abundance)	OUT (avg. abundance)
Photis longipes	16.92	4.25	59.50
Aricidea capensis bansei	8.50	11.25	22.50
Capitella capitata	6.77	20.00	0.08
Pisione remota	5.00	0.00	13.33
Polydora flava	3.82	11.67	1.25
Protodorvillea kefersteini	3.40	0.08	9.58
Magelona filiformis	2.32	4.58	6.08
Bathyporeia phaiophthalma	2.31	0.67	7.25
Lumbrineris latreilli	2.03	0.17	5.50

Tab. 2.8 Results of SIMPER analysis showing macrozoobenthic taxa contributing most (in order of decreasing percentage) to dissimilarity between IN and OUT areas and their average abundance during the second sampling of the second phase of the study (September 2007).

Species	Dissimilarity contribution	IN (avg. abundance)	OUT (avg. abundance)
Photis longipes	19.73	85.75	76.17
Linhomoeus sp.	12.62	2.67	74.25
Abludomelita aculeata	5.85	27.17	4.58
Chone duneri	5.49	5.50	26.33
Bathyporeia phaiophthalma	3.85	3.17	21.42
Aricidea capensis bansei	3.63	7.75	20.50
Viscosia sp.	2.93	15.33	3.67
Polydora flava	2.83	12.33	1.17
Capitella capitata	2.49	10.67	0.33
Ophryotrocha sp.	1.97	9.00	0.00

Tab. 2.9. Results of SIMPER analysis showing macrozoobenthic taxa contributing most (in order of decreasing percentage) to dissimilarity between IN and OUT areas and their average abundance during the third sampling of the second phase of the study (December 2007).

Species	Dissimilarity contribution	IN (avg. abundance)	OUT (avg. abundance)
Linhomoeus sp.	19.01	8.75	78.67
Photis longipes	13.47	53	13.92
Sebateria sp.	10.47	33	0
Chone duneri	5.32	4.17	19.42
Aricidea capensis bansei	3.93	3.42	16.42
Bathyporeia phaiophthalma	2.55	0.25	9.5
Paradoneis ilvana	2.29	8.5	1.67
Viscosia sp.	2.17	8.08	8.75
Ophryotrocha sp.	1.91	6.25	0

2.4 Discussion and conclusions

To date, the MERAMOD® model has already been validated for a number of fish farms along the Mediterranean coasts (Brambilla *et al.*, 2007b). The model has also been applied across a range of different scenarios in terms of the environmental conditions and farm size. Importantly, the model has also been tested for sites where a range of hydrodynamic conditions and bathymetry were measured.

As already pointed out by Cromey *et al.* (2002a) for DEPOMOD model from which MERAMOD® originates, however, testing the sensitivity of particle starting position in the cage was undertaken at an early stage of model development and, as a result, starting positions were assigned as random for benthic module validation. Although some observational evidence suggests that defecation from fish generally occurs at the surface directly after feeding, it is unlikely that any particular spatial distribution in the cage could be modelled accurately.

Furthermore, depending on cage design and biofouling (see Chapter 3 for details), food pellets may have a starting position close to the centre of the cage bottom due to the pellets rolling towards the centre before leaving the cage. Considering all these factors and the sensitivity of this parameter, it is prudent to assign random starting positions in the cage for continuity across all sites.

Nevertheless, some caution is required when assigning model parameters at the validation stage to obtain a best fit between predicted and observed data which subsequently cannot be changed by the user (*e.g.* critical threshold for resuspension, consolidation time parameter).

Although this fact implies that these parameters are site–specific, varying of the parameters within limits set according to literature values, subsequent sensitivity analysis and testing at other sites justifies cautious use of the same parameters across all sites. A requirement for the user to determine critical threshold for resuspension, for example, for every site modelled would severely restrict use of the model. Indeed, several studies available in the current literature showed that this has been measured for only a few fish farm sites globally (Sarà, 2007a, b and references therein cited).

Other parameters, such as the time consolidation parameter, could be used in a more site–specific manner if required. For example, decreasing its value to describe increased bioturbation would cause particulate material to be removed from the resuspendable fluff layer more quickly. Ideally, this parameter would be varied spatially across the model grid according to the abundance of different types of bioturbators

surrounding the farm, but would not easily be validated (Cromey *et al.*, 2002a).

From a general point of view, features of the cultivated animals in aquacultural activities (*i.e.* fish, shrimps and molluscs) and their metabolic processes, ecosystem type (mixed, marine and fresh water), typology of cultivation (ponds, cages in open waters, land–based, etc.), influence from receiving aquatic ecosystems (*e.g.* hydrodynamics of water bodies and water residence time), and management practices (*e.g.* annual biomass productions, feed supply, etc.) have been invoked as major factors affecting the area surrounding farms (Islam, 2005).

These high and complex sources of variability lead only to a very fragmented panorama, from which one can generalise only with great difficulty about the phenomenon of environmental effects of aquaculture.

Typical characteristics of the Mediterranean marine environment might result in considerable differences when compared with the patterns induced by the salmon industry, for which most of the models to evaluate environmental impacts were formulated. In fact, in this geographical area the microtidal regime can influence dispersion of settling organic material; the high temperature can affect sediment metabolism and oxygen availability; light availability because of more sunshine and water transparency allows for photosynthesis deeper in the water column; and the low–biomass and high–diversity benthic communities adapted to oligotrophic conditions can respond differently to sudden increases in organic content of the sediment (Karakassis *et al.*, 2000).

Therefore, in Mediterranean Sea the impacts of fish farms on the seabed can greatly vary both in terms of geochemistry and macrofaunal assemblages composition. As a general rule, the silty sediment site can show typical characteristics of the effects of organic enrichment, comparable to those observed in the vicinity of salmon cage farms. In coarse sediments, instead, the effects on geochemical properties can dramatically vary, even if macrofaunal assemblages may not quantitively decrease. In all cases diversity in the immediate vicinity of the farming facilities decreases, albeit the spatial extent of this effect can be quite limited (Karakassis *et al.*, 2000).

The results from the present study quantitatively report the effects of aquaculture loadings on the surrounding area of a caged fish farming facility in North Western Sardinia using the MERAMOD® model, although at present the effects of such aquacultural activities appear highly location–specific, and linked to the hydrodynamic regime, rather than to the cultivated biomass or number of cultivated species or type of

species.

On the other hand, a realistic application of a model like MERAMOD® would also have to consider: a) the diameter of the actual feed distributed to fish (Vassallo *et al.*, 2006), and b) seasonal variations of water temperature. In fact, even if some data about physical properties of feed pellets have been published in the framework of salmonid rearing, there is a complete lack of information related to the Mediterranean Sea, as regards typical values of temperature, salinity and feed composition for the main reared fish species like gilthead sea bream and sea bass.

Determination of the settling velocity of the uneaten feed pellets has been shown to be a key parameter in the accuracy of the prediction of models. Consequently, collaboration with farmers, essential for nutritional data collection and hydrological measurements, is also of primary importance to improve aquaculture impact predictions. In similar studies, therefore, this recommendation will be followed to develop a reliable waste dispersion model for Mediterranean marine aquaculture (Vassallo *et al.*, 2006).

In conclusion, this application of the MERAMOD® model in Sardinian waters confirms once more its role as a potential tool to enable better the predictive capability of impact from large marine cage fish farming on the seabed surface, and to improve objectivity in the regulatory decision–making processes.

As the benthic response module included in the model can also forecast the effects of the solids deposition on the benthic community (by predicting variations of the biodiversity indices), further research is needed to validate these putative variations of the benthic environment below and in the neighbourhood of the fish farming facilities investigated (Brambilla *et al.*, 2007b).

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Chapter 3

ASSESSMENT OF BIOFOULING ON CAGE NETS

3.1 Introduction

Biofouling occurs as a result of the settlement and growth of sedentary and semisedentary organisms on artificial structures placed in water (Venugopalan & Wagh, 1990). It is mostly composed of organisms but also has organic or mineral material trapped in the biological tangle. Fouling is a major problem for submerged surfaces (Read & Gordon, 1991) and particularly in aquatic culture (Porter, 1981; Huse *et al.*, 1990; Sarà *et al.*, 2007).

Multi–filament netting material is an ideal substrate for fouling. It is non–toxic, contains many crevices which can entrap and protect settling organisms and has a high surface–area to volume ratio (Dubost *et al.*, 1996).

The waters of fish farms are conducive to rapid fouling development because nutrient and organic loading from feed wastage, fish excretion and faecal production increase the growth of algae (Ruokolahti, 1988). Therefore, as already said in Chapter 1, biofouling represents a severe problem to mariculture activities (Hodson *et al.*, 1995, 1997; Swift *et al.*, 2006).

The communities of organisms that develop on suspended, aquaculture fish cages (Fig. 3.1) result in added weight and drag to the cage, thus reducing water flow (Fig. 3.2) and affecting cage behaviour during rough seas and high current conditions (Beveridge, 1996 and references therein; Swift *et al.*, 2006).

Fig. 3.1. Biofouling on a cage collar in the Mediterranean Sea.

Floating cage culture using nets is particularly vulnerable during the hot season (Moring & Moring, 1975; Milne, 1976). Environmental conditions at the site rapidly

deteriorate and become the cause of stress for the cultured fish (Inoue, 1972; Lovegrove, 1979). Studies of net cages fouling rate and organisms involved have been detailed for marine sites of many different parts of the world (Milne, 1970, 1976; Lovegrove, 1979).

A variety of methods, sometimes original and/or innovative (Lodeiros & García, 2004; Ross *et al.*, 2004; Braithwaite *et al.*, 2007; Forrest *et al.*, 2007; Sala & Lucchetti, 2008), have been developed to control biofouling, although it still remains a problem at culture sites worldwide (Hodson *et al.*, 2000; Relini & Merello, 2004; Braithwaite & McEvoy, 2005; Greene &, Grizzle, 2007).

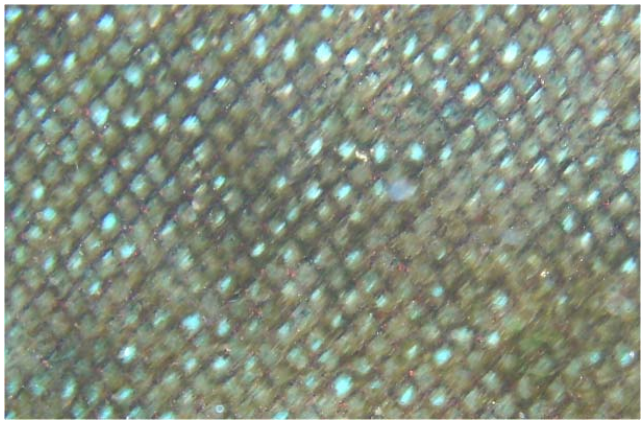

Fig. 3.2. Biofouling organisms on a cage net.

Ecological succession is a complex process and may involve a variety of potential causal mechanisms that influence species composition over time (Connell, 1978; Lubchenco, 1986; Hubbell, 1997; Sousa, 2001). In particular, the colonization of a new surface (Oliveira, 1997) is composed of 4 main phases, constituting an overlapping time sequence: 1) biochemical conditioning; 2) bacterial colonization; 3) unicellular; and 4) multicellular eukaryotic fouling (Wahl, 1989).

In the course of this sequence, the prevailing processes change progressively from purely physical to predominantly biological, even though the influence of near–bottom hydrodynamics, for example, can be important at the level of larval settlement (Butman, 1987).

It assumes primary importance of determining forces on individual elements caused by sea currents, which are heavily influenced by the intensity of biofouling on the net cages (Swift *et al.*, 2006). In fact, when the structure is in its depth, this phenomenon is responsible for different effects on the structure and mass bred at the

same time due to the increase in vertical stress for the cumbersome structure, the increase for both horizontal stresses that the hydrodynamic resistance, and the decline in the recirculating water with reduction of the exchange of oxygen and removal of toxic metabolites. Thus, in those environments where lateral drift is more pronounced than sedimentation, biofouling descriptors appear to be a promising tool to assess modifications induced by anthropogenic stress (Sarà *et al.*, 2007; Mannino & Sarà, 2008).

The present study, therefore, aimed to describe the settlement and development of biofouling organisms on cage nets of a fish farming facility in North Western Sardinia. This was done by investigating putative different ways of growth of both macrophytobenthic and macrozoobenthic species on net panels inside cages where big and small sea breams (*Sparus aurata*, Linnaeus 1758) were reared. Finally, controls outside cages were also considered in order to evaluate: 1) if there was a different grazing activity of different–sized sea breams on the cage nets, and 2) if this fact could affect the structure of biofouling communities on the cage nets.

3.2 Materials and methods

3.2.1 Field methods and experimental design

The activities in the field started in October 2007 off the North Western coast of Sardinia (Latitude 40°33'43.9"N; Longitude 8°16'09.0"E) at the fish farming facilities of "La Maricoltura Alghero" s.r.l., and, at the moment, the study is still in progress. This fish farm (already described in detail in Chapter 2) is located in the Alghero Bay at a distance of about 1 nautical mile from the coastline. It covers a quadrilateral area of about 2.15 hectares on a muddy/sandy bottom located at a depth of approximately 40 m (Brambilla *et al.*, 2007).

The experiment started with the installation of custom–made panels (suitable for the development of biofouling, Fig. 3.3) on the nets of floating cages in which sea breams of different size were reared. In detail, the aforementioned–panels were immersed inside 4 fish rearing cages, 2 of which containing large (*i.e.* >150 g) and 2 containing small (*i.e.* <50 g) sea breams, at a constant depth of 1 m and 5 m, respectively.

Two series of control panels were also placed outside the cages at the same depth levels. Overall, with the aim of sampling 3 panels per group every 3 months for a year (*i.e.* approximately every season), 12 panels were positioned at each depth level considered inside or outside the cages studied using cable ties (Fig. 3.4). Consequently, a total of 144 panels (*i.e.* 3 panels x 4 seasons x 2 depth levels x 3 experimental groups x 2 cages each) were used for the whole study period.

Fig. 3.3. A custom–made panel used in the present study.

In detail, each experimental panel was composed by a piece of polyamide net with a mesh size of 8 mm per side (the same used for fish cages), assembled on a 25x25 cm polyethylene frame of 1.6 cm outside diameter.

After they were assembled, all the panels were individually labelled by means of plastic labels with the following criterium: from 1 to 72 (white labels) for panels positioned at a depth of 1 m, and from 101 to 172 (yellow labels) for panels positioned at a depth of 5 m (Fig. 3.3).

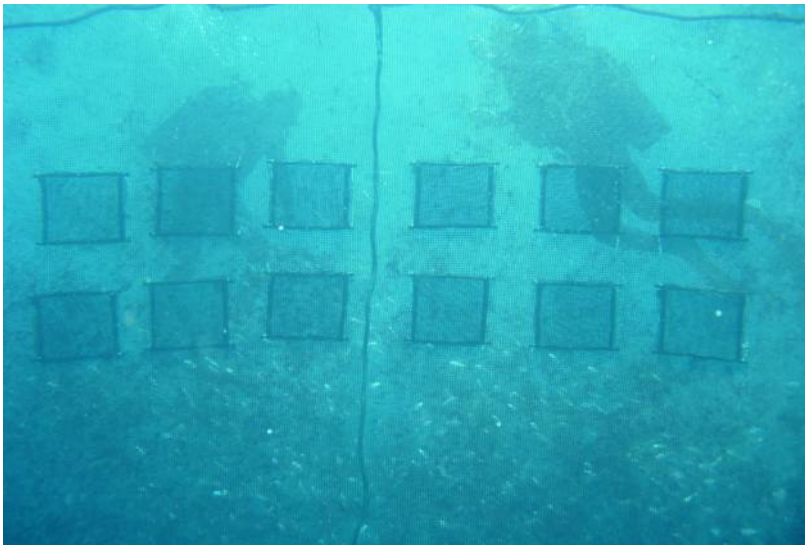

Fig. 3.4. Position of the panels inside a cage at the depth of 5 m.

In order to maximize sunlight exposure, all the panels were placed with the surface for biofouling organisms settlement oriented towards the South. This was done with the intent of giving to biofouling organisms the maximum chances to settle and to develop on the nets of the cages and, also, to evaluate if there where any differences between the 2 depth levels considered.

Before being positioned *in situ*, each net panel was weighed to determine its weight without biofouling and also photographed in its entirety using both front and back lighting to create large contrast between empty space and dark netting. All the experimental panels were positioned in the cages during November 2007.

Subsequently, panels from each experimental group of cages (*i.e.* large sea breams vs. small sea breams vs. controls) were removed at intervals of 3 months (almost seasonally).

SCUBA divers collected the experimental panels by placing each of them separately into a 35×45 cm plastic envelope, and sealing it underwater with a knot. Each

envelope was then brought to the surface, the netting was preserved with 4% formalin (40% aqueous solution of formaldehyde) solution, and returned to the laboratory for processing.

Here are reported and discussed the results from the first 2 phases of the study (i.e. February–May 2008). It is worth mentioning that, during this period, 6 control panels were lost.

3.2.2 Laboratory methods

In the laboratory, panels were carefully removed from the envelopes, placed in a transparent plastic tray filled with seawater, and photographed in their entirety with a digital camera, using both front and back lighting. The backlit shots created large contrast between the light background and the dark netting/biofouling. This technique was utilized to process the images in order to estimate the percentage of mesh occluded by biofouling organisms.

Images were then downloaded to a personal computer and the "Percentage Net Aperture" (PNA) was calculated for each image using both the software packages Paint Shop Pro 7 and Image–Pro Plus 4. The use of the software Paint Shop Pro 7 has allowed a chromatic reduction at a two–colour level and, consequently, to eliminate interferences due to the many nuances present after the selection of digital images because of different colours of algal felt.

The area to analyse was enclosed in a digital framework of 25x25 cm that was subsequently superimposed onto the real image (Fig. 3.5). Image–Pro Plus 4 software has allowed the cutting edge for an exact definition of a square 25x25 cm for analysis and selection of total square voids necessary for determination of covering percentage. Data were then transferred to a worksheet in order to compute the percentage of occlusion of the net panels.

Subsequently panels and any remaining organisms that had dropped off the panels were removed from the plastic envelope and placed back in the tray. The contents of each envelope were emptied into a plastic tray, where all organisms were removed from the netting under a microscope with tweezers and stored in 70% ethanol solution or 4% formalin solution as a function of taxa (Braithwaite *et al.*, 2007).

The netting was then examined under the dissecting microscope at 10× power and any remaining organisms were removed and placed in the fixative solution. All organisms of macrofitobenthos and macrozoobenthos were identified to the lowest

practical level, counted, and weighed (preserved wet weight).

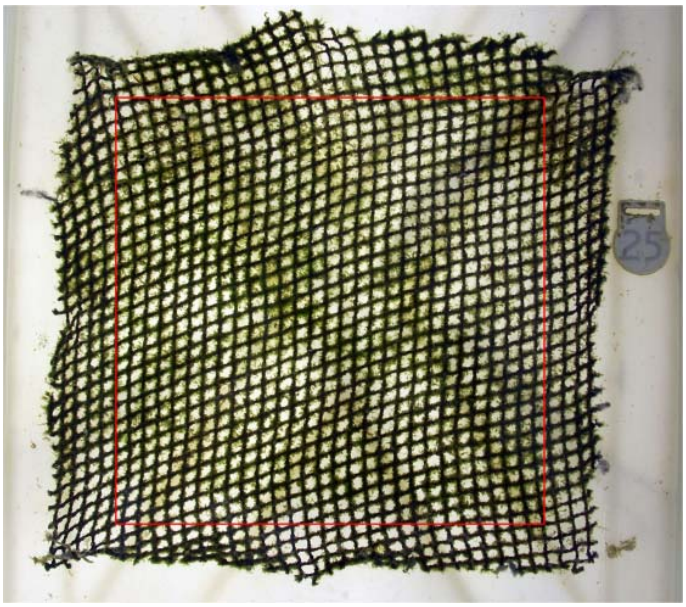

Fig. 3.5. Digital framework of 25x25 cm enclosing the area to analyse in each panel.

The total wet weight of biofouling per net was calculated by weighing empty nets before and after deployment (clean nets were immersed, and both clean and fouled nets, after 1 min of dripping, were placed on a rack to drain for 5 minutes before they were weighed).

3.2.3 Statistical analyses

Analysis of variance (ANOVA) was used to detect differences in biofouling covering percentage observed on the panels at the depth of 1 and 5 m for the factors "Time" (*i.e.* February vs. May) and "Cage" (*i.e.* large sea breams vs. small sea breams vs. controls).

The same statistical approach was used to detect differences in macrofitobenthic and macrofaunal assemblages found on the panels during the 2 sampling phases (Underwood 1997). ANOVAs were always performed using the STATISTICA software package.

Macrofitobenthic and macrofaunal assemblages structure was also analyzed by multivariate statistical techniques using the PRIMER software package (Plymouth Marine Laboratory; Clarke & Warwick, 2001). Similarity of biofouling assemblages between sampling stations was calculated using the Bray–Curtis coefficient (Bray & Curtis, 1957).

The data of macrofitobenthic and macrozoobenthic abundance for each monitoring period were then pooled and graphically represented in two–dimensional ordination plots by non–metric multi dimensional scaling (nMDS) and cluster analyses.

3.3 Results

During the study period (*i.e.* February–May 2008), the surface covering due to the biofouling assemblages on the experimental panels were higher at –1 m than at –5 m. In fact, the percentage values of empty space recorded on panels positioned at a depth of 1 m ranged between 30 and 50% (Fig. 3.6), while those detected on panels at the depth of 5 m ranged between 40 and 60% (Fig. 3.7). Looking at the histogram in Fig. 3.6, it is possible to see a clear dominance of empty surface (corresponding to a lesser amount of biofouling coverage) on the panels located inside small sea breams cages compared to those inside the bigger fish cages and the controls.

By considering Fig. 3.7, it can be noticed a comparable increase in terms of coverage (*i.e.* reduction of empty surface) between the winter (February) and the spring (May) periods for cages containing both large and small sea breams. As regards the controls, it is possible instead to observe an opposite trend due to a greater coverage in February than in May samples (Fig. 3.7).

ANOVA detected significant differences for the factor “Cage” at both the depth levels considered, while significant differences for the interaction “Time x Cage” were observed only at a depth of 5 m (Tab. 3.1).

As far as macrophytobenthic species richness, apparently comparable results were obtained for both the depth levels investigated (Figs. 3.8 and 3.9). Nevertheless, ANOVA detected significantly different values only for the factor “Cage” for both algal assemblages settled on the panels at a depths of 1 m and 5 m (Tab. 3.2). By contrast, no significant differences for the factor “Time” and for the interaction “Time x Cage” were found (Tab. 3.2).

Furthermore, histogram of species richness illustrated in Fig. 3.8 shows an equal number of macrophytobenthic species in control panels at –1 m in February as well as in May samples. On the other hand, it may be noted that panels inside rearing cages of both large and small sea breams had a considerably lower number of species than controls (Fig. 3.8).

As in the previous case, the graphic in Fig. 3.9 shows values of algal species richness higher in controls than in the other cages at a depth of –5 m. However, it is worth noting that the number of species in control panels was almost equal during the winter and spring sampling phases. It was also noticed that the panels inside rearing cages for large and small sea breams were characterized by a reduction of the number of species from winter to spring (Fig. 3.9).

Both nMDS ordination plot and cluster analysis for the depth of 1 m in the winter period (February, Fig. 3.10) also indicated weak variations in algal species richness among the 3 groups. In fact, panels used as controls tended to form a cluster rather separated from those positioned within the large and small sea breams cages. These latter, by contrast, showed a partial overlap.

Similarly, considering the same depth level (i.e. –1 m), both nMDS ordination plot and cluster analysis reported in Fig. 3.11 revealed no substantial differences among the 3 different groups of panels during the spring period (May), which are characterized by a complete overlap.

An analogous trend was also observed for the macrocrophytobenthic assemblages in all the panels investigated at the depth of 5 m in the winter period (February). Indeed, the results of multivariate analyses illustrated in the nMDS plots and cluster dendrogram reported in Fig. 3.12 detected no significant differences among the 3 experimental groups of panels.

Even in the case of macroalgal species richness recorded at a depth of 5 m during the spring period (*i.e.* May), both nMDS ordination plot and cluster analysis illustrated in Fig. 3.13 did not illustrate the existence of a clear separation pattern among the above–mentioned groups of panels.

As far as the composition of macrozobenthic assemblages is concerned (in terms of mean number of individuals detected on the panels at both the depth levels considered and during the 2 time period investigated), 8 principal taxa were identified in most of the samples examined: Peracarid Crustaceans, Decapod Crustaceans, Pantopods, Gastropod Molluscs, Bivalve Molluscs, Nematode Worms, Cnidarians, and Polychaete Worms.

In the context of this results report, it is important to clarify that because of the large number of individuals of Peracarid Crustaceans found, this taxon was considered apart both in the graphic representations and in the statistical analyses.

Looking at the graphs illustrated in Fig. 3.14 (related to the abundance of the other taxa), it is possible to observe a clear dominance of Nematode and Polychaete Worms during the first sampling phase (*i.e.* February) at a depth of 1 m. Moreover, it is worth noting that the higher abundance values for these 2 categories (and also for Bivalves) were recorded in panels positioned inside the small sea bream rearing cages. Peracarids, by contrast, were characterized by mean abundance values higher than 6,000 individuals panel^{-1}, in all the groups of panels (Fig. 3.15).

As in the case just described, during the spring sampling phase the most represented taxa in the panels positioned at a depth of 1 m were Nematodes and Polychaetes Worms, and Bivalve Molluscs (Fig. 3.16). More specifically, Nematodes were more abundant in control panels (with an average number of about 600 individuals panel^{-1}), while Polychaetes accounted for the same average number of individuals in the panels positioned inside large sea breams rearing cages.

For these 2 taxa, it is important to note that also the average abundance of individuals in the panels placed inside small sea bream cages was almost the same (*i.e.* about 400 individuals panel^{-1} in both cases). On the other hand, Molluscs were most abundant in the panels inside large sea breams rearing cages (Fig. 3.16). In the same sampling phase, Peracarids accounted for mean values higher than 4,000 individuals panel^{-1} (Fig. 3.17).

Looking at the individual cases, ANOVA detected significant differences for Decapods (as well as for Bivalves) only for the interaction "Time x Cage" (Tab. 3.3). As regards Pantopods, significant differences were instead found for both the factor "Cage" and the interaction "Time x Cage". ANOVA showed significant differences for the factor "Cage" for Nematodes and Cnidarians, this latter showing significant differences also for the factor "Time" as well as Polychaetes (Tab. 3.3). Finally, only 2 taxa (*i.e.* Peracarids and Gasteropods) did not show any significant difference among the 3 experimental groups at the depth of 1 m.

The abundances of the major taxa recorded in the panels at a depth of 5 m during the winter period (Fig. 3.18) showed, as before, higher values for Nematodes and Polychaetes. In particular, the former taxon was most abundant in the panels positioned in cages containing small sea breams, whereas the latter showed an evident prevalence (with an average value greater than 1,000 individuals panel^{-1}) in those inside large sea bream cages.

During the winter sampling phase, as for the panels positioned at a depth of 1 m, the values of abundance of Peracarids at –5 m illustrated in Fig. 3.19 were higher than 3,500 individuals panel^{-1} for all the 3 experimental groups, with an average peak value of about 5,000 individuals in control panels.

Similarly to all previous cases, as regards the abundances of macrozoobenthic assemblages at a depth of 5 m in the spring sampling phase, the higher values of abundance were recorded for the same taxa (*i.e.* Polychaetes, Nematodes and Bivalves; (Fig. 3.20). In particular, it is worth mentioning the great abundance of Polychaetes in

panels inside cages containing large sea breams, with mean values exceeding 1,500 individuals panel^{-1}. Peracarids were still very abundant in all the 3 experimental groups of panels, with mean values always higher than 4,000 individuals panel^{-1} (Fig. 3.21).

ANOVA performed on major taxa recorded at –5 m (Tab. 3.4) revelead significant differences for the factor "Cage" for all the taxa examined, except for Peracarids and Cnidarians, with the former showing significant differences for the factor "Time".

As far as multivariate analysis is concerned, macrozoobenthic assemblages at –1 m during the winter period in panels inside small sea bream rearing cages showed a similar structure (represented by a single cluster in Fig. 3.22), with respect to both assemblages in panels inside large sea bream cages and controls that were more scattered. By contrast, at the same depth level during the spring period (as shown in both the nMDS ordination plot and cluster dendrogram reported in Fig. 3.23), there was a substantial overlap among macrozoobenthic assemblages of the 3 experimental groups of panels.

Lastly, as regards panels positioned at a depth of 5 m, in the winter sampling phase (*i.e.* February) there were no substantial differences in the structural composition of macrozoobenthic assemblages in the 3 groups of panels (as illustrated by nMDS plot and cluster analysis in Fig. 3.24). On the contrary, during the spring sampling phase (*i.e.* May), a fairly clear–cut separation was evident for macrozoobenthic assemblages in control panels, whereas those relative to large and small sea bream cages were more interspersed (Fig. 3.25).

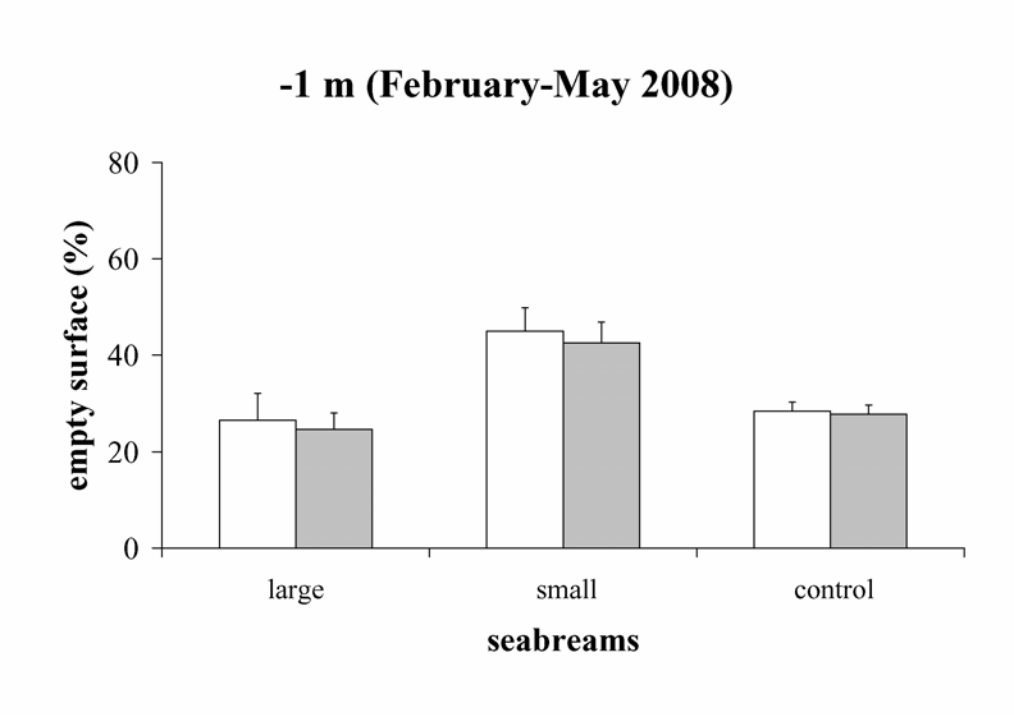

Fig. 3.6. Percentages of empty surface recorded on the panels at the depth of 1 m (white bars=February; grey bars=May).

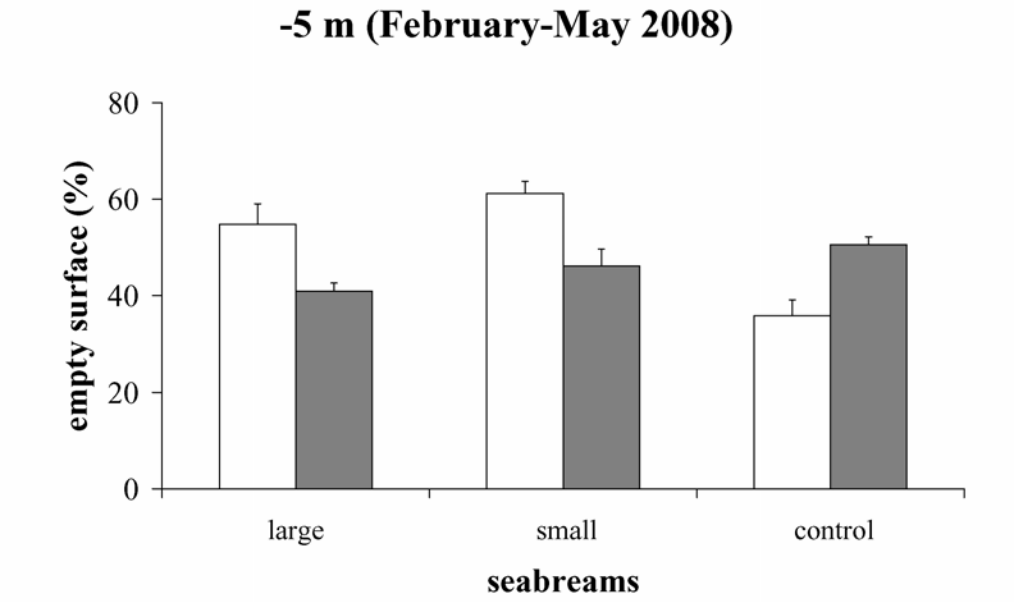

Fig. 3.7. Percentages of empty surface recorded on the panels at the depth of 5 m (white bars=February; grey bars=May).

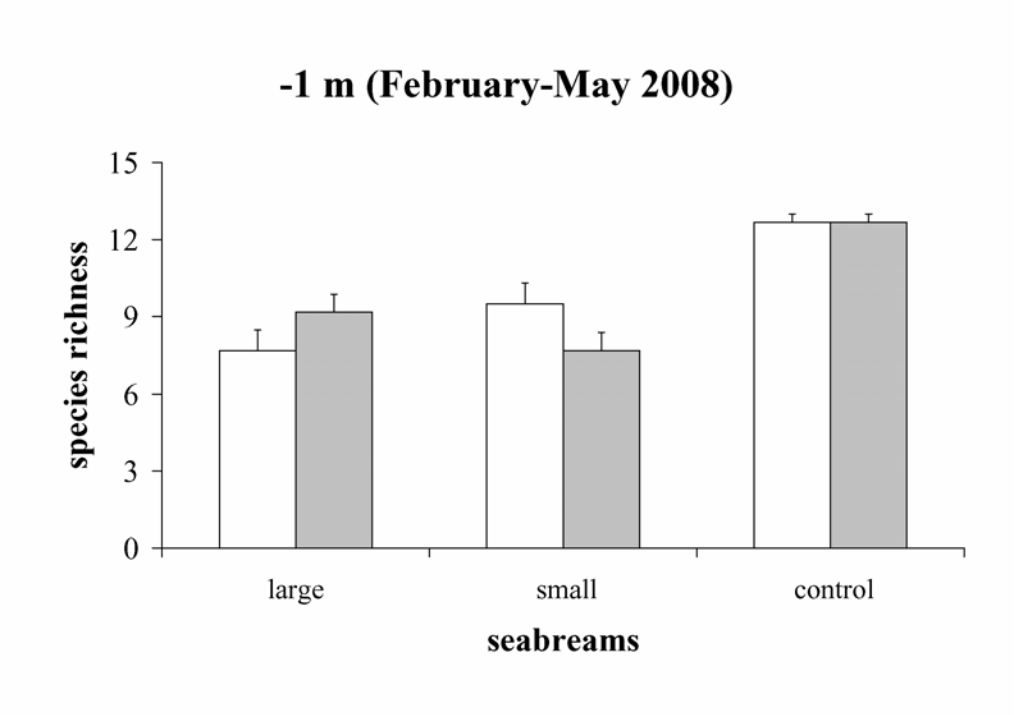

Fig. 3.8. Species richness of the macrophytobenthic assemblages recorded on the panels at the depth of 1 m (white bars=February; grey bars=May).

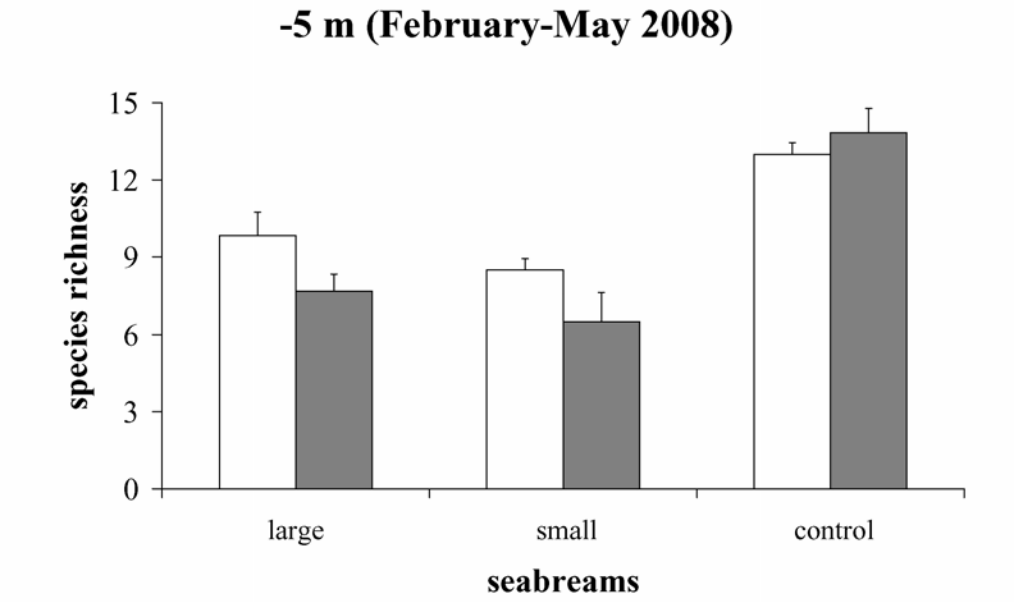

Fig. 3.9. Species richness of the macrophytobenthic assemblages recorded on the panels at the depth of 5 m (white bars=February; grey bars=May).

Tab. 3.1. Results of ANOVAs for the effects of time and cage type on biofouling covering percentage observed on the panels at the depth of 1 and 5 m, respectively (significant differences are marked in bold).

	–1 m				–5 m			
Source of variation	df	MS	F	p	df	MS	F	p
Time	1	18.88	0.17	0.681	1	208.62	3.97	0.055
Cage	2	1089.00	10.02	**0.001**	2	328.37	6.25	**0.005**
Time x Cage	2	1.77	0.02	0.984	2	844.12	16.07	**0.000**
Residuals	24	108.70			30	52.53		

Tab. 3.2. Results of ANOVAs for the effects of time and cage type on macrofitobenthic assemblages observed on the panels at the depth of 1 and 5 m, respectively (significant differences are marked in bold).

	–1 m				–5 m			
Source of variation	df	MS	F	*p*	df	MS	F	*p*
Time	1	0.08	0.03	0.868	1	11.11	2.92	0.098
Cage	2	41.75	14.25	**0.000**	2	116.70	30.71	**0.000**
Time x Cage	2	8.35	2.85	0.078	2	8.53	2.24	0.124
Residuals	24	2.93			30	3.80		

-1 m (February 2008)

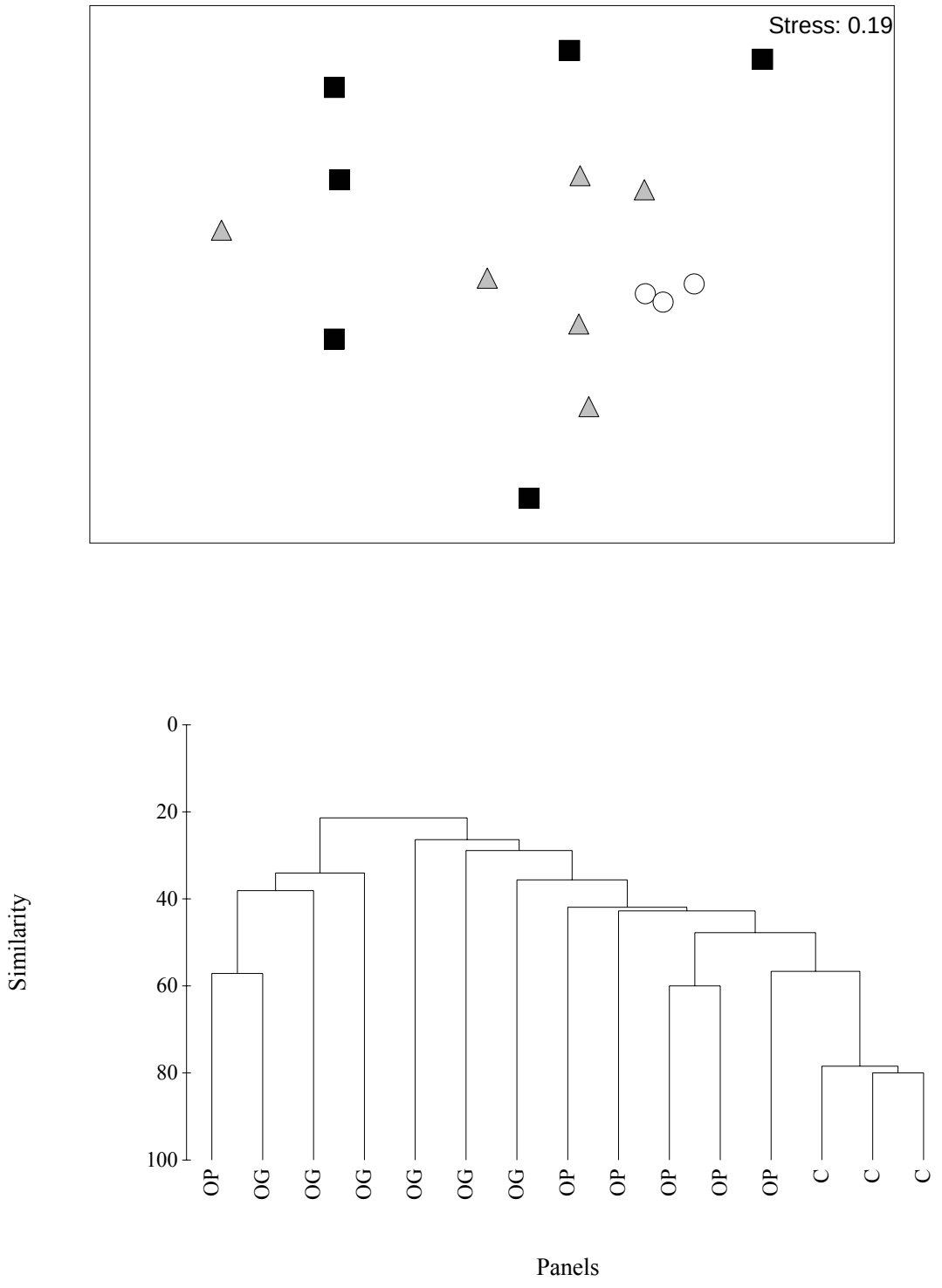

Fig. 3.10. Multidimensional scaling and cluster analysis performed on the Bray–Curtis similarity matrix to test the differences among macrophytobenthic assemblages on the panels inside sea bream rearing cages and controls (OG=big sea breams; OP= small sea breams; C=controls).

-1 m (May 2008)

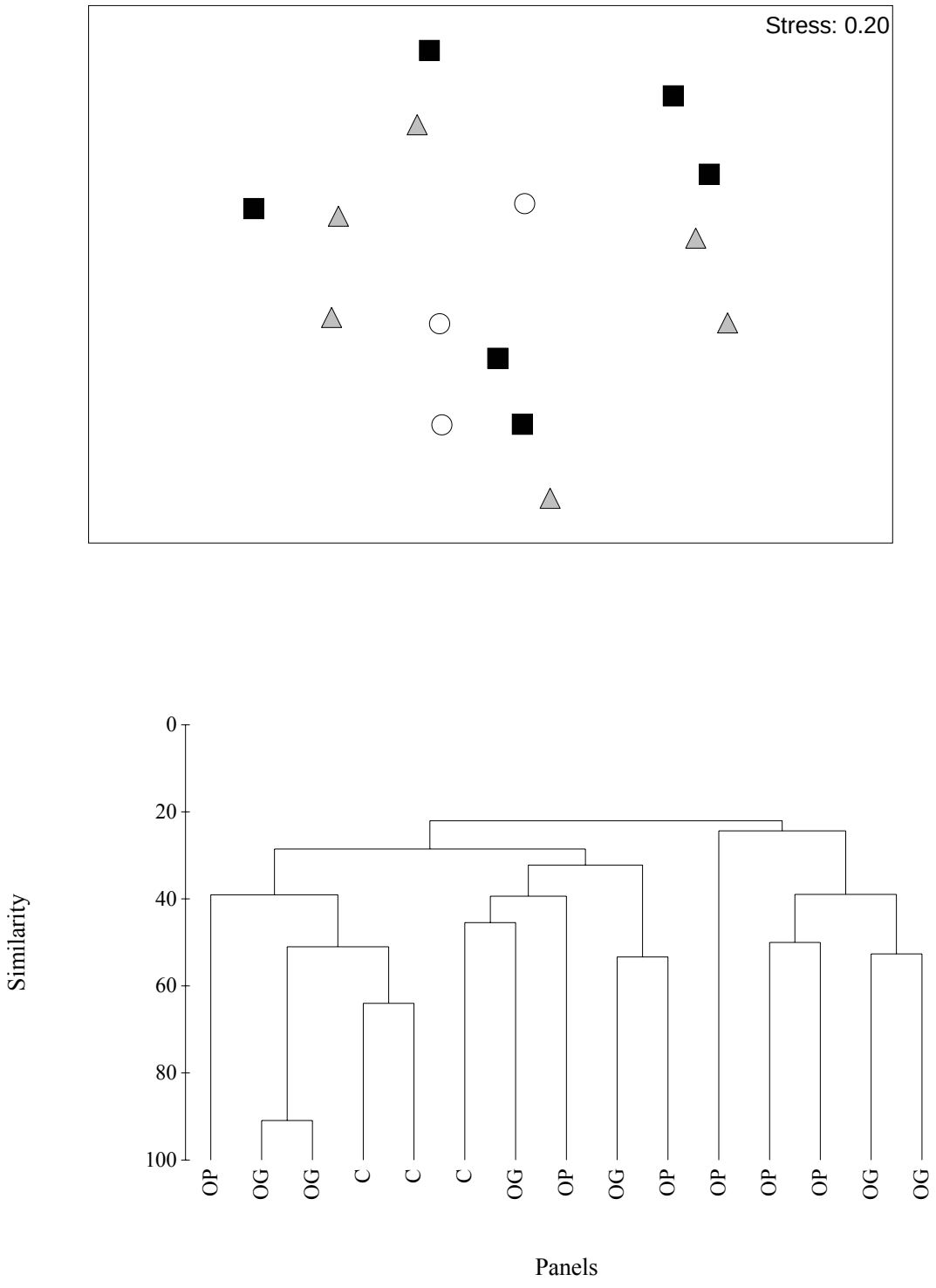

Fig. 3.11. Multidimensional scaling and cluster analysis performed on the Bray–Curtis similarity matrix to test the differences among macrophytobenthic assemblages on the panels inside sea bream rearing cages and controls (OG=big sea breams; OP= small sea breams; C=controls).

-5 m (February 2008)

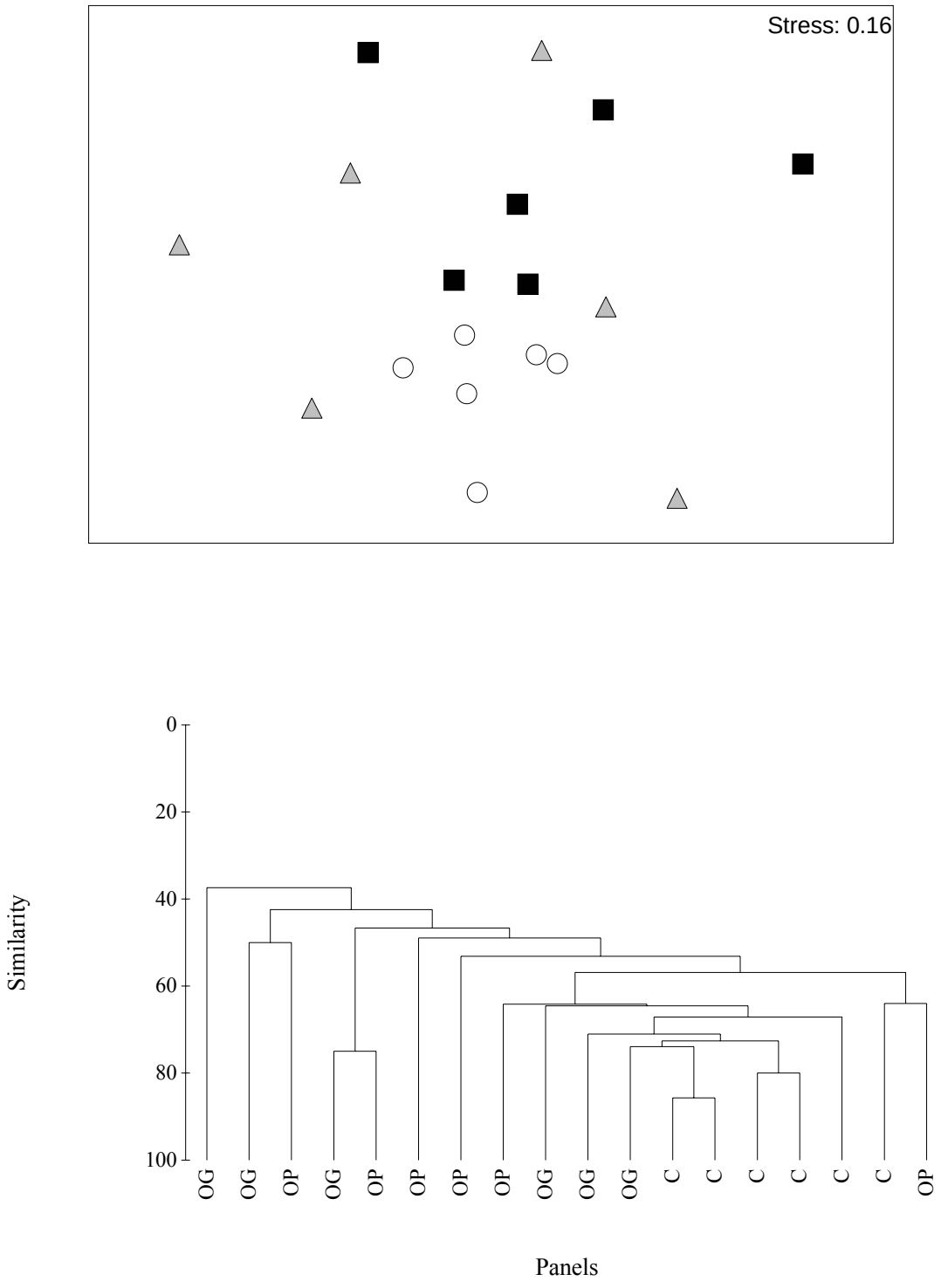

Fig. 3.12. Multidimensional scaling and cluster analysis performed on the Bray–Curtis similarity matrix to test the differences among macrophytobenthic assemblages on the panels inside sea bream rearing cages and controls (OG=big sea breams; OP= small sea breams; C=controls).

-5 m (May 2008)

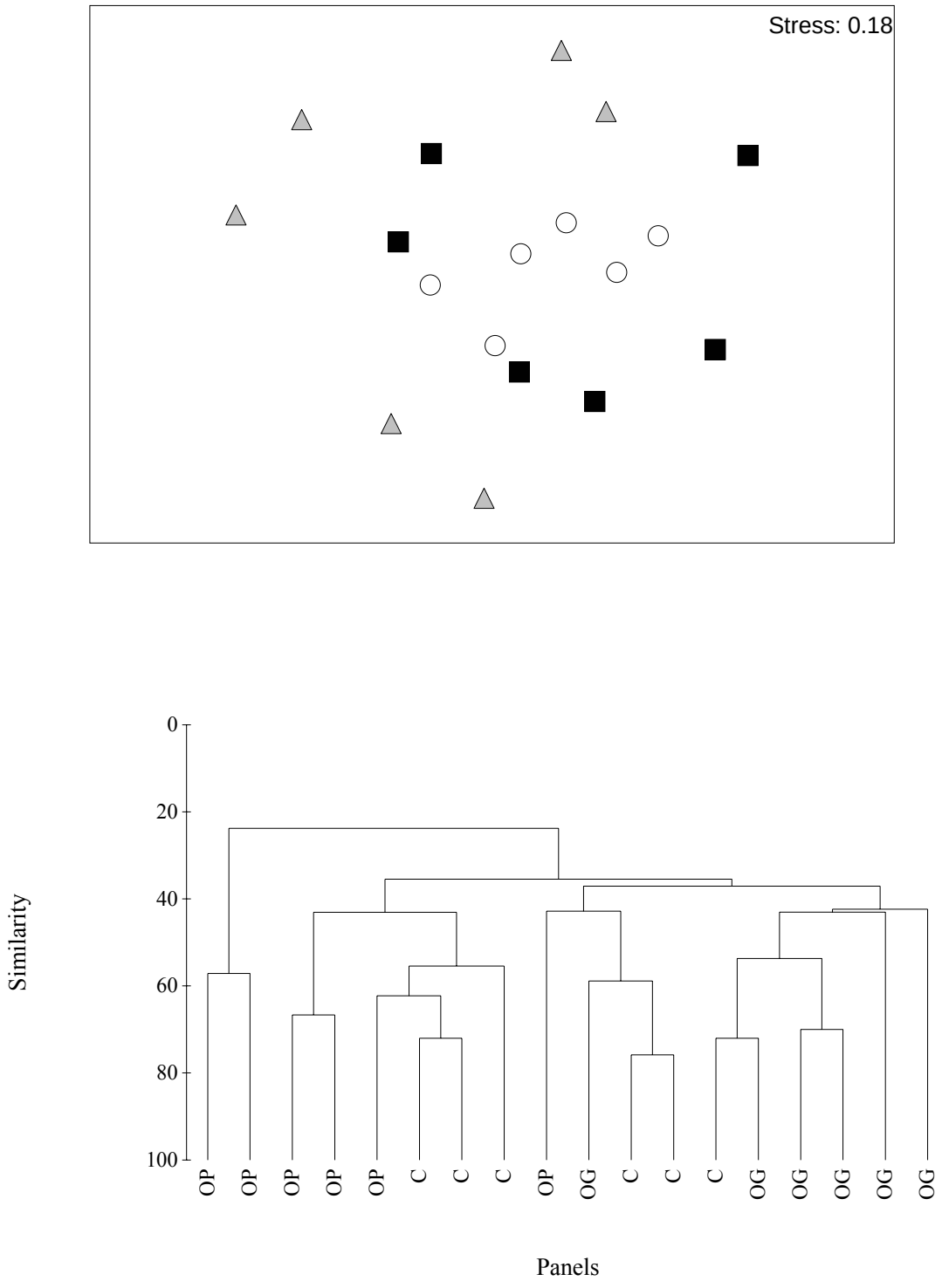

Fig. 3.13. Multidimensional scaling and cluster analysis performed on the Bray–Curtis similarity matrix to test the differences among macrophytobenthic assemblages on the panels inside sea bream rearing cages and controls (OG=big sea breams; OP= small sea breams; C=controls).

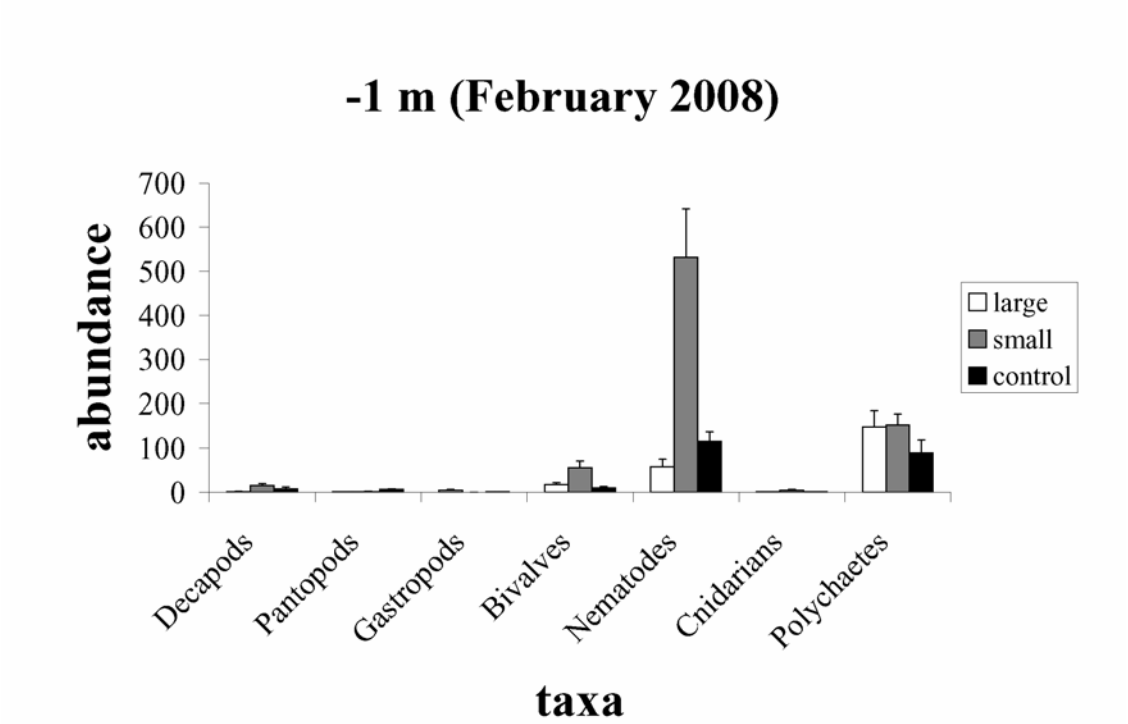

Fig. 3.14. Abundance of the most represented macrozoobenthic taxa (without Peracarids) recorded on the panels in the first phase of the study at the depth of 1 m.

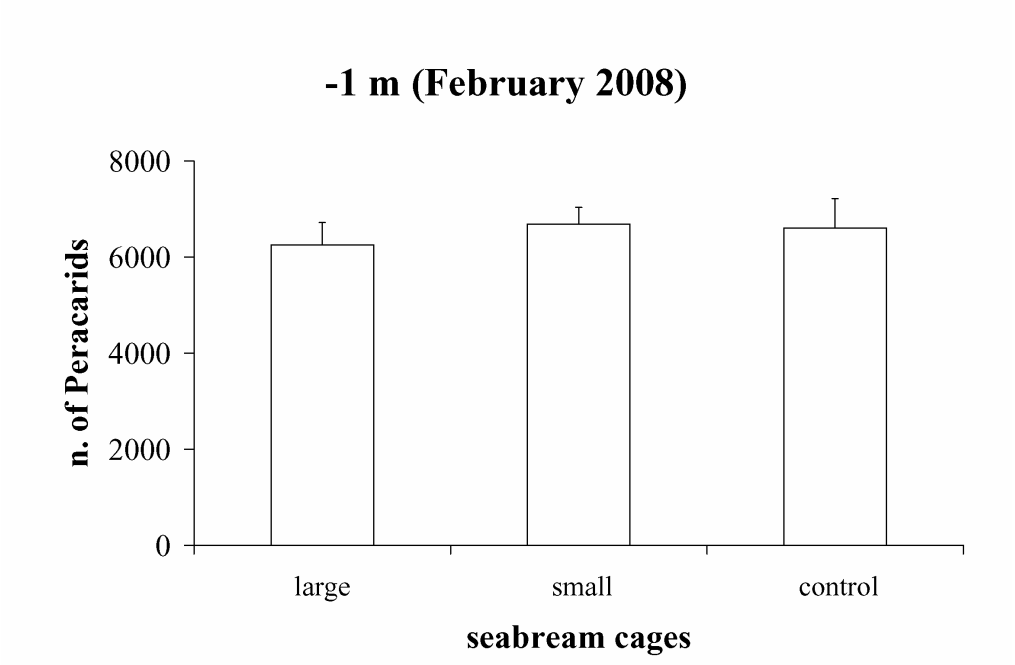

Fig. 3.15. Abundance of Peracarids recorded on the panels in the first phase of the study at the depth of 1 m.

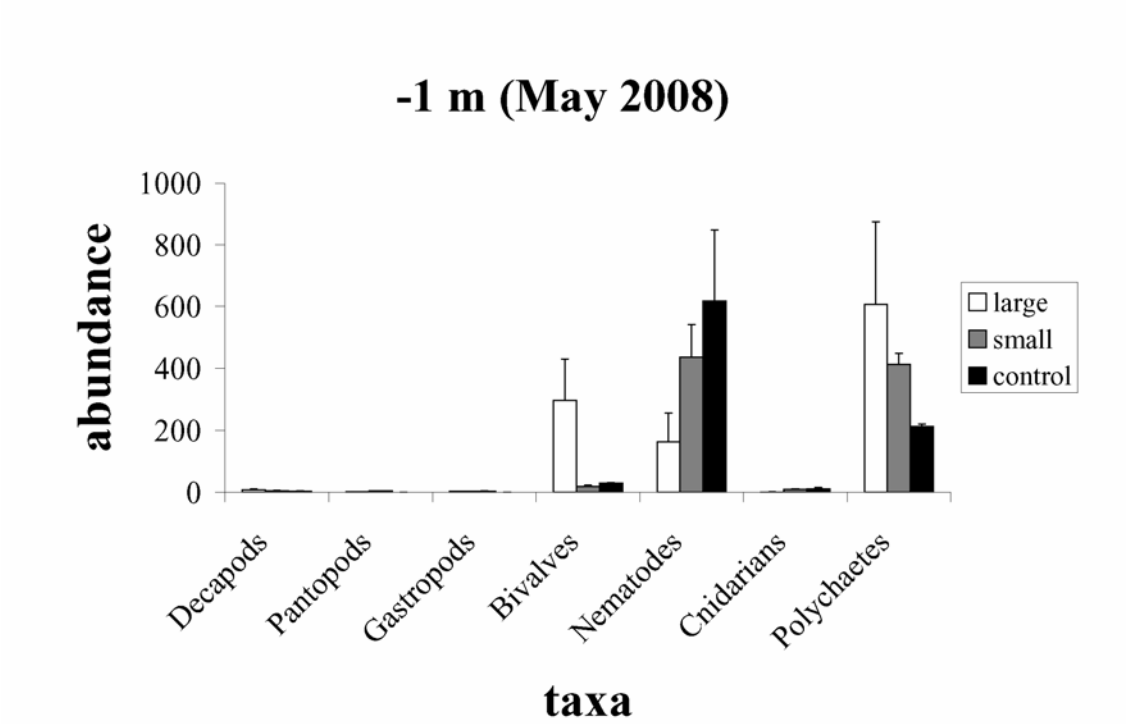

Fig. 3.16. Abundance of the most represented macrozoobenthic taxa (without Peracarids) recorded on the panels in the second phase of the study at the depth of 1 m.

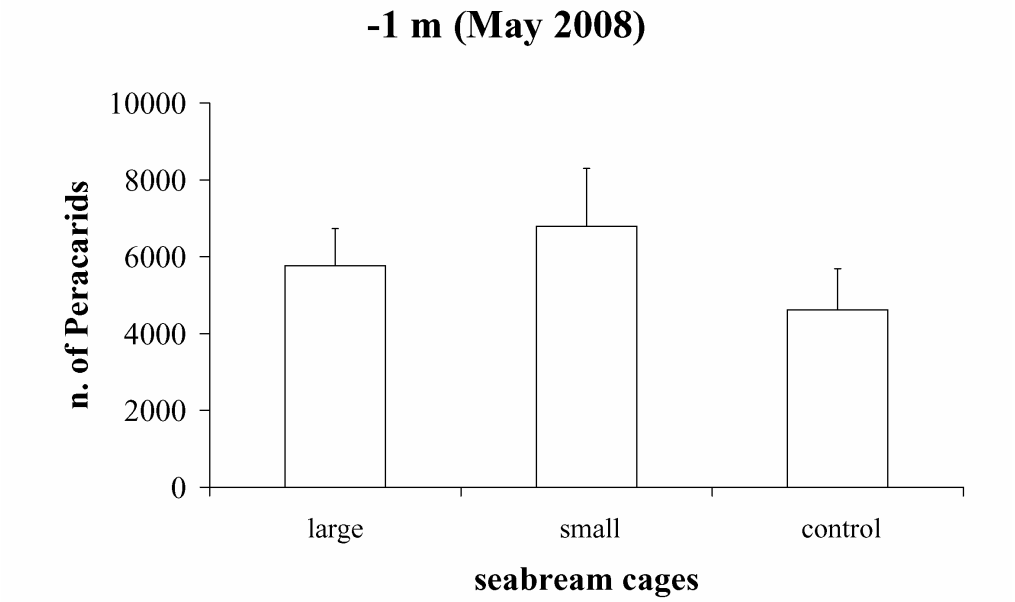

Fig. 3.17. Abundance of Peracarids recorded on the panels in the second phase of the study at the depth of 1 m.

Tab. 3.3. Results of ANOVAs for the effects of time and cage type on the main macrozoobenthic taxa observed on the panels at the depth of 1 m (significant differences are marked in bold).

		Peracarids			Decapods			Pantopods			Gastropods		
Source of variation	df	MS	F	*p*	MS	F	*p*	MS	F	*p*	MS	F	*p*
Time	1	4.20×10^{-6}	0.88	0.358	70.08	1.93	0.177	6.02	2.54	0.124	0.33	0.03	0.861
Cage	2	2.98×10^{-6}	0.63	0.544	96.77	2.67	0.090	13.73	5.80	**0.009**	20.82	1.95	0.164
Time x Cage	2	2.21×10^{-6}	0.46	0.635	225.60	6.22	**0.007**	36.19	15.28	**0.000**	19.02	1.79	0.189
Residuals	24	4.77×10^{-6}			36.29			2.37			10.65		
		Bivalves			Nematodes			Cnidarians			Polychaetes		
Source of variation	df	MS	F	*p*	MS	F	*p*	MS	F	*p*	MS	F	*p*
Time	1	51025.50	2.20	0.151	195713.00	3.63	0.069	147.00	9.76	**0.005**	531934.00	5.67	**0.026**
Cage	2	56854.00	2.45	0.107	430038.00	7.99	**0.002**	127.00	8.43	**0.002**	103842.00	1.11	0.347
Time x Cage	2	80450.50	3.47	**0.048**	179603.00	3.34	0.053	47.83	3.17	0.060	63150.00	0.67	0.519
Residuals	24	23189.20			53856.00			15.07			93755.00		

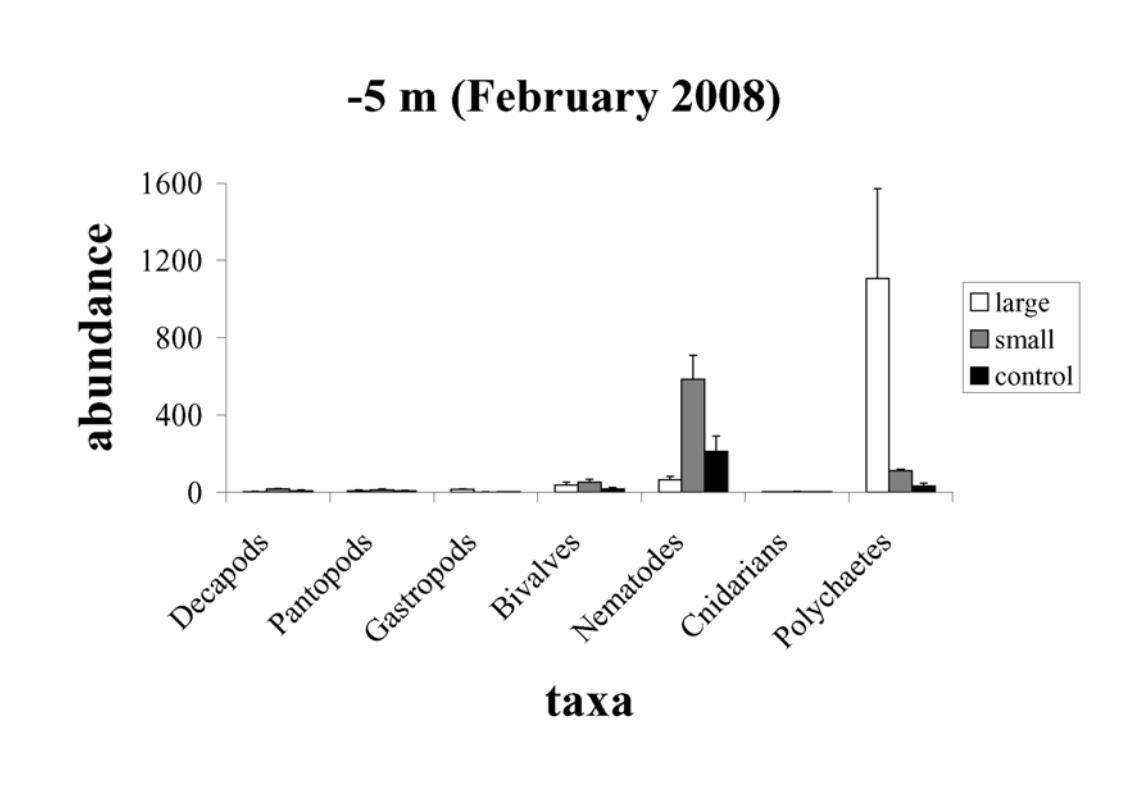

Fig. 3.18. Abundance of the most represented macrozoobenthic taxa (without Peracarids) recorded on the panels in the first phase of the study at the depth of 5 m.

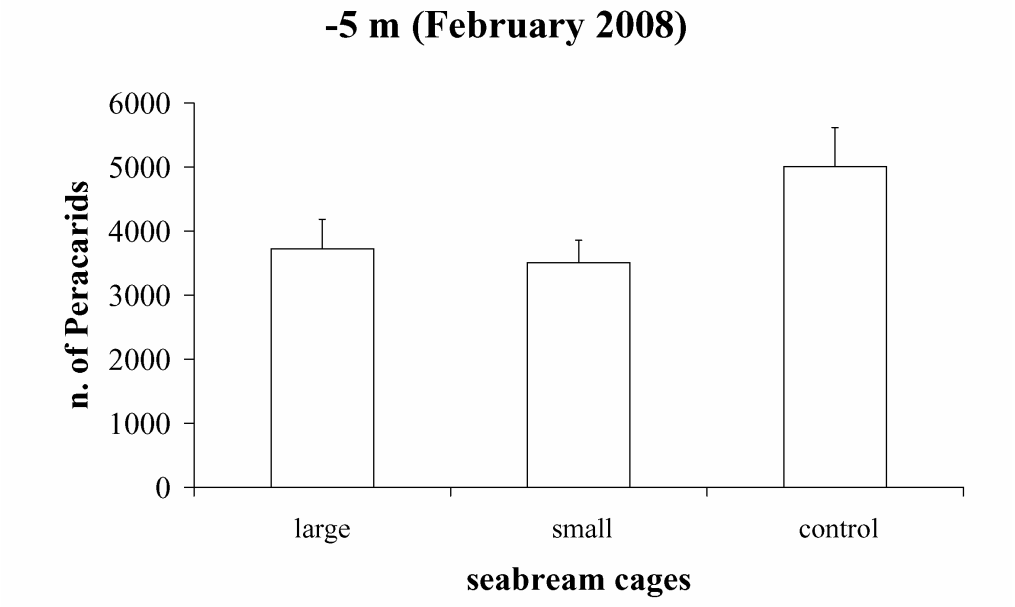

Fig. 3.19. Abundance of Peracarids recorded on the panels in the first phase of the study at the depth of 5 m.

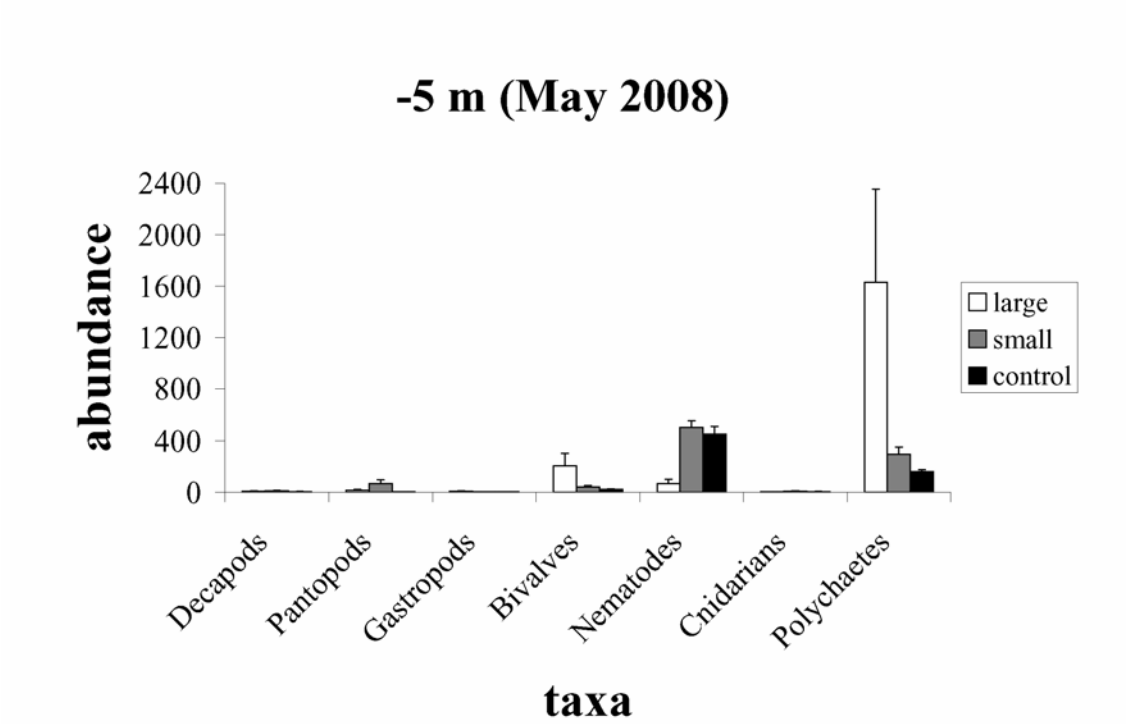

Fig. 3.20. Abundance of the most represented macrozoobenthic taxa (without Peracarids) recorded on the panels in the second phase of the study at the depth of 5 m.

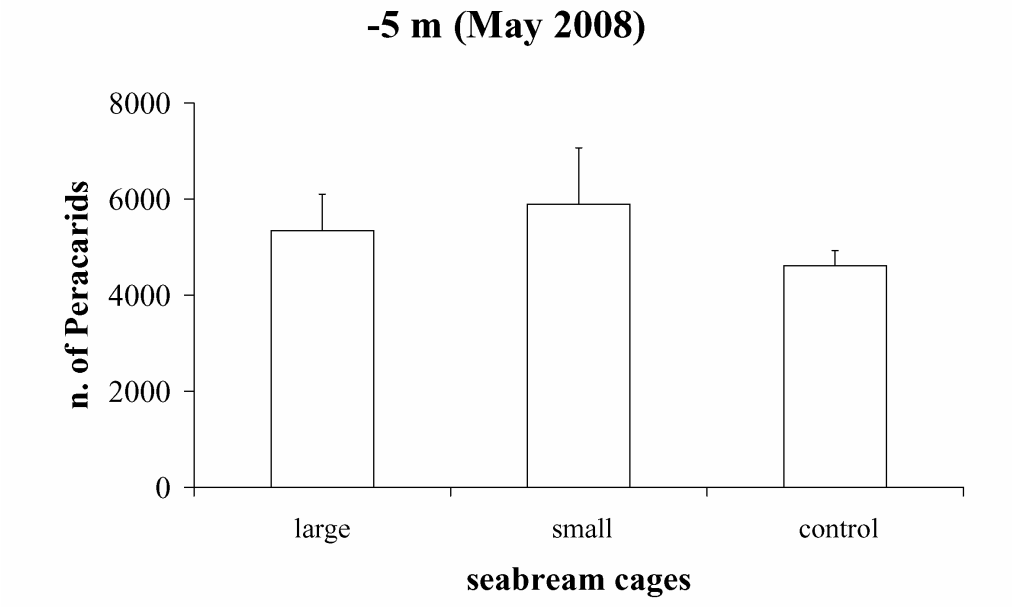

Fig. 3.21. Abundance of Peracarids recorded on the panels in the second phase of the study at the depth of 5 m.

Tab. 3.4. Results of ANOVAs for the effects of time and cage type on the main macrozoobenthic taxa observed on the panels at the depth of 5 m (significant differences are marked in bold).

		Peracarids			Decapods			Pantopods			Gastropods		
Source of variation	df	MS	F	*p*	MS	F	*p*	MS	F	*p*	MS	F	*p*
Time	1	13020069.00	4.35	**0.046**	34.03	1.19	0.285	3383.36	3.97	0.056	20.25	1.26	0.270
Cage	2	242698.00	0.08	0.922	229.08	7.98	**0.002**	4271.44	5.01	**0.013**	351.75	21.90	**0.000**
Time x Cage	2	6204560.00	2.07	0.144	94.69	3.30	0.051	3245.44	3.81	0.034	45.58	2.84	0.074
Residuals	30	2996405.00			28.71			852.53			16.06		
		Bivalves			Nematodes			Cnidarians			Polychaetes		
Source of variation	df	MS	F	*p*	MS	F	*p*	MS	F	*p*	MS	F	*p*
Time	1	25493.40	2.57	0.120	27005.00	0.90	0.349	78.03	3.35	0.077	688900.00	0.93	0.343
Cage	2	33781.00	3.40	**0.047**	685661.00	22.96	**0.000**	27.44	1.18	0.321	5986613.00	8.07	**0.002**
Time x Cage	2	29377.50	2.96	0.067	83676.00	2.80	0.077	5.78	0.25	0.782	138439.00	0.19	0.831
Residuals	30	9938.50			29862.00			23.26			741691.00		

-1 m (February 2008)

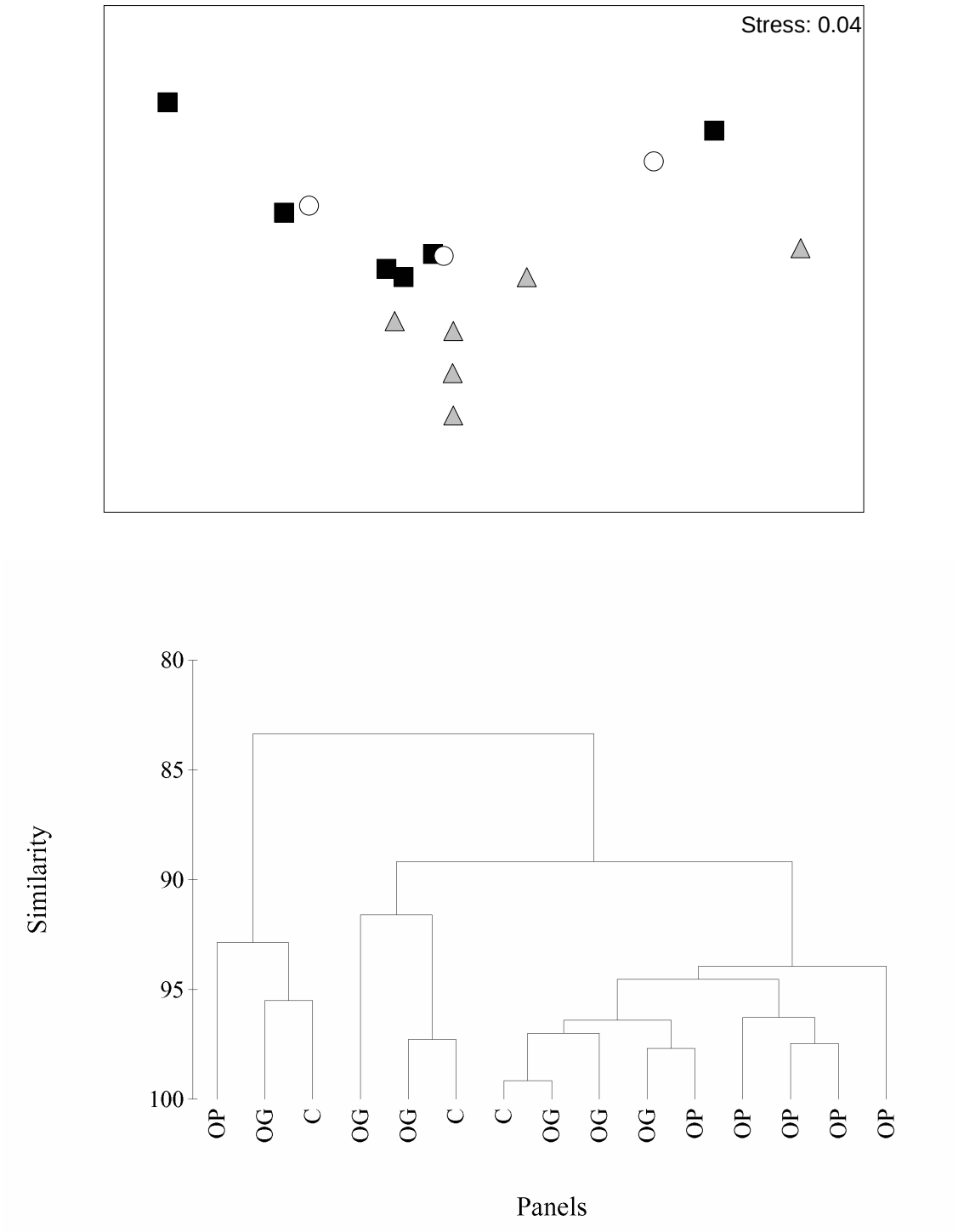

Fig. 3.22. Multidimensional scaling and cluster analysis performed on the Bray–Curtis similarity matrix to test the differences among macrozoobenthic assemblages on the panels inside sea bream rearing cages and controls (OG=big sea breams; OP= small sea breams; C=controls).

-1 m (May 2008)

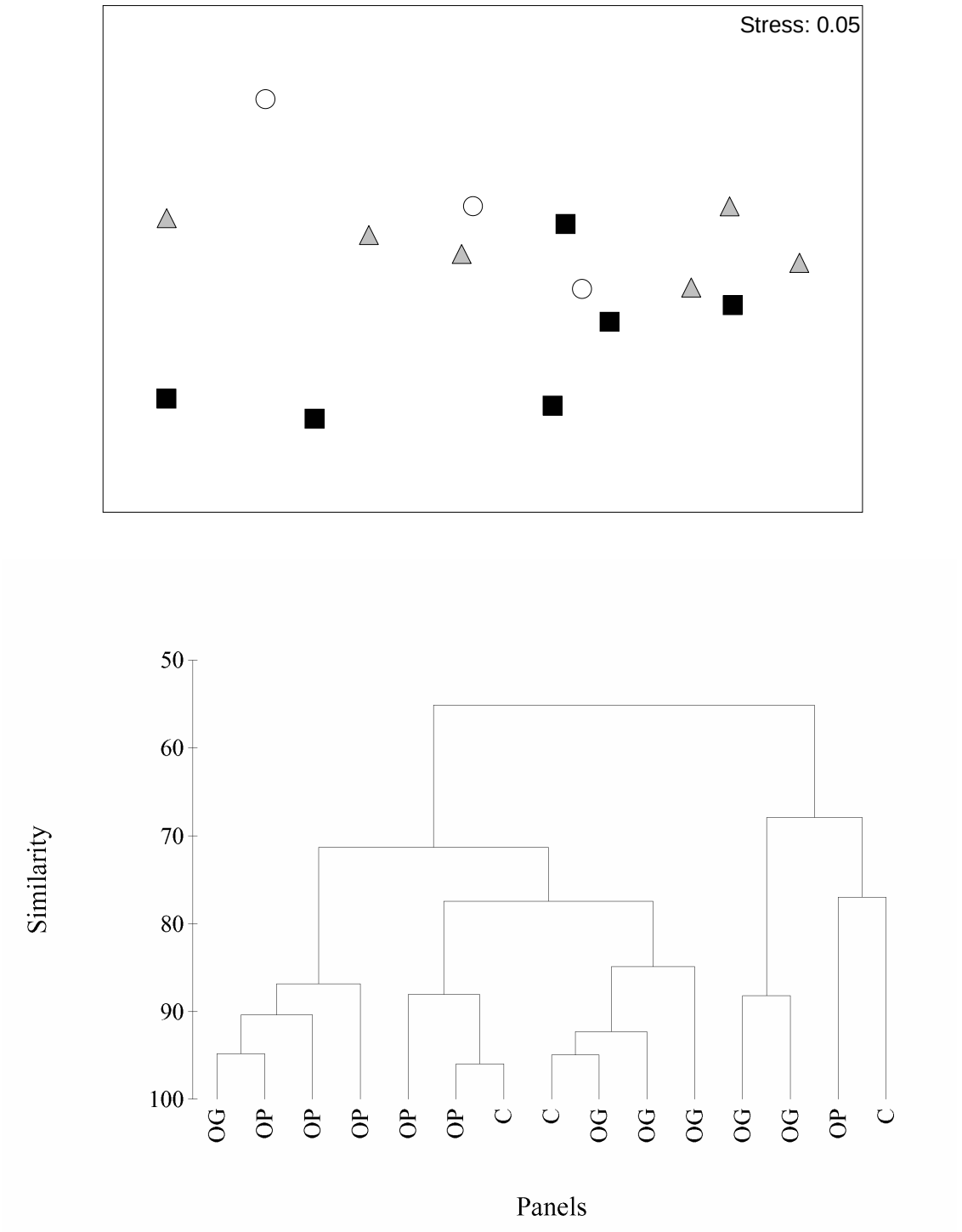

Fig. 3.23. Multidimensional scaling and cluster analysis performed on the Bray–Curtis similarity matrix to test the differences among macrozoobenthic assemblages on the panels inside sea bream rearing cages and controls (OG=big sea breams; OP= small sea breams; C=controls).

-5 m (February 2008)

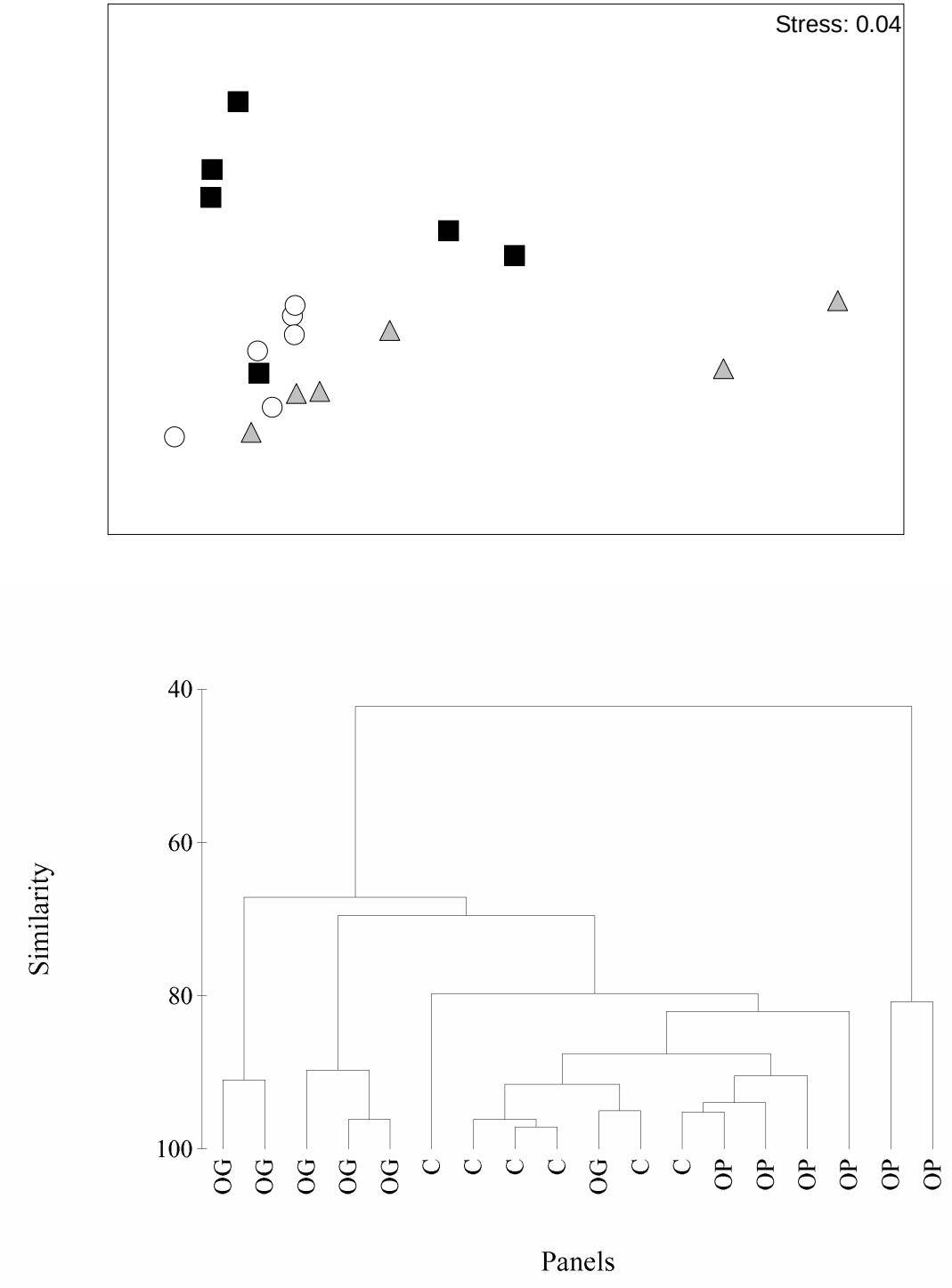

Fig. 3.24. Multidimensional scaling and cluster analysis performed on the Bray–Curtis similarity matrix to test the differences among macrozoobenthic assemblages on the panels inside sea bream rearing cages and controls (OG=big sea breams; OP= small sea breams; C=controls).

-5 m (May 2008)

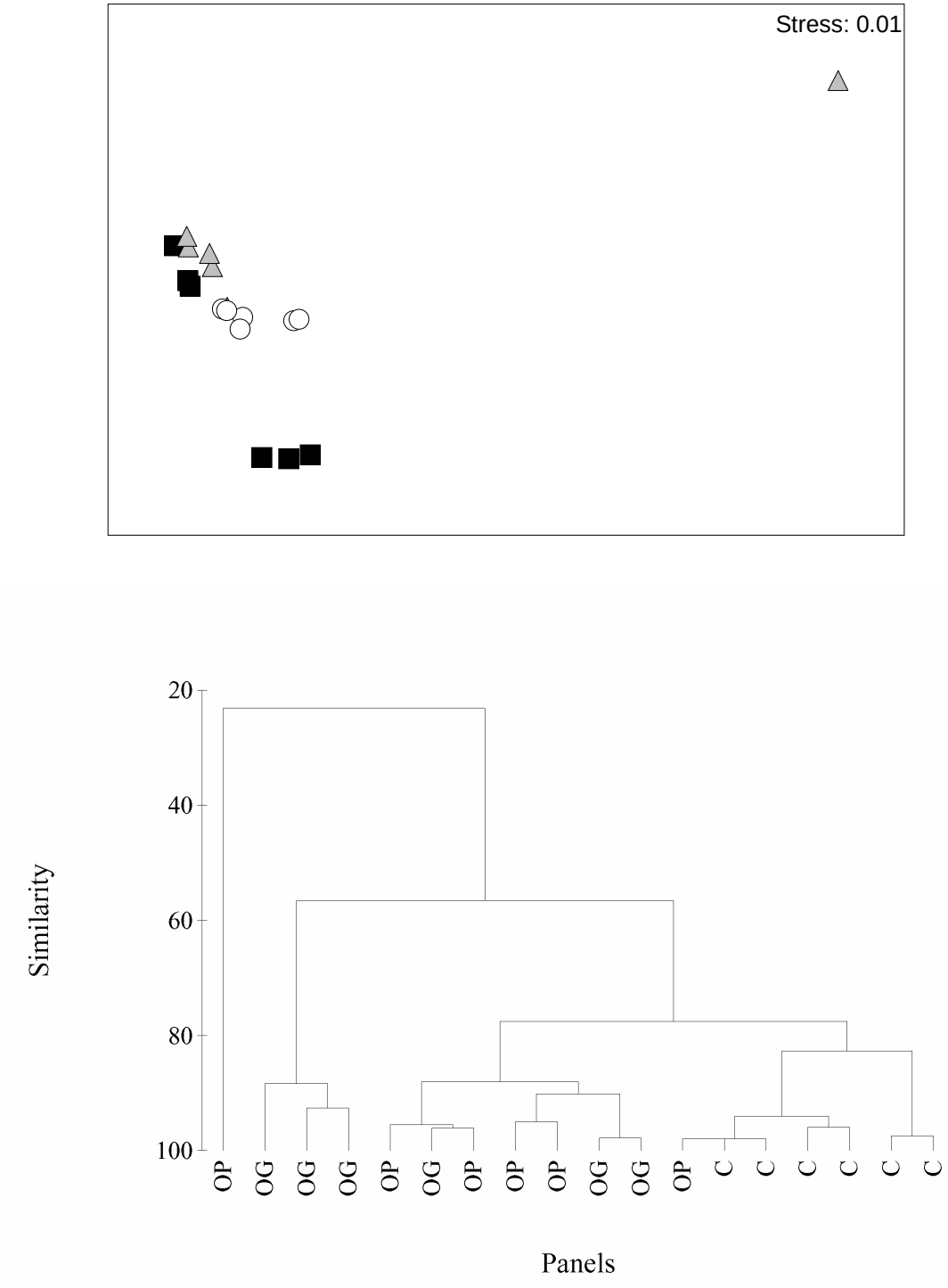

Fig. 3.25. Multidimensional scaling and cluster analysis performed on the Bray–Curtis similarity matrix to test the differences among macrozoobenthic assemblages on the panels inside sea bream rearing cages and controls (OG=big sea breams; OP= small sea breams; C=controls).

3.4 Discussion and conclusions

The rapid biomass increase of the biofouling organisms on all panels deployed inside the fish cages studied suggests that caged mariculture may have provided an enhanced food supply to epibiotic communities. This supports the results of a previous study carried out by Lojen *et al.* (2003), which hypothesized that a proportion of the diet of the epibiotic communities was associated with nitrogen derived from the fish farm. However, an enhanced plankton production stimulated by elevated nutrient levels in the close vicinity of the mariculture operation, may also play an important role in the diet (Cook *et al.*, 2006).

A number of studies carried out in the Mediterranean Sea have found that dissolved nutrients derived from fish farms are typically retained in measurable quantities around fish cages in areas of low dispersal (*e.g.* Pitta *et al.*, 1998; Modica *et al.*, 2006; Sarà *et al.*, 2006; Sarà, 2007a, b, and references therein). On the contrary, in highly dispersive environments (*e.g.* the Bay of Fundy, Canada), dissolved nutrients are undetectable above background levels within a short distance of the fish farm facilities (Wildish *et al.*, 1993).

To date, a large number of studies have assessed the influence of caged mariculture on the community assemblage of soft sediment macrobenthic communities in limited geographical ranges (see Chapter 2 and references therein reported).

On the other hand, only few studies provides results of the influence of caged mariculture on early development of hard–substrata biofouling communities over a wide geographical range (*e.g.* Cook *et al.*, 2006). Following these research results, it appears that caged aquacultural activities, through the provision of an enhanced food supply, have the potential to increase the biomass of biofouling assemblages, particularly in oligotrophic marine areas, and also have a greater influence on community structure in regions of low dispersion.

As an example of this, the relatively high current measurements recorded by Cook *et al.* (2006) near Piran (Slovenia, Mediterranean Sea) can be cited. Considering these outcomes it was hypothesized that the rapid dispersion of the dissolved nutrients released from the fish farm studied was related to the reduced growth rates of macroalgae observed at the reference site. Conversely, in the same paper, the low residual current speeds recorded near Eilat (Israel, Red Sea) were supposed to have prevented the dissolved nutrients from reaching the reference site before assimilation by both the pelagic auto– and heterotrophic communities (Cook *et al.*, 2006).

In general, we can say that fish–farm biodeposition can cause a number of changes in the chemical environment (Sarà, 2007a, b, and references therein). As already said in Chapter 2, the sites investigated inside and outside the fish farming facility area were significantly different as regards the organic nutrient content. These results were similar to the changes observed in other Mediterranean areas by several Authors (Pitta *et al.*, 1998; Pitta *et al.*, 2006, 2007; Sarà *et al.*, 2007). This fact may also induce changes in the characteristics of the mediolittoral benthic environment (Boyra *et al.*, 2007). Therefore, the results reported in this study are in line with one of the best–documented impacts of net pen fish farms (*i.e.* alteration of community dynamics and changes in biodiversity of local fauna; see Weston, 1990 for details).

To date, biofouling on hard artificial substrata and fish–cage netting has been investigated only as a negative factor affecting aquaculture productivity (Hodson *et al.*, 2000). In fact, biofouling communities are able to respond and adapt to the chronic input of allochthonous organic matter (*i.e.* fish waste and uneaten food), by exhibiting changes more or less consistently with regards to abundance, species composition, biomass and general community diversity (Sarà *et al.*, 2007).

In such aquatic environmental areas, characterized by high levels of anthropogenic organic enrichment, the local first response adopted by the system to the unnatural enhancement of food availability is a change in the total number of individuals per surface unit (Angel & Spanier, 2002). Consequently, if the attachment surface is not a limiting factor (Dayton, 1971), the main result is an increase in recruitment of new specimens that in turn leads to an increase in abundance.

A common solution to avoid biofouling is to make surfaces unsuitable for settlers. Surfaces are thus coated with antifouling paints containing toxic compounds (Terlizzi *et al.*, 2001). These biocides are present at the paint–water interface and affect settling organisms (Costlow & Tipper, 1984). Biofouling prevention requires a constant threshold concentration of biocides on the painted surface. The toxicant should be released from the paint matrix for sufficiently long periods. The so called antifouling paints can be classified into categories based on the chemical properties of the paint matrix and the mechanisms involved in releasing toxic compounds (Terlizzi *et al.*, 2001).

The knowledge of biofouling survival at various concentrations and exposure times of different chemical treatments has application in a number of facility management scenarios (*e.g.* in the sterilisation of infested nets and/or cage structures).

In such circumstances, decisions about whether or not to apply the treatments must balance treatment costs and benefits against the unmanaged risks and consequences of the development of biofouling. (Forrest *et al.*, 2007).

Nevertheless, although always seen as an important problem especially for marine aquacultural activities, biofouling can play a key–role in the so–called "bioremediation" process. In theory, for biofouling communities to be successful in reducing the environmental impact of caged fish culture, the position of the artificial structures relative to nutrient availability, light intensity, waste particle–settling, proximity to breeding populations, longevity, grazing pressure, and predation should be considered carefully in order to maximize the effectiveness of the "biofilters" in removing fine particulates derived from fish farms and dissolved nutrients from the water column (Cook *et al.*, 2006).

In practice, the scale of the biofiltering material required for significant retention of nutrient wastes over the whole industry is likely to remain extremely large, and an impractical number of biofilters would be needed to allow the application of this technology at a commercial scale (Cook *et al.*, 2004; but see also Angel & Spanier, 2002 and Angel *et al.*, 2002).

In conclusion, especially in the Mediterranean basin, biofilters could be used in specific cases where even a small reduction in loadings could be critical for the health of the environment, or the growth of commercially valuable species could assist in reducing the waste and provide a co–harvesting incentive for the industry to adopt a more environmentally sustainable approach to cage mariculture (Giangrande *et al.*, 2005; Pierri *et al.*, 2006; Stabili *et al.*, 2006, 2008).

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Chapter 4

RESPONSE OF CAPTIVE SEABREAM AS BEHAVIOURAL INDICATOR IN CAGE CULTURE

4.1 Introduction

4.1.1 Definition of animal welfare

The term “animal welfare” has been created by animal scientists in order to justify the continued use of animals in agriculture and experimentation. The farm animal welfare science movement traces its roots back to the findings of the Brambell Committee in 1965. These findings were reported in a report (Brambell, 1965) that established minimum standards for the treatment of livestock, including the following “5 freedoms”:

- freedom from hunger and thirst by ready access to fresh water and a diet to maintain full health and vigour;
- freedom from discomfort by providing an appropriate environment including shelter and a comfortable resting area;
- freedom from pain, injury or disease by prevention or rapid diagnosis and treatment;
- freedom to express normal behaviour by providing sufficient space, proper facilities and company of the animal's own kind;
- freedom from fear and distress by ensuring conditions and treatment which avoid mental suffering.

With the aim to discuss the “animal welfare” issues from a more specific point of view, however, we need to define it and this may not be easy, because its concept is complex and word can be used in different meanings (Dawkins, 1998; Appleby, 1999). Most definitions fall into one of three major categories (Duncan & Fraser, 1997; Fraser *et al.*, 1997) and it is important to note that none of which is right or wrong from a scientific point of view; rather they express different ideals about what we should be concerned about in our dealings with animals:

- Feelings–based definitions are set in terms of subjective mental states. Here, the requirement for good welfare is that the animal should feel well, being free from negative experiences such as pain or fear and having access to positive experiences, such as companionship in the case of social species. This use of the term welfare obviously depends on the animal concerned having conscious subjective experiences and our ability to interpret such experiences.
- Function–based definitions centre on an animal’s ability to adapt to its present environment. Here good welfare requires that the animal be in good health with

its biological systems (and particularly those involved in coping with challenges to stasis) functioning appropriately and not being forced to respond beyond their capacity. This definition is based on things that are relatively easy to observe and measure.

- Nature–based definitions arise from the view that each species of animal has an inherent biological nature that it must express. Here good welfare requires that the animal is able to lead a natural life and express its natural behaviour. This approach, which reflects a view that what is natural is inherently good, focuses on something we can measure, namely what animals do in the wild and in captivity. Because suffering, health problems and impairment of natural behaviour often accompany each other, in many cases the above mentioned three approaches will reach the same conclusions (Huntingford *et al.*, 2006).

4.1.2 Animal behaviour as a descriptor of the organism condition

The animals kept in captivity at large density and from several generations reproduced under controlled conditions may develop behavioural different traits from those usually selected individuals in the wild (Hammer, 1997; Canario *et al.*, 1998; Mendl, 2001; Sørensen *et al.*, 2001; Andrew *et al.*, 2004; Bégout Anras & Lagardere, 2004; Huntingford, 2004, 2007; Conte, 2004; Huntingford & Adams, 2005).

It is well know, for example, that the high density where animals are raised, combined with diet–induced forcibly promoting competition, may inadvertently select for high aggressiveness levels. Also, because of controlled, repaired and built where animals are raised, the absence of predators and wild prey, can lead the development of behavioural responses is not an effective defence by the predator (Malavasi *et al.*, 2004) and predation (Romano *et al.*, 2005). In this case deviation from captivity induced behaviour can be measured.

4.1.3 Behavioural responses to stress and ways of measuring fish welfare

In some respects, behavioural responses are an animal's first line of defence against adverse environmental change, often being triggered by the same *stimuli* that initiate the primary stress response (Huntingford *et al.*, 2006). In fish, as in other animals, individuals exhibit distinct behavioural strategies when faced with potentially threatening circumstances, and the type of behavioural response initiated, and the magnitude of the neuroendocrine response to the stressor, can be expressed as

individual traits (Schjolden *et al.*, 2005).

The exact behavioural response depends on the stressor concerned (FSBI, 2002). Based on knowledge of the natural responses of fish to adverse conditions, the physiological, health and/or behavioural status of individual fish have been used as indicators of compromised welfare, though the link between components of the stress response and welfare is not simple (Rose, 2002; Braithwaite & Huntingford, 2004; Huntingford *et al.*, 2006, 2007; Arlinghaus *et al.*, 2007; Øverli *et al.*, 2007). Stress responses represent a fish natural reaction to challenging conditions and these are often used as indicators of impaired welfare, so studies of physiological stress feature prominently in welfare research (Pickering & Pottinger, 1989; Barton & Iwama, 1991; FSBI, 2002; Huntingford *et al.*, 2006).

It is important, however, to recognize that physiological stress is not synonymous with suffering (Dawkins, 1998; but see also Sneddon, 2002; Sneddon *et al.*, 2003a, b). There is no particular reason to suggest that the temporary physiological activation that prepares fish for activity is detrimental to welfare and in some contexts short–term stress responses (for example, in anticipation of feeding) may well be beneficial (Moberg, 1999). If an individual fish shows disease symptoms, it seems reasonable to infer that it is in a poor state of welfare, as a direct result of disease.

Nevertheless, behavioural studies have been important in welfare research for a number of reasons. Since altered behaviour is an early and easily observed response to adverse conditions, specific responses to natural stressors (such as 'freezing' in the presence of a predator or rubbing to remove ectoparasites) can be used as an indicator of impaired welfare. Likewise, since animals pay attention to those *stimuli* that are currently important for fitness, changes in attentional state can be used to highlight welfare problems. For example, trout exhibit strong avoidance responses when exposed to a novel object (Sundstrom *et al.*, 2004). Such responses are suppressed if the fish has been exposed to a noxious *stimulus*. The fact that exposure to noxious *stimuli* interferes with the normal neophobic responses suggests that fish give a high priority to such *stimuli* (Sneddon *et al.*, 2003a). Additionally, since animals may suffer if prevented from performing their full behavioural repertoire, behavioural deficits have been used to identify conditions that compromise welfare (Mench & Mason, 1997).

The range of behavioural responses exhibited by fish to deal with stressors of varying magnitude is diverse (Wedemeyer *et al.*, 1990; Wendelaar Bonga, 1997). Altered patterns of swimming (changes in speed and direction) are shown in response to

many stressors (Juell & Fosseidengen, 2004). After an attack by another fish of the same species, fish may flee and hide or take up a submissive posture, often with altered body colour (O'Connor *et al.*, 2000; Sutor & Huntingford, 2002). When attacked by a predator, fish may respond by shoaling (Pitcher & Parrish, 1993), freezing (Goodey & Liley, 1985) or taking shelter (Brown & Warburton, 1999) and may change colour in this context as well (Endler, 1986). Feeding may be suppressed following an encounter with a predator, or inefficient feeding strategies may be adopted (Hart, 1993) and fish may avoid areas in which they have been attacked (Lima, 1998). The specific adaptive behaviour patterns are observed in response to parasitic disease (Furevik *et al.*, 1993) and to tissue damage (for example, carp that are hooked in the mouth show rapid darting, spitting and shaking of the head (Verheijen & Buwalda, 1988).

4.1.4 Aim of the study

Fish culture is one of the foodstuffs production sector with the most rapid growth in the world (FAO, 2006, 2007). In this state of affairs, however, the "animal welfare" issues are still considered of minor importance, because the farmed species are relatively "new" in terms of livestock exploiting (Duarte *et al.*, 2007). In fact, the biological needs of a species whose nervous system is simpler than that of mammals and birds are often unknown. Bearing this consideration in mind, it can be easily assumed that the response to captivity of fish may lead to levels of stress which are very different from those of more evolved organisms (Griffin & Gauthier, 2004).

In the Mediterranean basin, the spread of intensive fish farming in cages can produce behavioural patterns that are quite dissimilar from those usually observable in the wild (Sarà *et al.*, 2006, 2007a). This is because the new environmental conditions, due to captivity, are very different from those in the wild (*e.g.* the absence of predators and the relative small volume in which fish live).

The main objective of this study, therefore, was to improve the knowledge of fish behaviour in rearing conditions by investigating the captive behaviour of different–sized individuals of the gilthead sea bream (*Sparus aurata* Linnaeus, 1758) cultured in floating cages. With this aim, the most common behavioural patterns of this species were observed *in situ* at a fish farm facility sited in Sardinia (central western Mediterranean) during different times of the day (*i.e.* a.m. vs. p.m.) and in the presence or absence of food (*i.e.* before, during and after the feeding phase).

4.2 Material and methods

4.2.1 Study site and fish farming features

The study was carried out in October 2006 off the North western coast of Sardinia (Latitude 40°33’43.9’’N; Longitude 8°16’09.0’’E) at the fish farming facilities of “La Maricoltura Alghero” s.r.l. At the time of the study, this fish farm (already described in detail in Chapter 2) was located in the middle of Alghero Bay at a distance of approximately 1 nautical mile from the coastline. It covered a quadrilateral area of about 2.15 hectares (215 x 100 m) on a muddy/sandy bottom located at a depth of about 38 m (Sarà *et al.*, 2007b).

The location of the facilities was determined according to the criteria set by the Autonomous Region of Sardinia in order to ensure the protection of *Posidonia oceanica* seagrass meadows (that are very abundant all around the coast of Sardinia, particularly inside the Alghero Bay; Scardi *et al.*, 2006), and to keep a reasonable distance from coastal areas of major importance for tourism. In order to reduce the effects of prevalent winds (which mainly blow from western to eastern quarters), the fish farm had an East–West orientation.

From a technological point of view the facilities consisted of semisubmerged cages “TLC” (Tension Leg Cage with tension stays) *REFA* structured as conventional cages overturned, with the most vulnerable to wave (modules moorings, floats, core network) seats at a depth to minimize stress (Fig. 4.1).

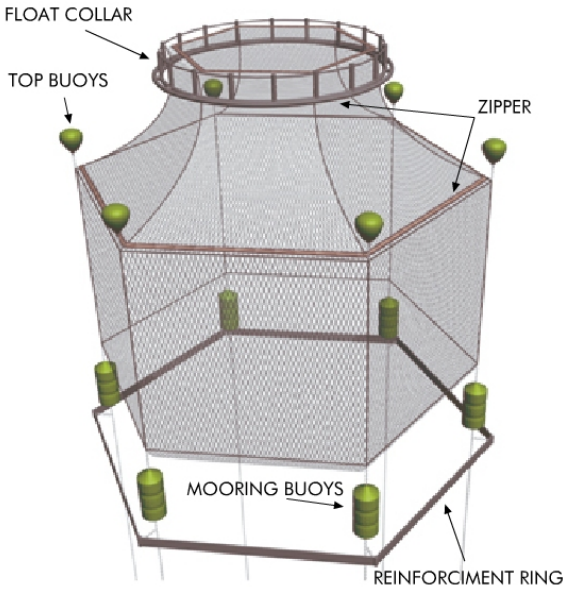

Fig. 4.1. Schematic design of a “TLC” *REFA*.

Four cages were of the "*REFA TLC 600 P*"–type (volume 600 m^3 each), and served for the seeding of juveniles. Beside these, 4 other cages of the "*REFA TLC 2200 M*" type (with a capacity of about 2200 m^3 each) were use to feed fishes up to commercial size. There were 4 more cages (with a volume of around 2,500 m^3 each) which were employed to rearing fish fry from the sowing level up to the commercial size (Fig. 4.2).

Fig. 4.2. *REFA* floating cages in Alghero Bay.

The "Tension Leg Cage" (TLC) concept is based on the dispersion of wave energy in the sea (Fig. 4.3). With increasing depth the waves are sequentially filtered; the sea is virtually calm at a depth corresponding to half the wavelength. The "TLC" cage is flexible and small in the upper section where the waves hit hardest, while its supporting structure is positioned at depth. In storm conditions the cage does not oppose the marine forces, but moves in synergy with the waves almost like seaweed, thus minimizing the strains on all cage components.

With conventional cages, the buoyancy is concentrated at the surface. The net–pen and associated weights is supported by the flotation collar on which the wind, current and wave forces all act. The floating collar and respective moorings are thus subject to violent stress, while the net-pen deforms. In high currents the net-pen can be compressed to below 30% of its original volume, while the fish are confined to the severe sea surface conditions, resulting in damage and mortality.

The principal net–pen of the "TLC" remains stable under all conditions, retaining its volume (Fig. 4.3), without any violent motion, thanks also to the effective anti-

fouling treatment of the net. This ensures a stress–free environment for the fish which continue feeding and growing, without any breaks in production. “TLC” farms can be sited at considerable depths. The vertical moorings occupy only the area of the net–pen and do not interfere with navigation or fishing and tourism interests. Each cage forms an independent unit.

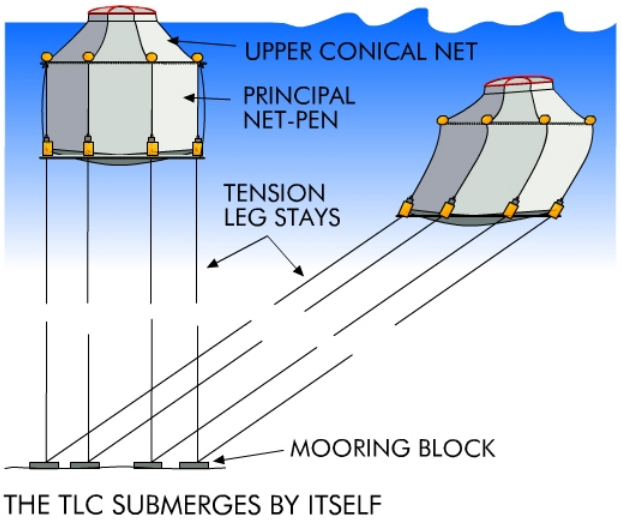

Fig. 4.3. “TLC” *REFA* in floating and submerged positions.

A “TLC” facility, mooring included, will require an installation area at least 10 times smaller than a facility with conventional cages. The cages can be installed over irregular and steep sea-floors, with mooring lines shorter than the sea depth.

The “TLC” consists of:

- the mooring module (clump weights, tension legs, mooring buoys, reinforcement ring);
- the cage-net module (net-pen, top buoys, float collar).

The mooring module is permanently installed, while the cage net module can be released to the surface for fish handling, towage, etc., just like any surface cage. The net-pen consists of a lower cylindrical part (principal net–pen) and an upper conical part. These are jointed with heavy–duty zippers, for fast and convenient removal.

On the surface the orbit diameter is equal to H, wave height, while decreases exponentially with depth as follows:

$$DZ\ H = \exp(2z / L)$$

where: DZ = diameter of orbit; z = depth; L = wavelength. At the depth L/9 (equal to 1/9 of wavelength) DZ has already halved, and the depth L/2 (half the wavelength) DZ

is reduced to only 4% of wave height on the surface. Owing to this the stress on the structure of a “TLC” cage type are equal to one fifth of those exposed in a cage moored surface. Moreover, the action of considerable size waves has the effect of completely submerge the cage below the water level, thus lowering the hydrodynamic impact on the structures.

The cages are moored by the tension forestays that keep them upright, such method has been tested over a long time on oil platforms. The forestays are made of galvanized long link chain or *spectrafibra* rope of S–Urethane, depending on the depth and the specific site characteristics.

The ballasts, weighing 4 tonnes each, are made of reinforced concrete and are disposed (6 per cage) in the circle determined by on bottom projection of the cage area (Fig. 4.1). In this way, the anchors occupy only the seabed area directly under the cage, considerably reducing mooring area than that occupied by conventional cages.

4.2.2 Description of the species studied

The Mediterranean gilthead sea bream (*Sparus aurata* Linnaeus, 1758) belongs to the Sparidae family (Tortonese, 1970). The body has an oval shape, very high and laterally compressed (Fig. 4.4). The head profile is convex with small eyes. The cheeks are covered with scales and the pre–opercular bone is scaleless. The mouth has the mandible shorter than the maxilla. Both jaws show canine (4–6) and molariform teeth, in 2–4 series in the upper jaw and 3-4 series, of which 1–2 are notably bigger, in the lower jaw. The gill rakers are short, 11–13 on the first branchial arch and 7–8 on the lower part. The lateral line has 75–85 scales. The dorsal fin presents 11 hard and 13 soft rays, the anal fin 3 hard and 11–12 soft rays. The pectoral fins are long and pointed, while the ventral ones are shorter. The caudal fin has pointed lobes. The gilthead sea bream colour is silver–grey with a big dark spot at the beginning of the lateral line that covers also the upper part of the opercular bone. A gold and a black band is found between the eyes, the golden one always narrow in the central part. The dorsal fin is blue–grey with a median black line. The caudal fin is grey–greenish white with black tips.

Sparus aurata is common in the Mediterranean Sea, it is present along the Eastern Atlantic coasts from Great Britain to Senegal, and is rare in the Black Sea. Due to its euryhaline and eurythermal habits, the species is found in both marine and brackishwater environments such as coastal lagoons and estuarine areas, in particular

during the initial stages of its life cycle. Born in the sea during wintertime, the fingerlings typically migrate in early spring towards protected coastal waters in search for abundant food and milder temperatures (trophic migration). Very sensitive to low temperatures (lower lethal limit is 4°C), in late autumn they return to the open sea, where the adult fish breed. The gilthead sea bream is usually found on rocky and seaweed bottoms, but it is also frequently caught on sandy grounds. Young fish remain at low depth (up to 30 m), whereas adults can reach deeper waters (maximum depth of 150 m) (Tortonese, 1970; Bauchot & Hureau, 1986).

Fig. 4.4. Gilthead sea bream specimens reared in cages.

This fish is a protandric hermaphrodite with a breeding season ranging from October to December. In the first two years of its life the gilthead sea bream is a functional male, while it becomes female at sizes over 30 cm. After spawning, the eggs, which are spherical and transparent, have a diameter of slightly less than one mm and present a single large oil droplet.

4.2.3 Feeding of the farmed fish

In order to meet both the biological rhythms and the physiological needs of the species reared (as well as a reduction of food waste), in the facility examined fish were manually fed (Fig. 4.5), with frequencies depending on:

- season (depending on the water temperature): *i.e.* 2 daily doses during the summer, only 1 dose during the rest of the year;
- size of fish (the smaller ones have a faster metabolism);
- fish response to food.

The average length of the rearing cycle needed to obtain a marketable product (*i.e.* sea breams weighing between 250 and 300 g) is about 12–15 months. At the end of the rearing cycle, and feeding sea breams with extruded feed of variable size depending on fish size, the estimated value of fish biomass in cages is around 15 kg m^{-3}. Actually, at the moment, extruded feed is nearly universal in the farming of a number of fish species such as several kinds of salmonid, cod, sea bass and sea bream. In particular, the high quality of fish produced at the facility investigated in the present study is guaranteed by a diet based on a granulated feed produced by Aller Aqua (which does not contain flour obtained from genetically modified organisms) and, even more, through a particularly efficient system of traceability.

Fig. 4.5. Manual distribution of the pellet at the facility studied.

4.2.4 Video sampling phases

In order to study the main behavioural traits of gilthead sea breams in captivity, videos were recorded inside rearing cages containing:

a) juvenile sea breams with a weight ranging from 30 to 40 g;

b) adult sea breams with a weight ranging from 200 to 300 g.

All the shots were recorded by a high resolution digital video camera (Panasonic NVDS 28), enclosed within a PVC housing mounted on sturdy aluminium stay slot trays to easily manage the apparatus under the water surface (Fig. 4.6).

Furthermore, with the purpose of reducing the putative disturbance of fish during the sampling phases, a particular bracket to support the whole recording equipment was employed. This allowed to use the video camera inside a cage without the need of an operator (Fig. 4.7) at a depth of about 1.5 m beneath the surface.

Fig. 4.6. PVC housing for the video recording apparatus employed.

The *in situ* sampling protocol followed a procedure which consisted of 30 minutes of shooting inside every cage, and in dividing the collection of video images into 3 phases of 10 minutes each in the following way:

1) a first phase, hereafter called PRE (*i.e.* before the meal), where sea breams, after a period of adaptation to the presence of the camera, did not feed;
2) a second phase, hereafter called DURING (*i.e.* during the meal), in which an operator positioned above the cage fed the fish;
3) a third phase, hereafter called POST (*i.e.* after the meal), in which sea breams returned to their original status.

Fig. 4.7. The video recording apparatus inside a cage.

The recording phases were planned to reduce any interference given by human

presence and were repeated inside the cages for 2 consecutive days, both in the morning (AM) and in the afternoon (PM). Moreover, during the second phase (DURING), the extruded pellet was supplied to both sea bream size classes at regular intervals (almost every 2 minutes), differently in quantity and diameter depending on fish size. In particular, 50 kg of pellet per day (25 kg in the morning and 25 kg in the afternoon) were distributed in the cage containing the smaller fish (about 100,000 individuals), while 100 kg per day (50 kg in the morning and 50 kg in the afternoon) were delivered in the cage with the adult (about 80,000 individuals).

Subsequently, the digital images recorded were framed and analysed in the laboratory, using a Personal Computer equipped with a software for image processing (Windows Media Player®). In detail, 4 movie sessions of 30" were haphazardly extracted from each of the above–mentioned phases (i.e. PRE, DURING and POST) and, for every session, the main behavioural traits of 30 sea bream specimens were observed and recorded (Figs. 4.8. and 4.9)

Fig. 4.8. Photogram recorded during the feeding phase of big gilthead sea breams.

After watching several random videos, we could identify the most frequent behavioural categories of sea bream in captivity and, consequently, the ethogram reported in Tab. 4.1 was then defined. This preliminary analysis made it possible to identify the major behavioural patterns by means of the so–called "*instantaneous sampling*" technique (Martin & Bateson, 1993).

The "*instantaneous sampling*" consisted in analyzing the individual behaviours of fish and in reporting it on a previously prepared matrix containing various behavioural categories. Their duration (in terms of frame number) after fish appearance in the

monitor until its total disappearance (*i.e.* the crossing passage) was also exactly recorded.

Fig. 4.9. Photogram recorded during the feeding phase of small gilthead sea breams.

Finally, a number of so–called "events" (exhibited by both big and small sea breams) were identified and categorized in a number of behavioural patterns whose description is reported in Tab. 4.2.

Tab. 4.1. Description of the main behavioural categories observed.

Behavioural category	Description
Horizontal swimming	Fish swims horizontally throughout all the field of view
Swimming towards surface 45°	Fish swims vertically towards the higher part of the field of view with an inclination of 45°
Swimming towards surface 90°	Fish swims vertically towards the higher part of the field of view with an inclination of 90°
Swimming towards bottom 45°	Fish swims vertically towards the lower part of the field of view with an inclination of 45°
Swimming towards bottom 90°	Fish swims vertically towards the lower part of the field of view with an inclination of 90°
Steady state	Fish stays in front of the video camera for more than 10 consecutive frames

Tab. 4.2. Description of the main events observed.

Event	Description
Direction change	During swimming fish changes direction suddenly
Collision	Physical contact between 2 fish, whose swimming was not directed towards each other
Taking food	Fish eats the pellet
Burst	Fish bursts abruptly due to a disturbing event

4.2.5 Data processing and statistical analysis

Starting from a worksheet containing all the data collected during the sampling phase, a three–way Analysis of Variance (ANOVA) was used to test the null hypothesis that there were no differences in the main behavioural categories due to fish size (2 levels: big vs. small sea breams), daytime (2 levels: AM vs. PM) and feeding phase (3 levels: PRE vs. DURING vs. AFTER). The interactions between factors was also computed. The same statistical procedure was used to test possible differences between the main events observed for the same above–mentioned factors. Cochran's C test was used to check the assumption of the homogeneity of variances and, whenever necessary, data were transformed to $\log(x+1)$. Where data transformation did not correct violations in the assumption of homogeneous variances, an alpha–level adjustment to 0.01 was used to compensate for increased type I errors (Underwood, 1997). All ANOVAs were performed using the STATISTICA® software package.

4.3 Results

For both sea bream size classes, a preliminary analysis of the video recordings inside the rearing cages allowed the identification of certain well–represented behavioural categories (6 in all), whose detailed description is summarized in the ethogram illustrated in Tab. 4.1. All these categories (*i.e.* horizontal swimming, swimming towards the surface at 45° and 90°, swimming towards the bottom at 45° and 90°, and steady state) were always observed within 3 larger behavioural activities of the fish, hereafter labelled as:

- free swimming (FS);
- swimming towards the video camera (STV);
- swimming towards the food (STF).

Furthermore, a preliminary screening of the images recorded during the sampling phase permits to clearly distinguish the 4 most recurrent behavioural patterns (*i.e.* direction change, collision, taking food and burst) of the captive sea breams investigated at the Sardinian fish farm.

As far as the importance of the behavioural category labelled as FS is concerned, ANOVA revealed significant differences in the horizontal swimming between the 2 sea bream size–classes and also among the 3 different phases considered (Tab. 4.3). In fact, as illustrated in Figs. 4.10 and 4.11 for both big and small *Sparus aurata* specimens, the number of observations of this activity in the phase DURING was fairly low if compared to those made in the phases PRE and POST. No significant differences were observed between the morning (AM) and afternoon (PM) sampling periods (*i.e.* factor "Daytime").

When considering instead the variable "swimming towards surface at 45°", ANOVA evidenced significant differences only among phases (Tab. 4.3). Furthermore, by examining Figs. 4.12 and 4.13, a remarkable reduction of the number of observations for this behavioural trait can be observed between AM and PM for both fish size–classes, except for big sea breams during the PRE and POST phases.

ANOVA found significant differences for the variable "swimming towards bottom at 45°" for all the factors (Tab. 4.3). Big sea breams always exhibited higher values in the AM period, even if a slight decrease in the phase DURING was noted (Fig. 4.14). In the same way, small sea breams showed higher values in the PRE and POST phases, with a comparable mean number of observations during AM and PM periods (Fig. 4.15).

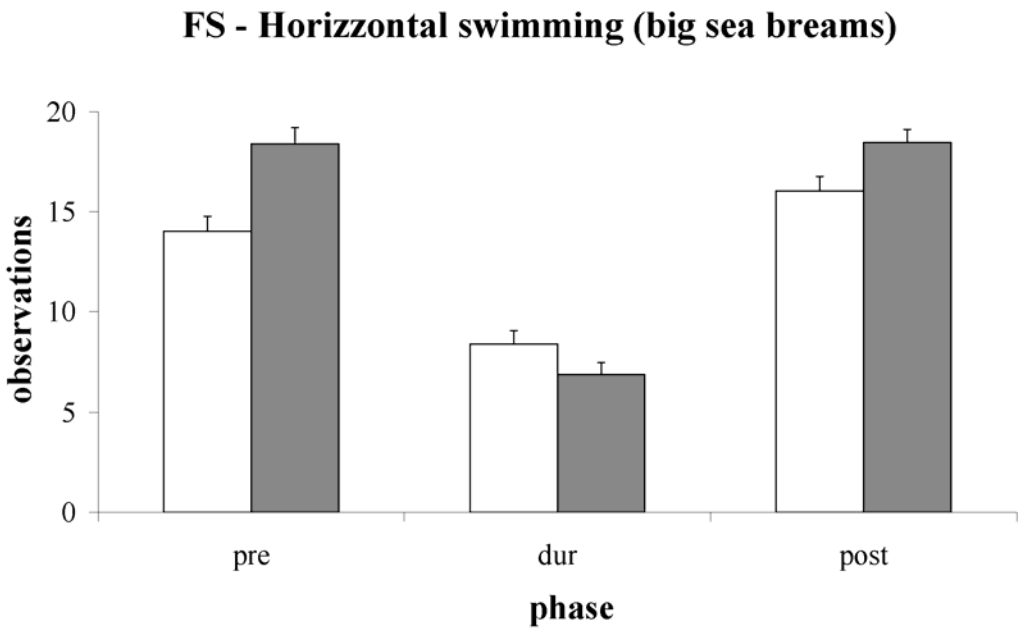

Fig. 4.10. Free swimming: mean ± SE (white=AM; grey=PM).

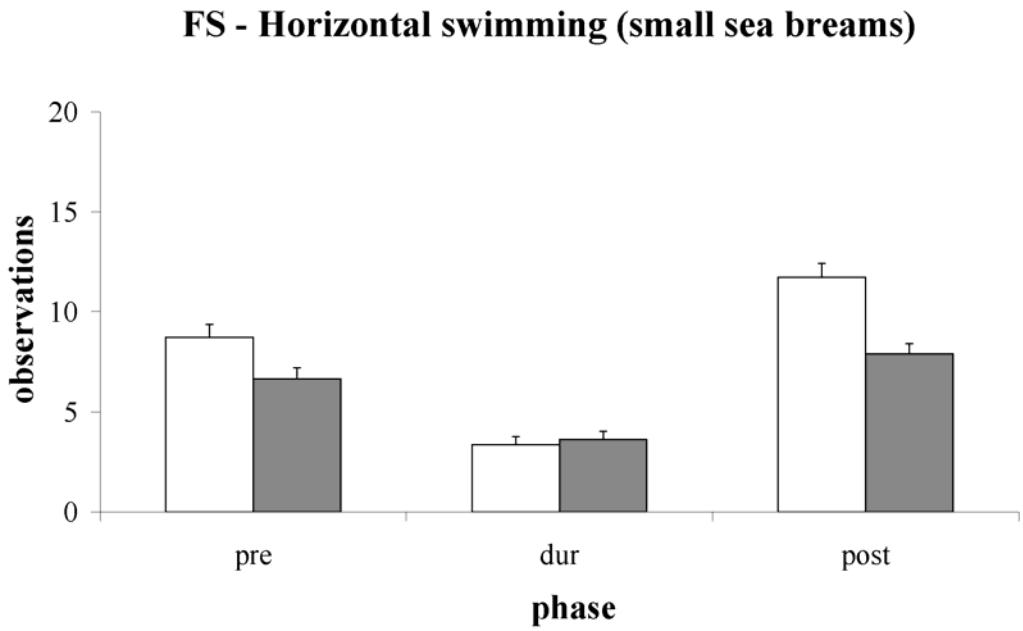

Fig. 4.11. Free swimming: mean ± SE (white=AM; grey=PM).

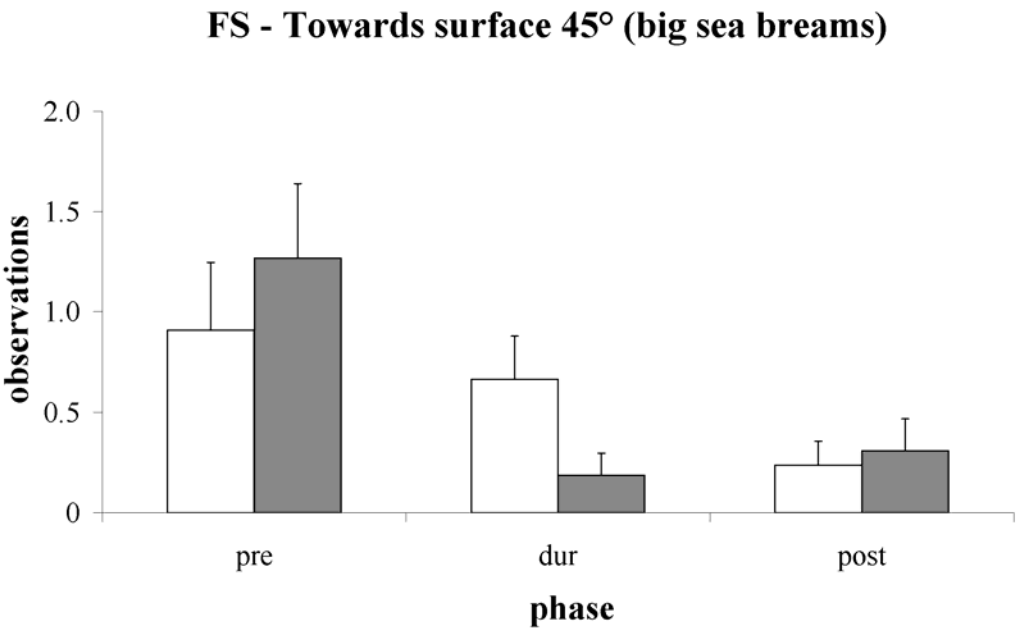

Fig. 4.12. Free swimming: mean ± SE (white=AM; grey=PM).

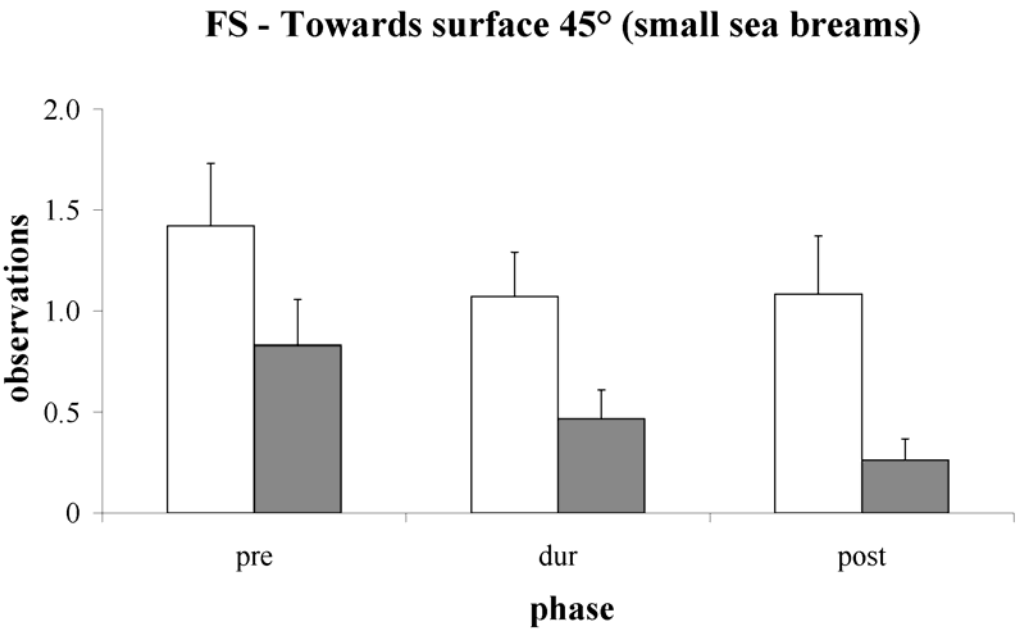

Fig. 4.13. Free swimming: mean ± SE (white=AM; grey=PM).

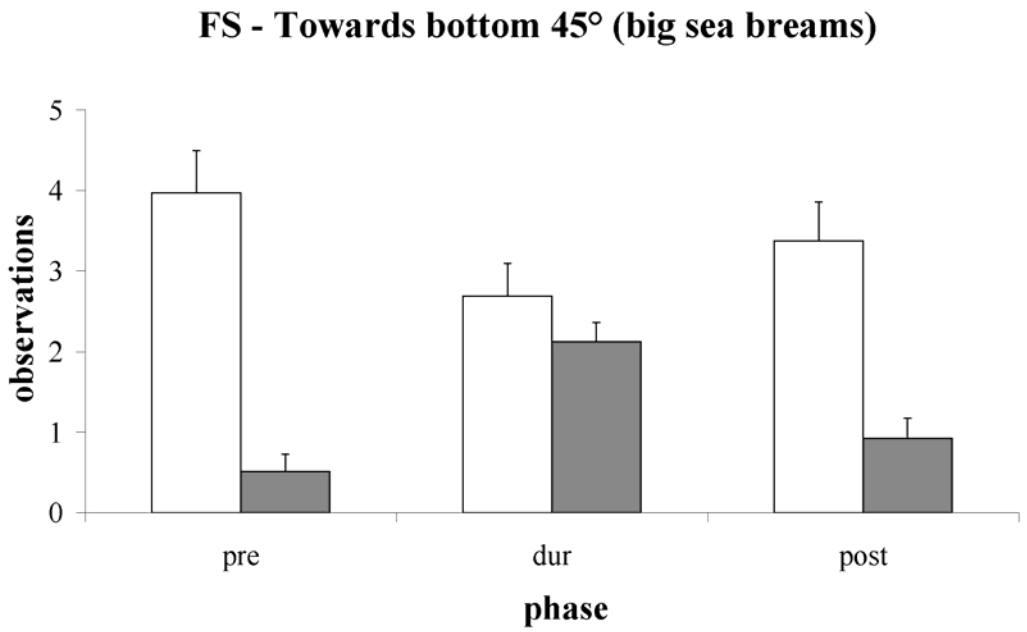

Fig. 4.14. Free swimming: mean ± SE (white=AM; grey=PM).

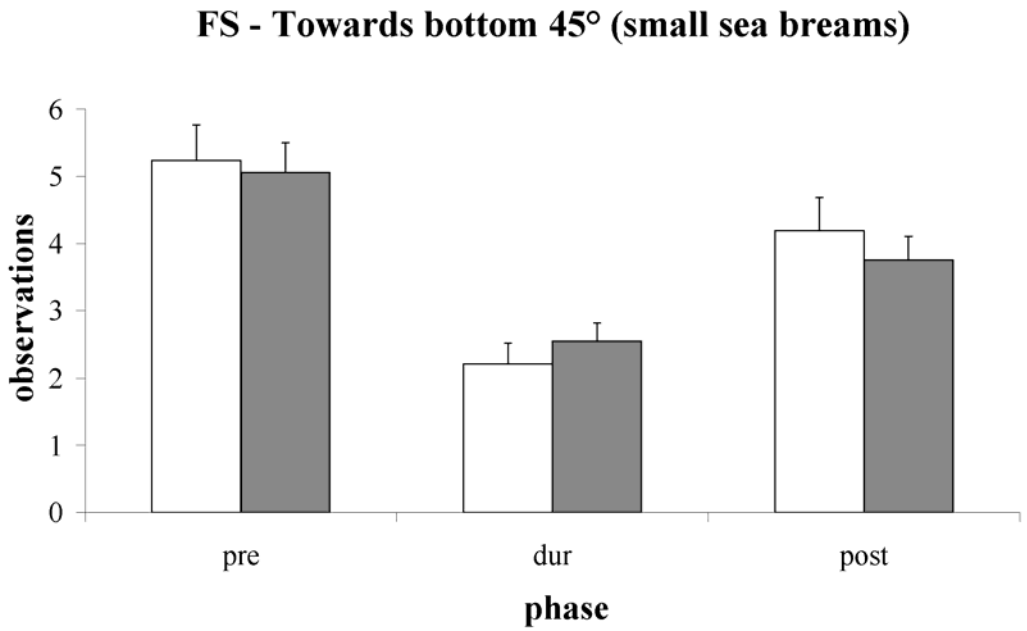

Fig. 4.15. Free swimming: mean ± SE (white=AM; grey=PM).

As regards “swimming towards bottom at 90°”, always within the behavioural category labelled as FS, ANOVA detected significant differences for both fish size–classes and phases (Tab. 4.3). In particular, big sea breams showed a clear dominance of this behavioural trait in the DURING phase (4.16), with a percentage incidence of recorded observations of approximately 67% during AM and 90% during PM respectively.

On the other hand, small sea breams showed a substantial correspondence between morning and afternoon observations, with the exception of the phase PRE during PM hours, in which a considerable increment of this kind of activity pattern was detected (4.17). In the afternoon samples, furthermore, it is noteworthy to mention a progressive reduction of this swimming activity from the period before (PRE) to that after meal consumption (POST).

With respect to the behavioural category labelled as “swimming towards the video camera (STV)”, ANOVA results are reported in Tab. 4.4. As can be seen by examining Figs. 4.18 and 4.19, both sea breams size–classes showed a significant prevalence of the horizontal swimming during phases PRE and POST during the morning as during the afternoon. It is also worth mentioning a slight decrease of this swimming movement pattern between the phase before the food supply (PRE) and that after (POST) for both big and small fish.

As regards big sea breams, a complete absence of the “swimming towards surface at 45°” in the phase of food consumption (DURING) was observed (Fig. 4.20). On the other hand, the percentage of observations for this activity was equal to about 40 and 60% in the afternoon (PM) phases PRE and POST, respectively, whereas accounted for the total (*i.e.* 100%) in the morning (AM) phase.

Always within the behavioural category labelled as STV (*i.e.* “swimming towards the video camera”), both fish size–classes showed the movement pattern “swimming towards bottom at 45°” only during the phase preceding the meal (PRE), as illustrated in Figs. 4.21 and 4.22. Moreover, it is important to stress that big sea breams showed this pattern exclusively during the AM (Fig. 4.21).

By considering the “steady state” pattern, ANOVA detected significant differences for both the factors “Size” and “Phase” (Tab. 4.4). In addition, big and small performed this kind of behavioural response in particular during the phase before the food supply (PRE), both in the morning (AM) as well in the afternoon (PM), as clearly shown in Figs. 4.23 and 4.24.

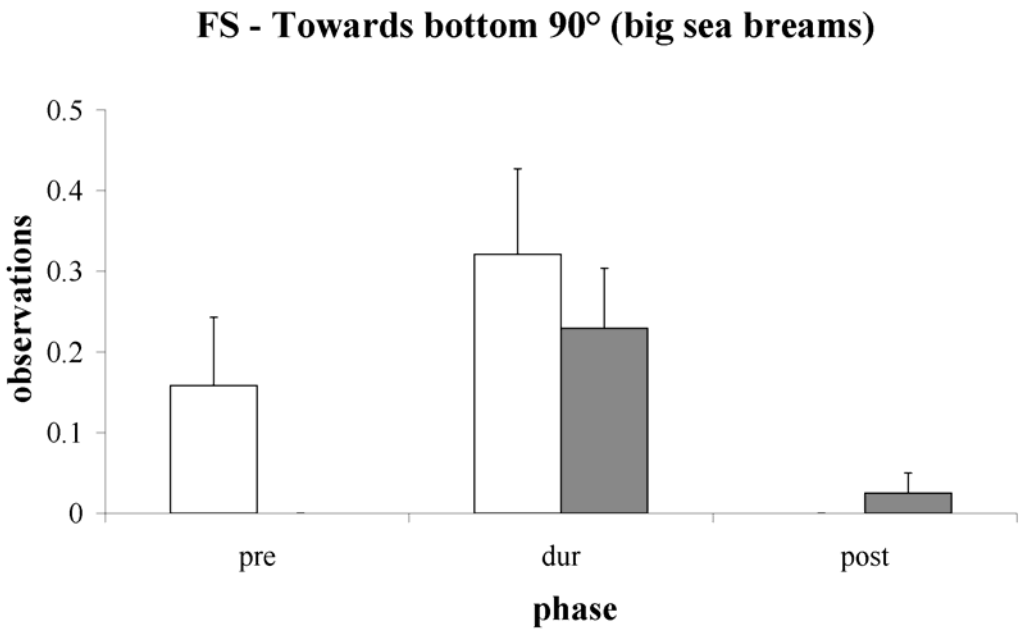

Fig. 4.16. Free swimming: mean ± SE (white=AM; grey=PM).

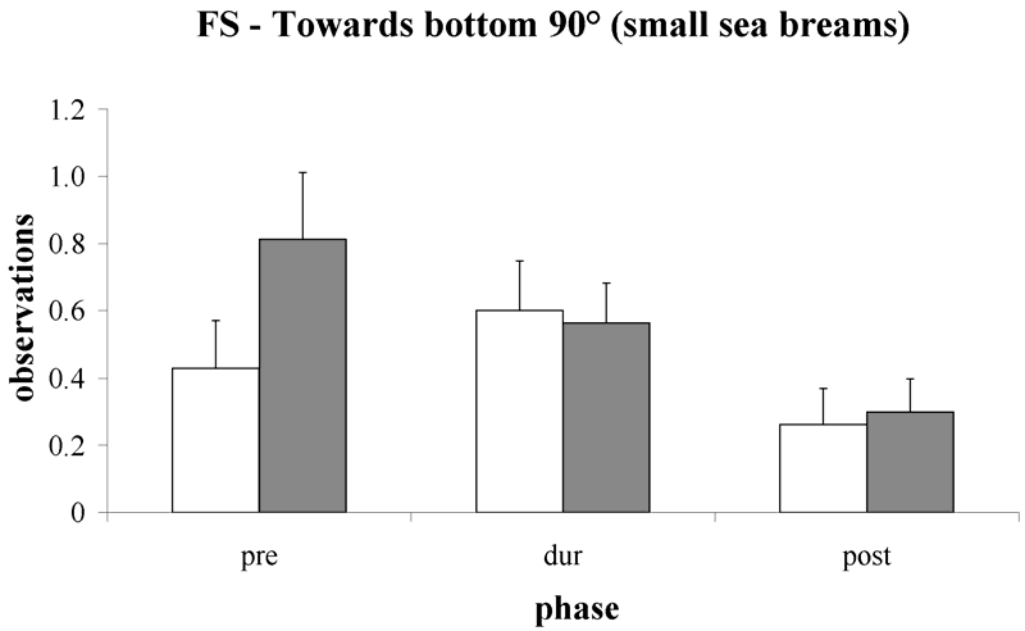

Fig. 4.17. Free swimming: mean ± SE (white=AM; grey=PM).

Tab. 4.3. ANOVA results for the behavioural pattern called "Free Swimming" (significant differences at $p<0.01$ are marked in bold).

		Horizontal swimming			Towards surface 45°			Towards bottom 45°			Towards bottom 90°		
Source of variation	df	MS	F	*p*	MS	F	*p*	MS	F	*p*	MS	F	*p*
Size	1	79.25	231.50	**0.000**	48.57	3.68	0.055	1,770.33	47.82	**0.000**	99.76	35.20	**0.000**
Daytime	1	0.00	0.01	0.908	85.42	6.47	0.011	913.50	24.67	**0.000**	0.50	0.18	0.674
Phase	2	55.03	160.76	**0.000**	108.02	8.18	**0.000**	408.95	11.05	**0.000**	20.23	7.14	**0.001**
Size x Daytime	1	3.20	9.34	**0.002**	77.36	5.86	0.016	766.73	20.71	**0.000**	7.40	2.61	0.106
Size x Phase	2	1.71	4.99	**0.007**	9.09	0.69	0.502	527.23	14.24	**0.000**	5.25	1.85	0.157
Fase x Daytime	2	0.29	0.86	0.424	11.01	0.83	0.434	190.63	5.15	**0.006**	1.89	0.67	0.514
Size x Daytime x Phase	2	2.80	8.18	**0.000**	12.72	0.96	0.382	85.03	2.30	0.101	5.19	1.83	0.160
Residuals	2,868	0.34			13.20			37.02			2.83		
Cochran's C test			0.100	ns		0.210	<0.01		0.152	<0.01		0.281	<0.01
Transformation				log(*x*+1)			none			none			none

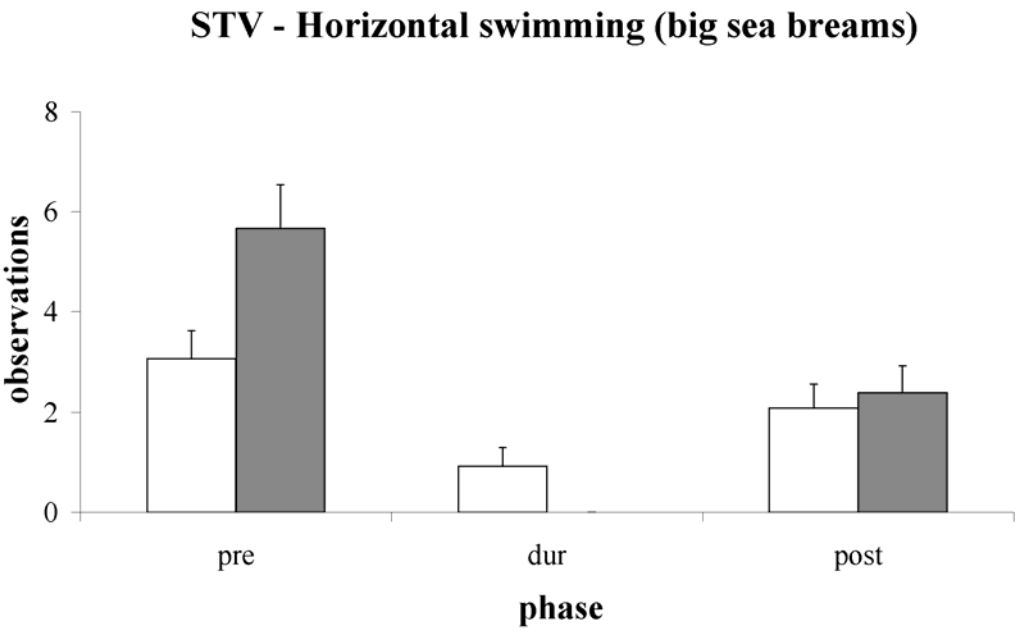

Fig. 4.18. Swimming towards videocam: mean ± SE (white=AM; grey=PM).

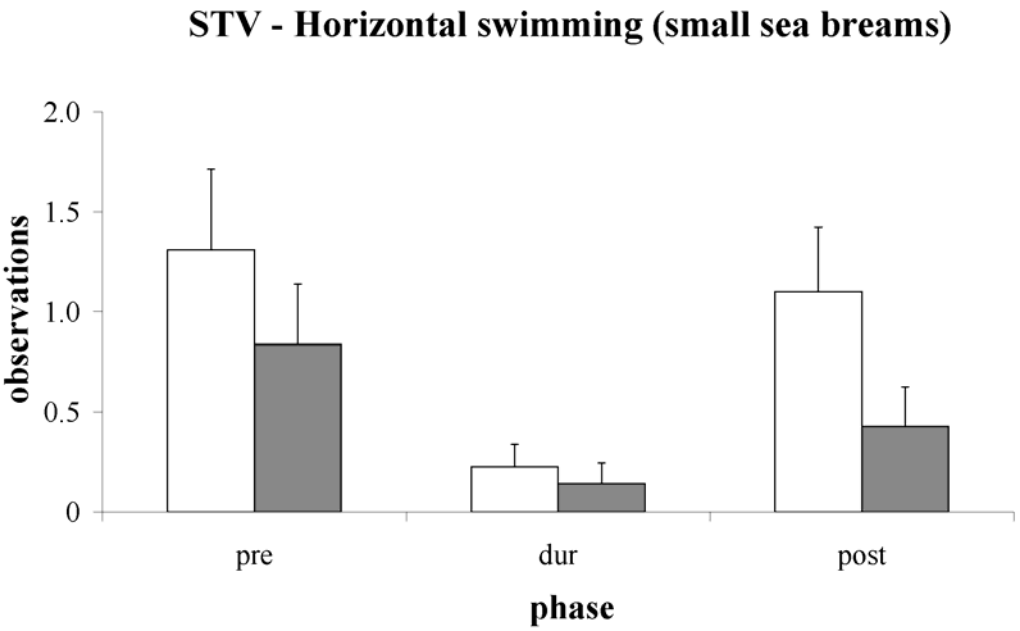

Fig. 4.19. Swimming towards videocam: mean ± SE (white=AM; grey=PM).

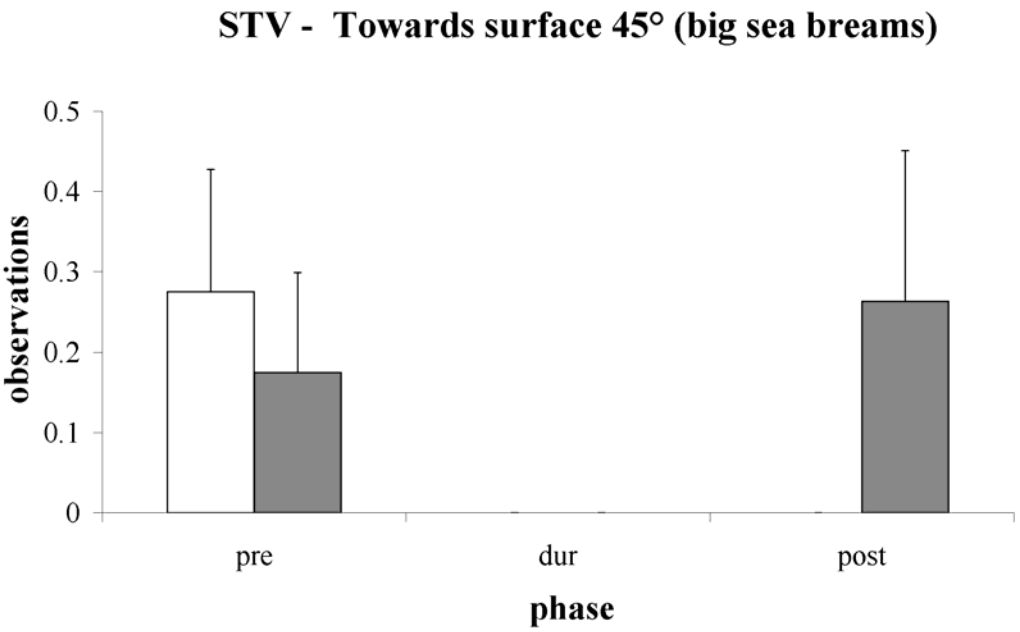

Fig. 4.20. Swimming towards videocam: mean ± SE (white=AM; grey=PM).

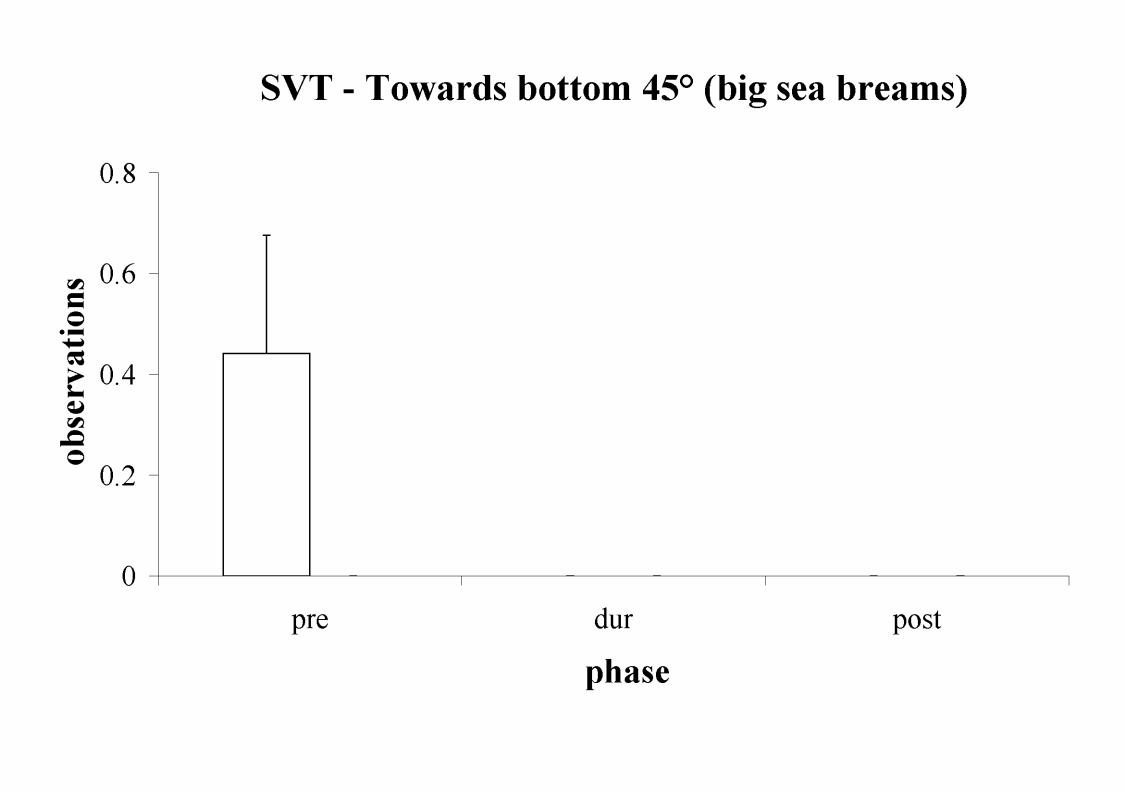

Fig. 4.21. Swimming towards videocam: mean ± SE (white=AM; grey=PM).

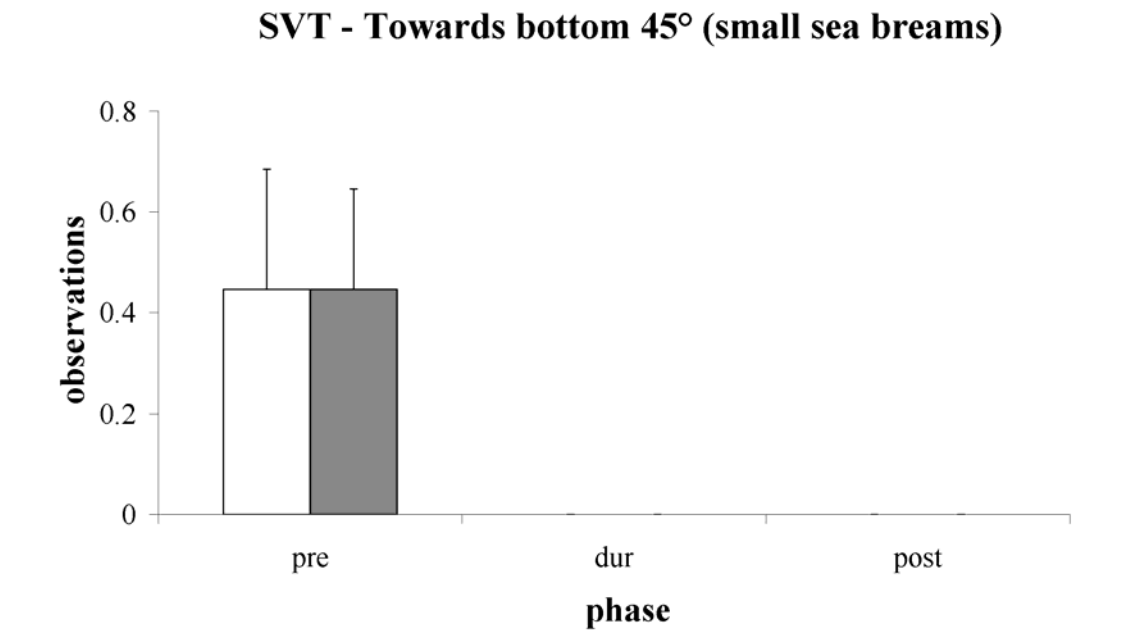

Fig. 4.22. Swimming towards videocam: mean ± SE (white=AM; grey=PM).

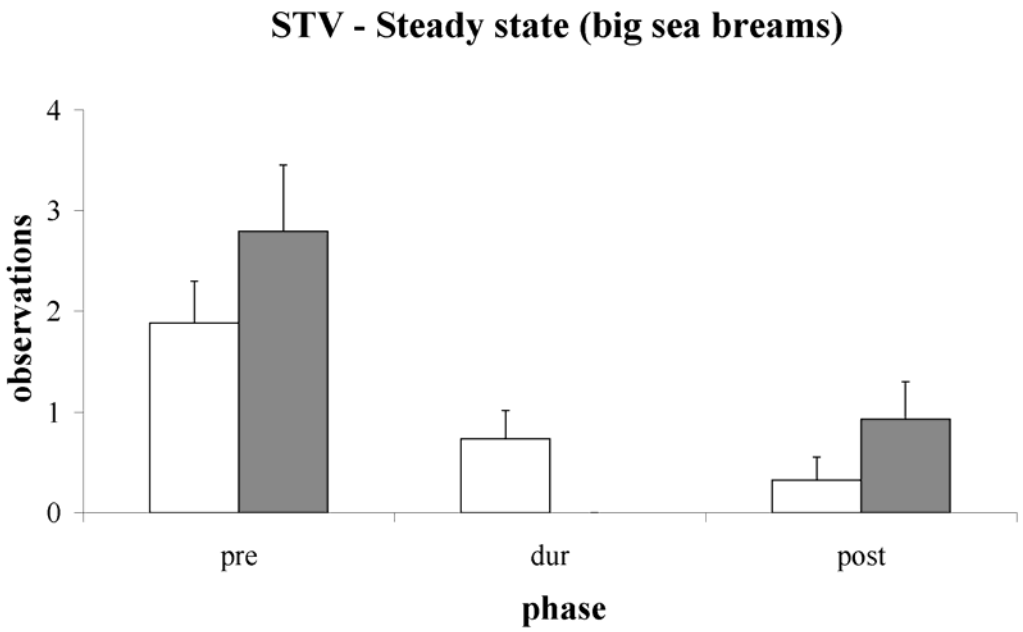

Fig. 4.23. Swimming towards videocam: mean ± SE (white=AM; grey=PM).

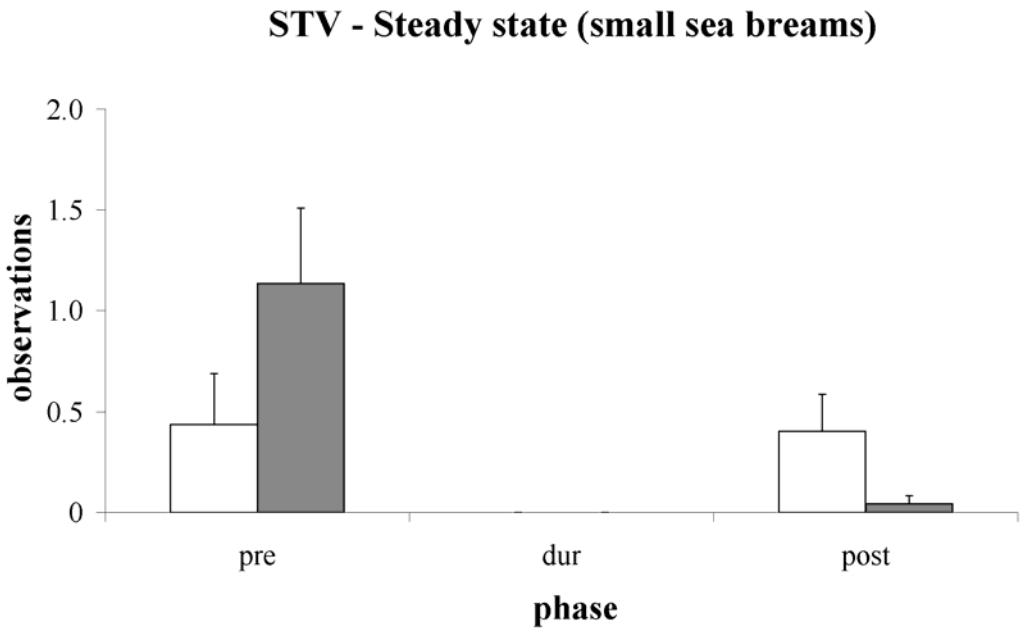

Fig. 4.24. Swimming towards videocam: mean ± SE (white=AM; grey=PM).

Tab. 4.4. ANOVA results for the behavioural pattern called "Swimming Towards Videocam" (significant differences at $p<0.01$ are marked in bold).

		Horizontal swimming			Towards surface 45°			Towards bottom 45°			Steady state		
Source of variation	df	MS	F	*p*	MS	F	*p*	MS	F	*p*	MS	F	*p*
Size	1	2,030.11	47.40	**0.000**	6.61	3.57	0.059	4.05	1.33	0.249	431.68	19.29	**0.000**
Daytime	1	11.76	0.27	0.600	0.01	0.01	0.935	3.90	1.28	0.258	25.13	1.12	0.289
Phase	2	1,381.35	32.25	**0.000**	5.20	2.80	0.061	35.56	11.68	**0.000**	519.87	23.23	**0.000**
Size x Daytime	1	206.94	4.83	0.028	1.80	0.97	0.325	3.90	1.28	0.258	4.13	0.18	0.668
Size x Phase	2	554.74	12.95	**0.000**	1.69	0.91	0.402	4.05	1.33	0.264	108.97	4.87	**0.008**
Fase x Daytime	2	164.07	3.83	0.022	3.75	2.02	0.132	3.90	1.28	0.278	83.33	3.72	0.024
Size x Daytime x Phase	2	229.03	5.35	**0.005**	1.21	0.65	0.521	3.90	1.28	0.278	43.57	1.95	0.143
Residuals	2,868	42.83			1.85			3.04			22.38		
Cochran's C test			0.355	<0.01		0.382	<0.01		0.376	<0.01		0.385	<0.01
Transformation				none			none			none			none

The results of ANOVA for the behavioural category labelled as “swimming towards the food” (STF) are illustrated in Tab. 4.5. It is important to observe that for this movement activity, a statistically significant difference was evidenced only for the factor “Phase” for all the 3 variables examined (*i.e.* “horizontal swimming”, “swimming towards the surface at 45°”, and “swimming towards the surface at 90°”). In particular, as far as the “horizontal swimming” is concerned, both fish size–classes showed this pattern almost exclusively in the phase where food was distributed (DURING), in the morning (AM) as well in afternoon (PM) daytime periods (Figs. 4.25 and 4.26), Nevertheless, a greater number of observations was always carried out within this latter period. Instead, by examining the graphic representation of the “swimming towards surface at 45°” (Figs. 4.27 and 4.28), and similarly in the case of “swimming towards surface at 90°” (Figs. 4.29 and 4.30), it can be noted that there was a substantial uniformity of this behavioural feature in the phase DURING for both big and small fish, either in the morning or afternoon.

The results of ANOVA for the most recurrent behavioural patterns observed in captive sea breams (the so–called “Events”) are reported in Tab. 4.6. Among them, the one labelled as “direction change” was characterized by statistically significant differences for all the factors considered (i.e. “Size”, “Daytime”, and “Phase”). For both the size–classes, the histogram illustrated in Figs. 4.31 and 4.32 show a dramatic reduction of this pattern in the phase DURING, between the observations carried out in the AM recording sessions and in the PM ones. A remarkable decrease can be also observed for big sea breams in the phase POST (Fig. 4.31).

The behavioural event defined as “collision” did not show any significant difference for the factors “Size” and “Phase” (Tab. 4.6), while significant differences were detected for the factor “Daytime”. By observing the graphic representations of this event illustrated in Figs. 4.33 and 4.34, a similar behavioural trend for both fish size–classes can be noted, with a higher number of collisions within the phase DURING of the AM recording sessions. For the activity event labelled as “taking food”, instead, significant differences were observed for both the factors “Size” and “Phase” (Tab. 4.6), although this behavioural pattern was shown exclusively in the phase DURING (Figs. 4.35 and 4.36). Finally, for the event defined as “burst” significant differences were detected only between fish size (Tab. 4.6). While for big sea breams this movement pattern was quite similar in all the phases (Fig. 4.37), small fish show an evident increase during the morning food distribution (Fig. 4.38).

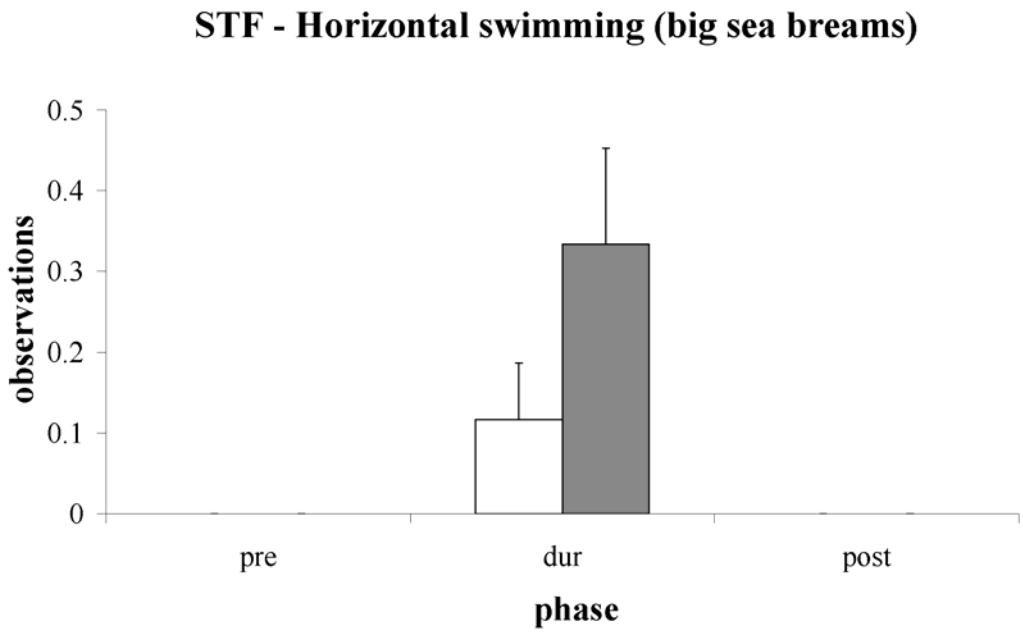

Fig. 4.25. Swimming towards food: mean ± SE (white=AM; grey=PM).

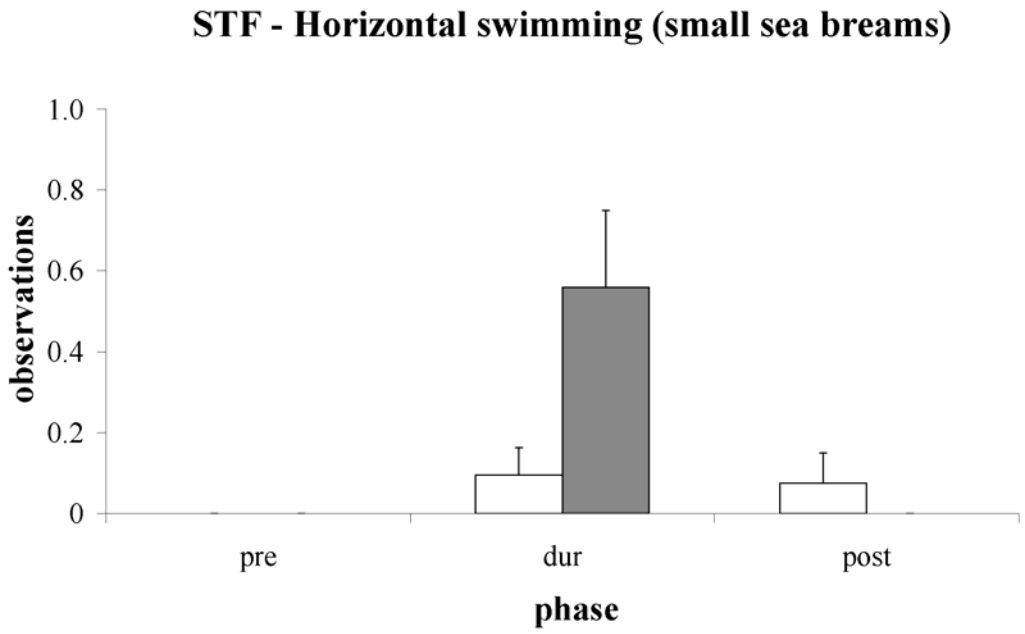

Fig. 4.26. Swimming towards food: mean ± SE (white=AM; grey=PM).

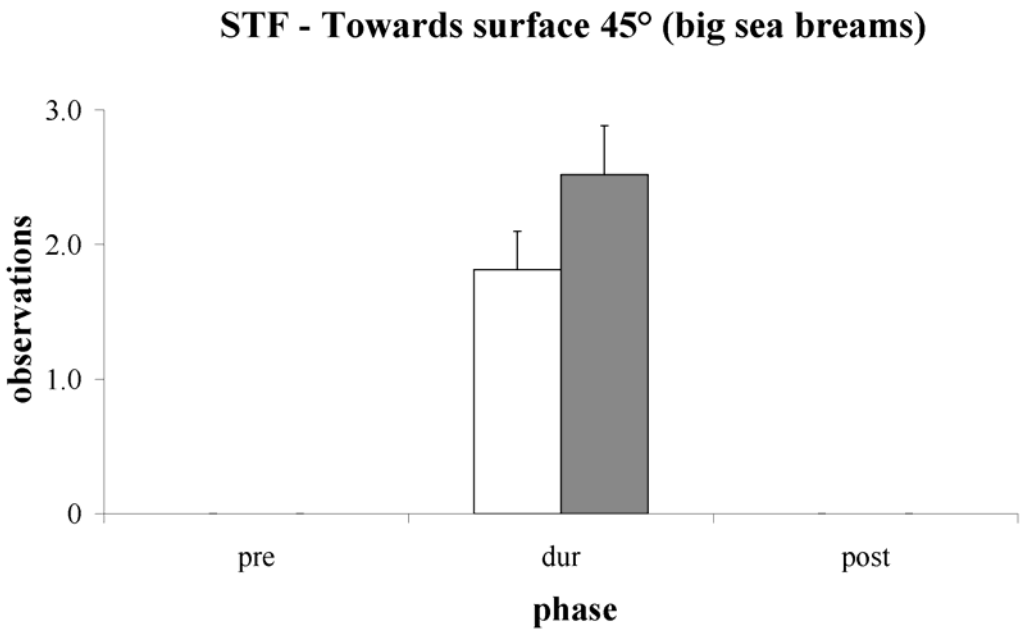

Fig. 4.27. Swimming towards food: mean ± SE (white=AM; grey=PM).

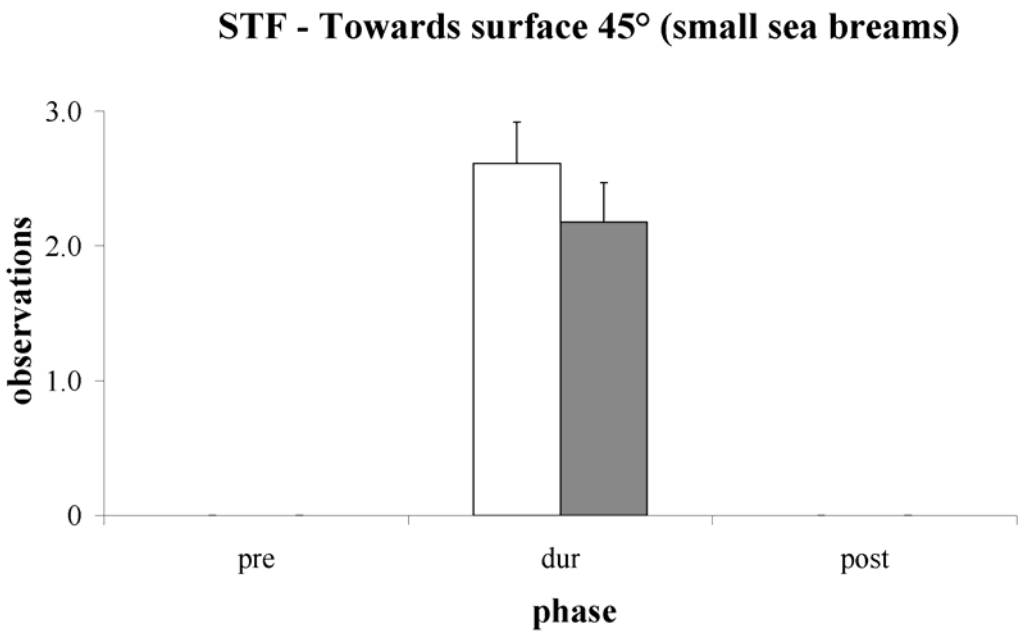

Fig. 4.28. Swimming towards food: mean ± SE (white=AM; grey=PM).

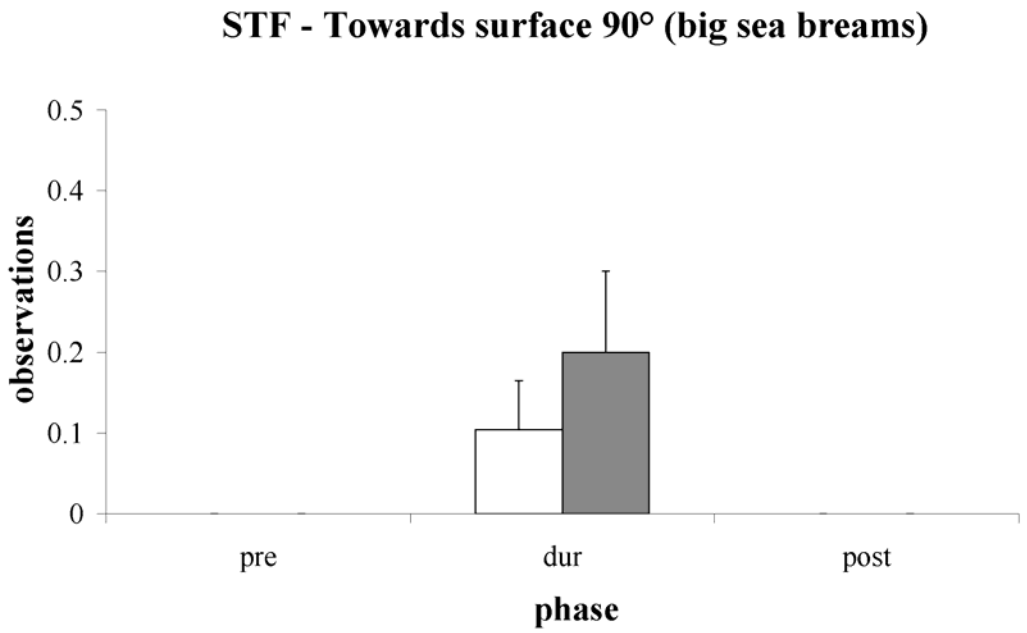

Fig. 4.29. Swimming towards food: mean ± SE (white=AM; grey=PM).

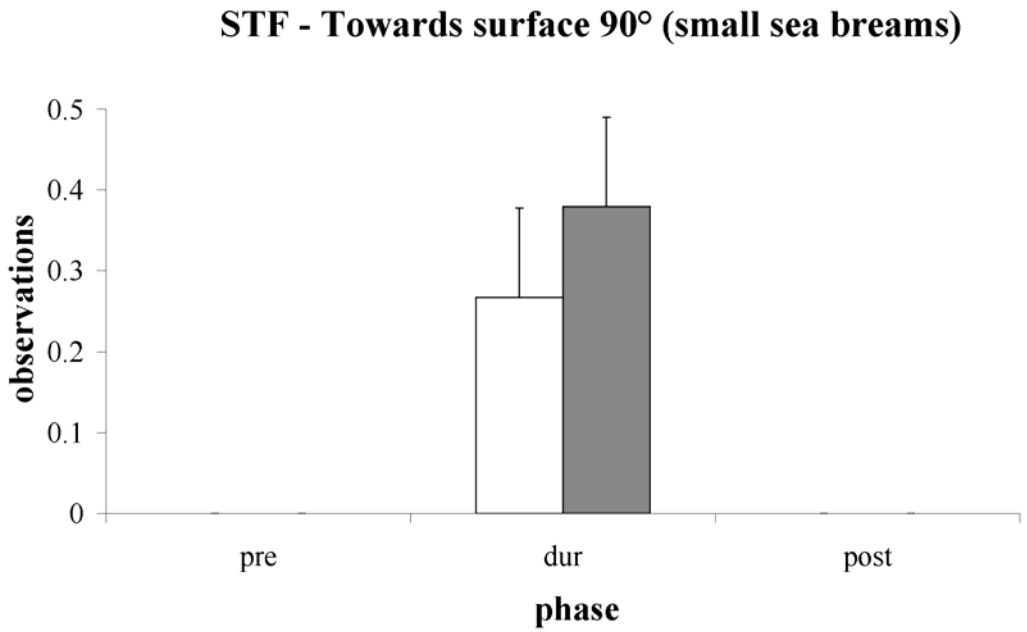

Fig. 4.30. Swimming towards food: mean ± SE (white=AM; grey=PM).

Tab. 4.5. ANOVA results for the behavioural pattern called "Swimming Towards Food" (significant differences at $p<0.01$ are marked in bold).

		Horizontal swimming			Towards surface 45°			Towards surface 90°		
Source of variation	df	MS	F	*p*	MS	F	*p*	MS	F	*p*
Size	1	1.56	1.18	0.277	4.13	0.52	0.470	2.33	3.04	0.081
Daytime	1	7.30	5.54	0.019	1.47	0.19	0.666	0.87	1.13	0.288
Phase	2	22.84	17.34	**0.000**	1663.79	210.82	**0.000**	18.05	23.53	**0.000**
Size x Daytime	1	0.58	0.44	0.506	26.26	3.33	0.068	0.01	0.01	0.932
Size x Phase	2	0.64	0.49	0.615	4.13	0.52	0.593	2.33	3.04	0.048
Fase x Daytime	2	10.36	7.86	**0.000**	1.47	0.19	0.830	0.87	1.13	0.323
Size x Daytime x Phase	2	1.69	1.28	0.277	26.26	3.33	0.036	0.01	0.01	0.993
Residuals	2,868	1.32			7.89			0.77		
Cochran's C test			0.556	<0.01		0.336	<0.01		0.322	<0.01
Transformation				none			none			none

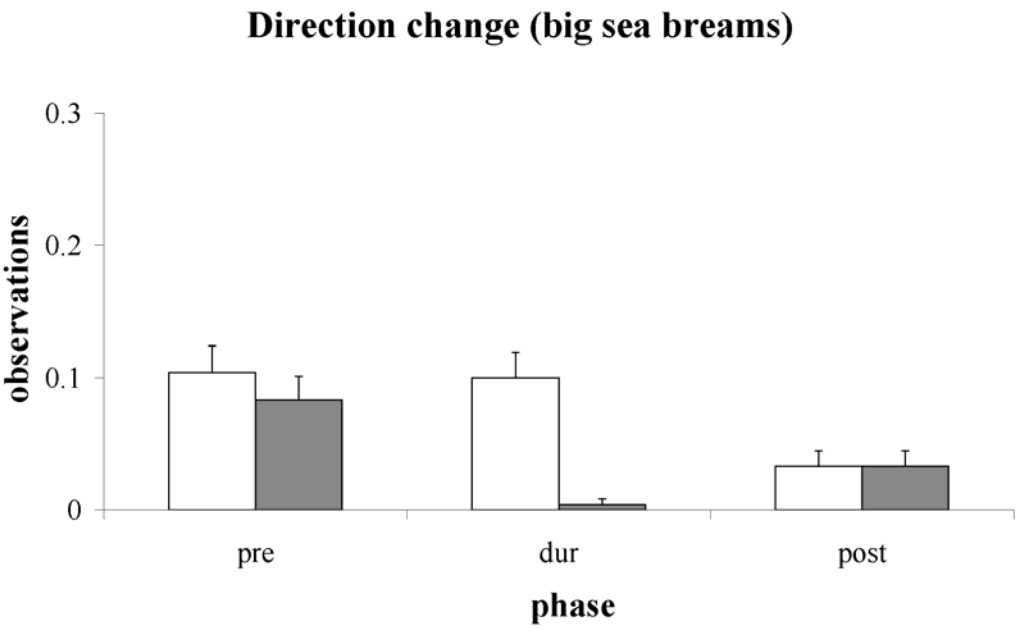

Fig. 4.31. Directional change event: mean ± SE (white=AM; grey=PM).

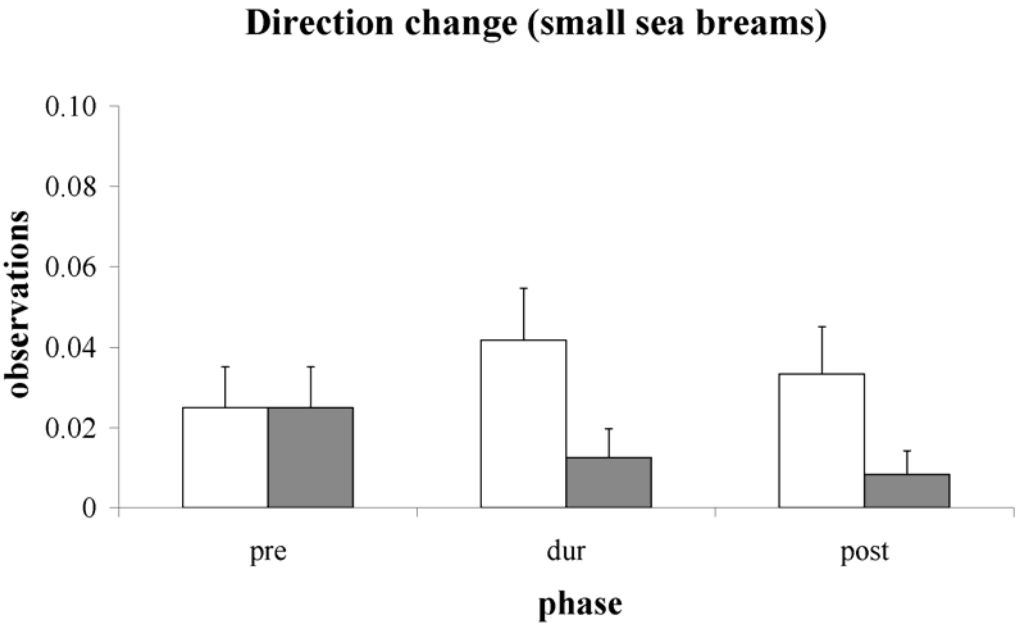

Fig. 4.32. Directional change event: mean ± SE (white=AM; grey=PM).

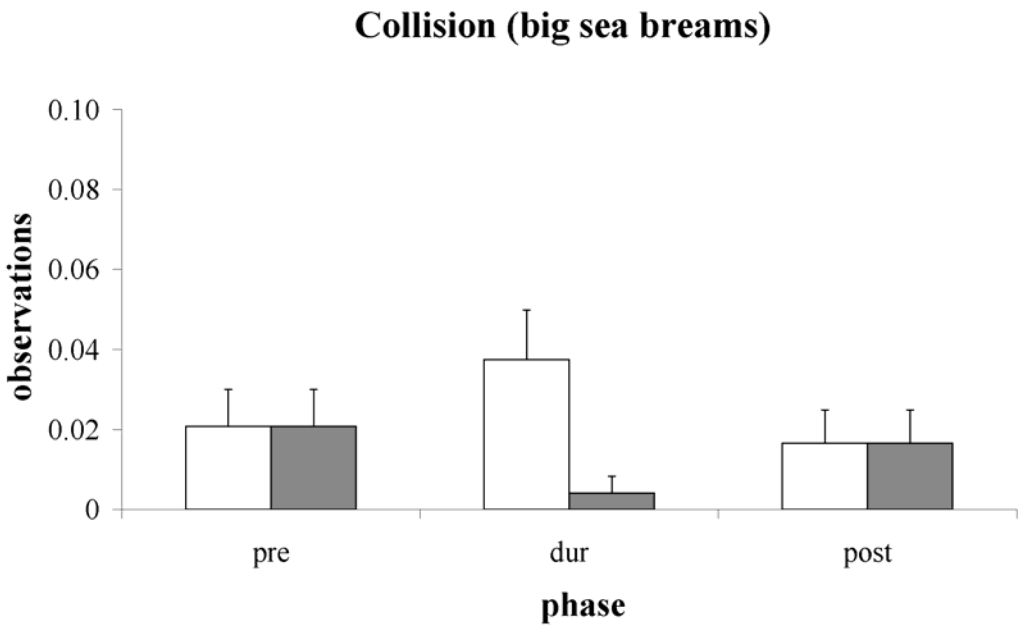

Fig. 4.33. Collision event: mean ± SE (white=AM; grey=PM).

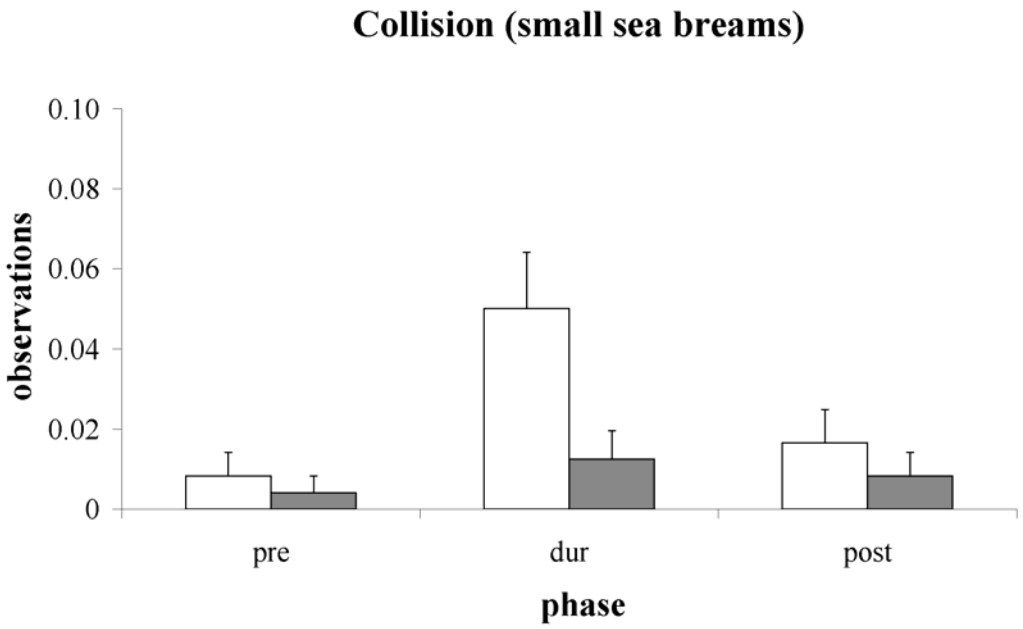

Fig. 4.34. Collision event: mean ± SE (white=AM; grey=PM).

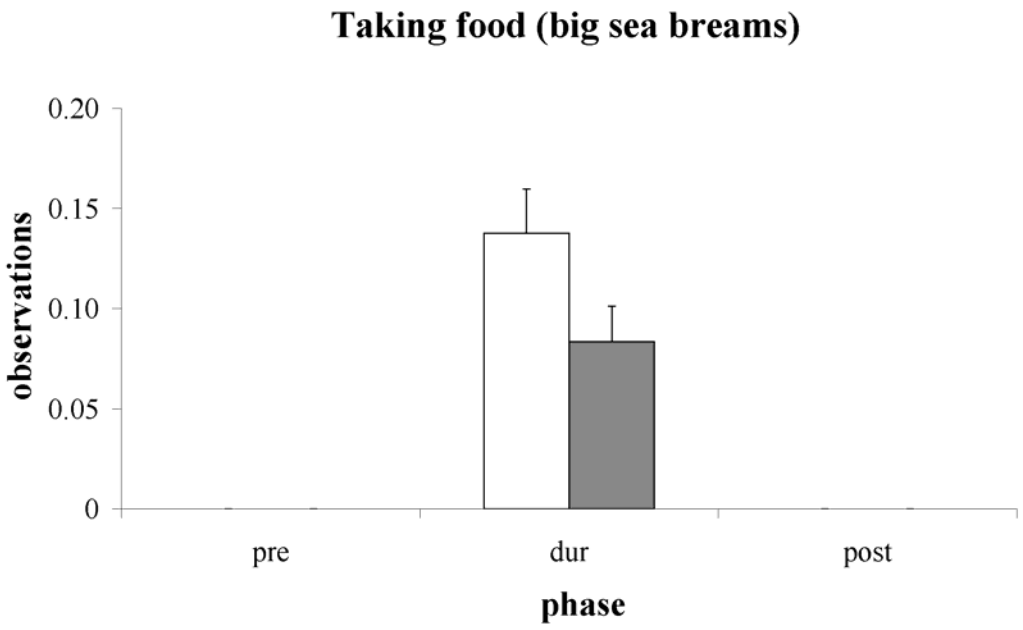

Fig. 4.35. Feeding event: mean ± SE (white=AM; grey=PM).

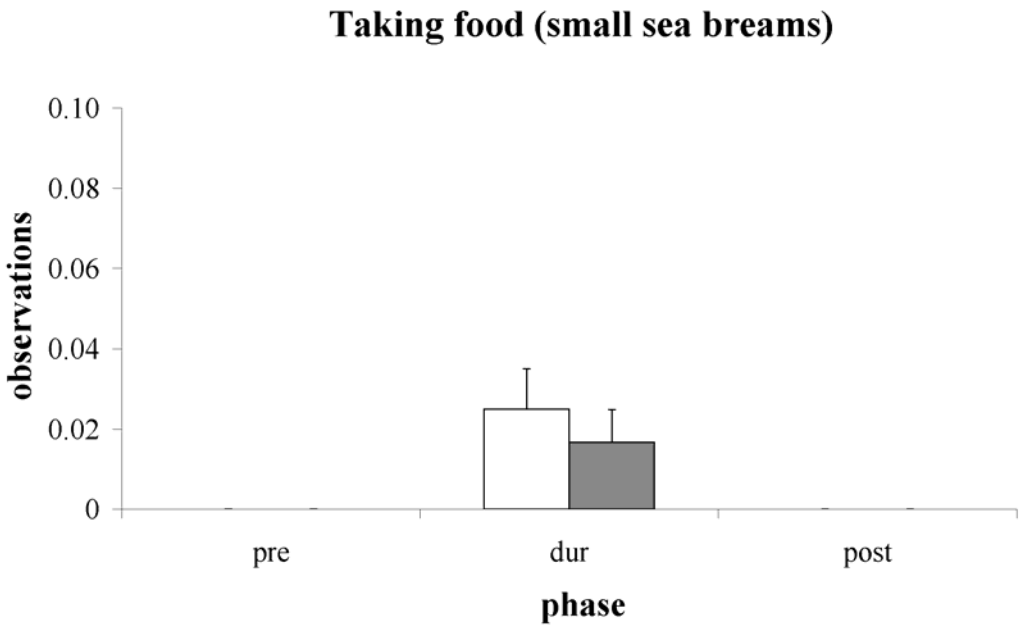

Fig. 4.36. Feeding event: mean ± SE (white=AM; grey=PM).

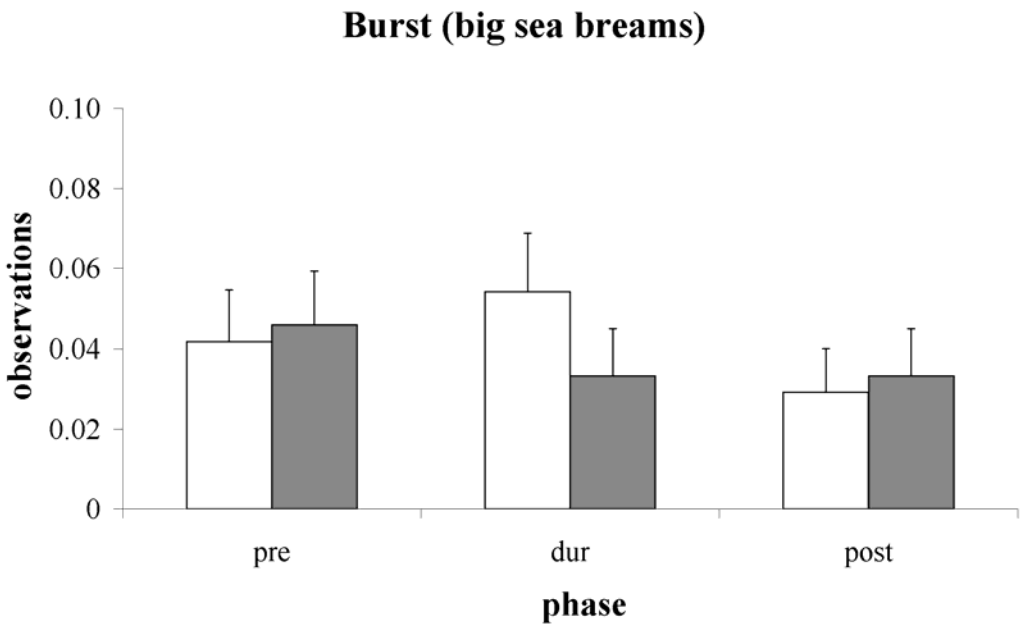

Fig. 4.37. Burst event: mean ± SE (white=AM; grey=PM).

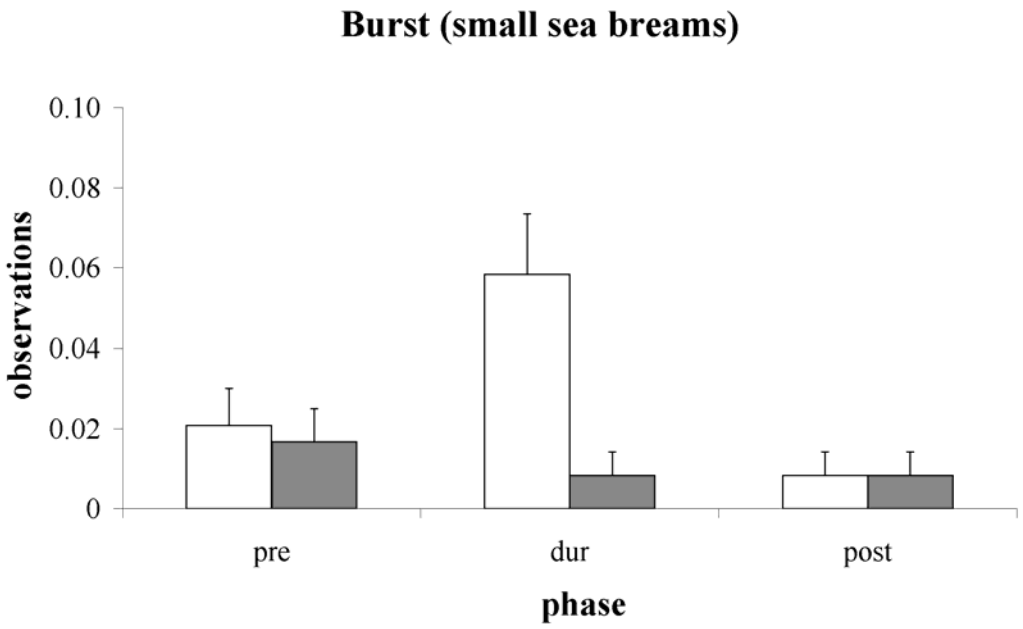

Fig. 4.38. Burst event: mean ± SE (white=AM; grey=PM).

Tab. 4.6. ANOVA results for the "Events" observed (bold indicates significant difference at $p<0.01$).

		Direction change			Collision			Taking food			Burst		
Source of variation	df	MS	F	*p*	MS	F	*p*	MS	F	*p*	MS	F	*p*
Size	1	0.90	22.97	**0.000**	0.01	0.32	0.575	0.64	32.54	**0.000**	0.27	9.45	**0.002**
Daytime	1	0.58	14.85	**0.000**	0.14	7.88	**0.005**	0.08	3.96	0.047	0.09	3.09	0.079
Phase	2	0.25	6.47	**0.002**	0.05	2.62	0.073	1.38	69.86	**0.000**	0.09	2.98	0.051
Size x Daytime	1	0.08	1.99	0.159	0.01	0.32	0.575	0.04	2.13	0.145	0.03	1.21	0.272
Size x Phase	2	0.21	5.33	**0.005**	0.04	2.15	0.117	0.64	32.54	**0.000**	0.01	0.52	0.596
Fase x Daytime	2	0.21	5.31	**0.005**	0.08	4.75	**0.009**	0.08	3.96	0.019	0.11	3.70	0.025
Size x Daytime x Phase	2	0.13	3.21	0.041	0.00	0.02	0.981	0.04	2.13	0.119	0.01	0.37	0.688
Residuals	2,868	0.04			0.02			0.02			0.03		
Cochran's C test			0.199	<0.01		0.225	<0.01		0.503	<0.01		0.160	<0.01
Transformation				none			none			none			none

4.4. Discussion and conclusions

Through the present study it was possible to collect a considerable, albeit preliminary, amount of data on the main behavioural patterns of a finfish species of high commercial value (*i.e.* the gilthead sea bream *Sparus aurata*) bred in offshore floating cages in North Western Sardinia (Sarà *et al.*, 2007b). In particular, and for the first time in this region of the Mediterranean, it was directly observed: 1) how sea breams are able to become accustomed to captivity conditions; 2) how their behavioural patterns in taking food may (to some extent) be altered by the artificial conditions of food distribution; and, above all, 3) if the human factor can play a key–role in determining behavioural responses of some species of Teleost fish in offshore aquaculture activities (Bégout Anras & Lagardere, 2004).

The observations carried out in this study on the main ethological traits of reared sea breams revealed that the breeding conditions had a noteworthy influence on both the fish size–classes examined, with a cyclical temporal recovery of the most common behavioural features. Indeed, it is important to note that for fish reared inside cages the water volume available is relatively low and constant over time, boundaries do not change, competition between individuals for space and resources is extremely high, population density remains relatively constant and food is regularly administered in fixed quantities (Canario *et al.*, 1998). All the above–mentioned factors may certainly contribute to cause some deviation from the behaviour of a species in its natural environment (*i.e.* stereotypes) and, consequently, to have a direct influence on fish welfare (Andrew *et al.*, 2004).

The results of this study also showed how gilthead sea bream specimens (both large and small) are able to express their main behavioural traits according to a definite sequence made up of a small number of events, in relation to both the period of the day (*i.e.* AM vs. PM) and the phases considered (*i.e.* BEFORE vs. AFTER the food distribution), respectively.

In terms of horizontal swimming, sea bream bigger specimens showed a significantly higher frequency, while for smaller ones it was registered a much higher tendency to swim towards the bottom. Statistical analysis confirmed a general significant difference in captive behaviour between the 2 size–classes.

In this regard, however, it is important to underline that during the whole study the main direction of the current at the site investigated was from North–West, and whose intensity could have significantly affected the swimming performance of

different–sized sea breams.

However, the behaviour of both size–classes of *S. aurata* did not appear to be responsive to the limited availability of food, but to the high unpredictability of where the food was supplied (*i.e.* the exact point on the water surface where pellet was dropped). This fact seems to be closely related to the manual distribution of the food that, although performed by expert technicians with a high degree of attention, is to some extent capable to influence the behavioural responses of fish, even if they are reared within offshore facilities.

In order to improve this study, however, it is also worth mentioning the usefulness of evaluating how and in what degree various sources of noise pollution (especially those caused by boats and vessels near the offshore facilities) could exert a direct influence on the ethological patterns of reared fish (Sarà *et al.*, 2007a).

Despite this fact, however, the general behaviour of most of the sea bream specimens resembled that of random searching of food in the wild. The position in the water column inside the cages was not predictable and fish remained close to the surface, continuously swimming with significant levels of turning behaviour (Sarà *et al.*, 2007b). As an example of this ethological trait, turning behaviour patterns have been observed on many occasions in the wild and were associated with different activities like: 1) hunting (Domenici *et al*., 2000); 2) reduction of ration (Hammer, 1997); 3) predation risk (Pitcher & Parrish, 1993).

The results achieved during the present research, therefore, led us to conclude that the behaviour of fish reared in offshore floating cages in the Alghero Bay is strongly affected by the feeding rhythms in captivity (Schjolden *et al.*, 2005). The behavioural differences detected between the 2 size–classes investigated, however, seemed mainly attributable to the distinctive characteristics of big and small sea bream specimens in natural conditions (Sarà *et al.*, 2007b).

Starting from a few years, several ethological studies have already been undertaken on the main behavioural traits of some of the most important finfish species reared in open sea conditions in Italian waters (Romano *et al.*, 2002; Sarà *et al.*, 2006, 2007a, b). Nevertheless, in order to better define the main behavioural patterns of fish living in captivity in comparison to those living in the wild, detailed studies on a large number of species are still lacking.

Indeed, although during the last decades there has been a significant increase in the number of the so–called “domesticated species” (*sensu* Duarte *et al.*, 2007) for

aquacultural activities, supplementary information on the behavioural patterns of fish is of primary importance with the aim of proposing an universally recognized definition of "fish welfare". In fact, through this concept we should be able to assess the real degree of the welfare (or of the intensity of the stress) of species subjected to fishing activities and/or to rearing conditions not only from an anthropocentric point of view (Griffin & Gauthier, 2004; Huntingford, *et al.*, 2006, 2007; Arlinghaus *et al.*, 2007).

4.5. References

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