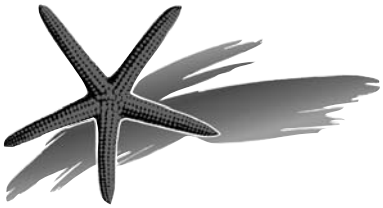
# CIESM Workshop Monographs



# Impact of mariculture on coastal ecosystems

*Lisboa, 21-24 February 2007*

**CIESM Workshop Monographs ◊ 32**.

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This collection offers a broad range of titles in the marine sciences, with a particular focus on emerging issues. The Monographs do not aim to present state-of-the-art reviews; they reflect the latest thinking of researchers gathered at CIESM invitation to assess existing knowledge, confront their hypotheses and perspectives, and to identify the most interesting paths for future action.

A collection founded and edited by Frédéric Briand.
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# I - EXECUTIVE SUMMARY

*This synthesis was drafted by all participants of the workshop under the coordination of Bella Galil and Kostas Stergiou. Frédéric Briand reviewed and edited the entire volume, assisted by Paula Moschella and by Valérie Gollino who took care of the physical production process.*

## 1. INTRODUCTION

The workshop took place from 21 to 24 February 2007 in Lisboa, Portugal. Scientists from nine countries (see list at the end of the volume) attended the meeting at the invitation of CIESM. In his welcoming presentation, Dr Frédéric Briand, Director General of CIESM, encouraged the participants to tackle the environmental issues on the agenda with scientific rigour and the most sensitive, with a full independence of mind. He then introduced Dr. Carlos Vale, Vice-Director of the Institute of Fisheries and Sea Research (IPIMAR), and Representative of Portugal on CIESM Board, thanking him warmly for hosting the seminar. After a brief overview by Dr Vale of IPIMAR structure and ongoing projects, Drs Kostas Stergiou and Bella Galil, co-Chairs of the Committee of Living Resources and Marine Ecosystems, presented the background and the main scientific challenges of the meeting as follows.

Annual global fisheries landings have been declining since the late 1980s despite the increase in fishing effort (Watson and Pauly, 2001). We now know that we are 'fishing down' the marine food webs everywhere, including in the Mediterranean, both at large (Pauly *et al*., 1998; Figure 1) and small scales (NW Mediterranean: Pinnegar *et al*., 2003; Greece: Stergiou, 2005). 'Fishing down' implies a gradual reduction in abundance of large, long-lived, high trophic level organisms and a replacement by smaller, short-lived, low trophic level invertebrates (e.g. jellyfish) and fish.

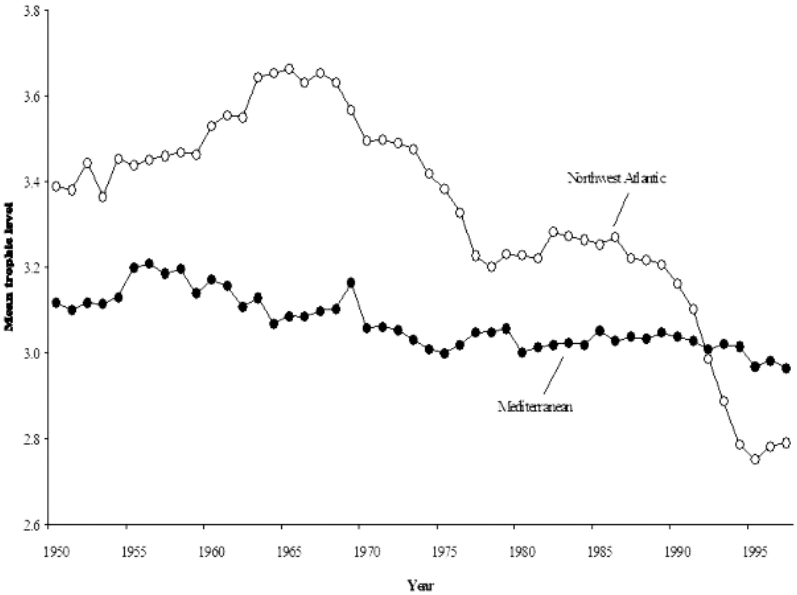


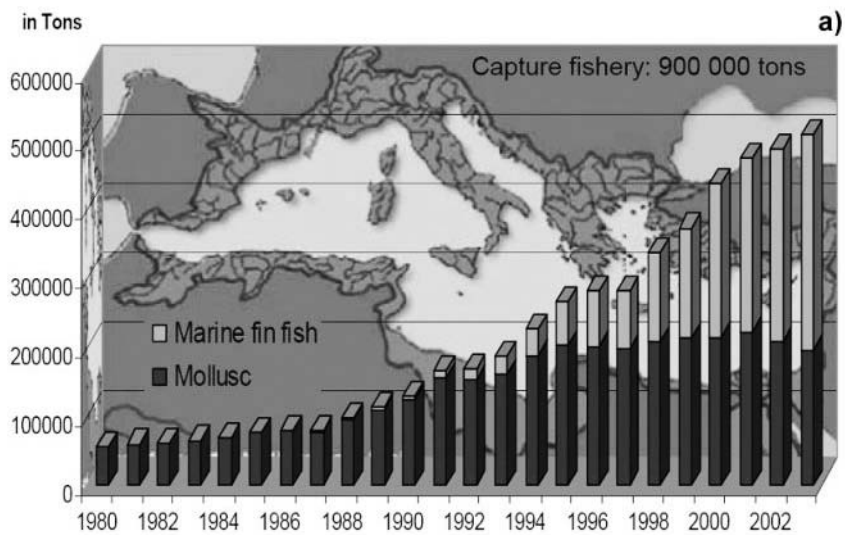

Fig. 1. Mean trophic level of the fisheries landings ('fishing down') in the Mediterranean and NE Atlantic during 1950-2000 (from Pauly and Palomares, 2000).

Indeed, the biomass of high trophic level species declined drastically (i.e. as much as 80-90%) over different spatial and temporal scales (e.g. Christensen *et al*., 2003; Myers and Worm, 2003). As a result, many stocks are threatened by biological or economic extinction. Thus, global catch per person is predicted to constantly decline from a peak in the 1990s of about 16 kg/person to less than 8 kg/person around 2020 (Pauly *et al*., 2002).

The fisheries crisis and the rising demand for animal protein have led many to increasingly consider marine farming (mariculture) as a replacement for wild fisheries. Yet "aquaculture is … also a contributing factor to the collapse of fisheries stocks around the world" (Naylor *et al*., 2001). Indeed mariculture has potentially deleterious impacts at all levels of biological organization (individuals, populations, communities, and ecosystems). Such impacts are directly or indirectly related to, among other things, the use of food, hormones, chemicals, antibiotics, degree of crowding in farming facilities, and geographic origin and ecological function of the species cultured. Both ecological and ethical aspects must be taken into account; mariculture must be considered as one of many integrated activities within ecosystem management frameworks.

Historically, the culture of marine species in the Mediterranean was small-scale and conducted *in situ* by raising local species. In the latter part of the 20$^{th}$ century, market-driven demands for finfish and shellfish rose with the increasing affluence of Mediterranean countries. This, coupled with the crisis in wild fisheries, that are either fully exploited, overfished, or depleted, has created a surge in the development of large-scale marine aquaculture (mariculture) farming along Mediterranean shores over the last twenty years. Modern mariculture in the Mediterranean was effectively launched in the 1980s, focusing on shellfish and two carnivorous finfish species with low volume capture fisheries – sea bream and sea bass. Scientific and technological advances in the past decade have led to a dramatic increase in intensive fish farming along the coastlines of the Mediterranean Sea. In 1990, total production was estimated at 100,000 tons (t), mostly shellfish; whereas in 2003 it reached 500,000 t, with finfish making up 2/3 of the total (Figure 2a). The main producing countries, in descending order, are Egypt (140,000 t, finfish), Greece (100,000 t, finfish and shellfish), Italy (100,000 t, mostly shellfish), Turkey (50,000 t, mostly finfish, 303 farms, 80,000 t production capacity), Spain (32,000 t, mostly finfish), France (Mediterranean coast only) (13,000 t, mostly shellfish), and Croatia (10,000 t finfish and shellfish) (Figure 2b). Over the past twenty years, the production of marine finfish has expanded greatly, reaching nearly 300,000 t in 2003 (Figure 2c) (Kirsch, 2006, including estuarine and lagoonar mariculture). The industry production value in the Mediterranean and the Black Sea topped 1 billion US$ in 2000, of which finfish production alone was worth 810 million US$ (GFCM, 2002).

To accommodate the demand, fish farming evolved from land-based and inshore installations to offshore cage farming, including submersible cages. It is expected that much of that growth will take place offshore. Already, over 80% of the sea bream and sea bass farms consist of sea-cage on-growing units, with land operations limited to hatcheries and pre-on-growing units. Since the



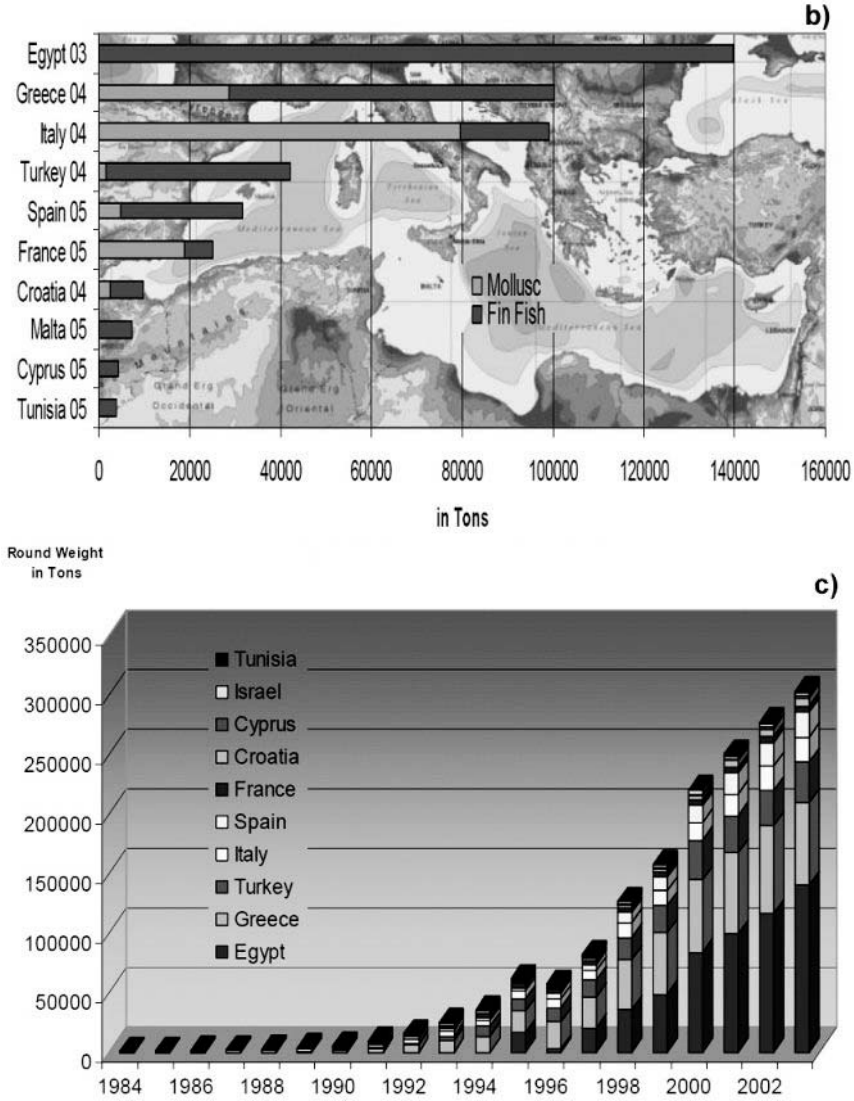



Fig. 2. Main Mediterranean aquaculture statistics: a) Marine aquaculture production in the Mediterranean Sea; b) Main Mediterranean producing countries; c) Main finfish production in Mediterranean countries (read graph in same vertical sequence of countries as listed in boxes).

development of large-volume intensive marine farming in the Mediterranean is a recent phenomenon, with a technology in relative infancy, we have only little knowledge of its impact on the local marine ecosystems. The few projects completed to date show mostly negligible, or inconclusive impacts on the environment. However, these studies essentially surveyed small-scale operations for short periods. Their application to large-scale concentrated fish farming, with possible cumulative, secondary and synergistic impacts, requires careful deliberation.

Aquaculture is increasingly based on species high in the food web, which implies that that these industries are direct fish-consuming rather than fish-producing activities. This can be illustrated using the trophic level concept, which expresses the position of an organism in the food web. In marine ecosystems it ranges from 2, for herbivores and detritivores, to 5.5 for specialised predators of marine mammals, such as the polar bear (Pauly *et al*., 2001). Indeed, the mean trophic level of mariculture production has increased with time ('farming up') in the Mediterranean with important ecological, socio-economic and ethical repercussions. This results from the fact that the vast majority of new species farmed are high-trophic level species (see perfect example of tuna fattening in section 3 below). Such a trend could be reversed by culturing species that are found low in the food web (i.e., low-trophic level species), based mainly on local resources. In addition, by-catch and fish by-products should be used more efficiently.

The workshop brought together researchers from different disciplines so as to tackle, as best as possible, the following issues related to Mediterranean mariculture:

a) effects of mariculture on ecosystem structure and function;
b) ecological effects of fattening high-trophic level species such as tuna;
c) possible effects of alien species intentionally and unintentionally introduced via mariculture on the biodiversity and ecology of the Mediterranean;
d) loss of genetic diversity through interaction with restocked or escaped cultured stock conspecific with native populations in the Mediterranean;
e) potential effects of introducing alien pathogens; disease transfer from cultured stocks to native wild fish.

## 2. THE MEDITERRANEAN ENVIRONMENT

The participants began by discussing certain characteristics of the Mediterranean Sea that need to be taken into account when reviewing the possible environmental impacts of mariculture.

The following attributes were identified as critical: (a) a low trophic potential, with the northwestern basin generally less oligotrophic than the eastern; (b) high seawater temperatures; (c) absence of a strong tidal regime; (d) phosphorus-limited primary production; (e) high biodiversity, with a large number of endemic species; (f) a highly variable coastal morphology and generally low sedimentation rate; (g) an extension of seagrass meadows into deeper waters; (h) diversity of socio-economic conditions; and (i) high volume maritime traffic (i.e. 30% of the international seaborne trade volume and 20% of the petroleum – Galil, 2006).

Mediterranean aquaculture operations are often installed over biogenic sediments and in close vicinity to seagrasses. High transparency in the water column allows this benthic flora to grow at depths of 30-40 meters. As Mediterranean biogenic sediments are generally oligotrophic, even small organic enrichments have profound effects on sediment chemistry, modifying in turn the flora and fauna. Consequently Mediterranean benthic environments will tend to be more sensitive to aquaculture operations than Atlantic terrigenic sediments, and it is not possible to directly transfer Atlantic monitoring and management approaches to the Mediterranean.

## 3. TUNA FATTENING

Tuna fattening refers to the direct catch of tunas with purse-seine nets and their live transportation within floating cages to 'farms' where they are kept in large fixed cages. In the farms, tuna are fattened using small pelagic fish (e.g. anchovies, sardines, mackerels), and then exported mainly to the Japanese market (see the official EU site <http://ec.europa.eu/fisheries/faq/resources_en.htm>). The tuna fattening industry is driven by high profitability. For instance, the highest amount paid for a bluefin on the Japanese fish market was US$180,000, close to the amount paid for a luxury car like a Ferrari. The large prices paid by the Japanese market for farmed tunas has led to a rapid, exponential growth of tuna fattening production over the last ten years (see <http://www.faosipam.org/>), with exports to the Japanese market growing accordingly (Figure 3).

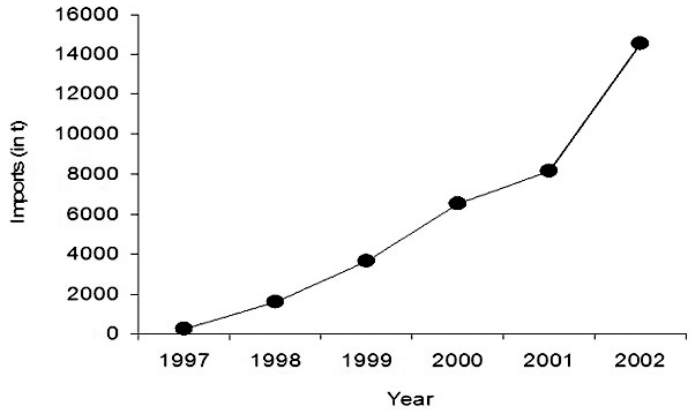



Fig. 3. Annual trend in the amount of Japanese imports of Mediterranean farmed bluefin tuna (mt). The graph refers to estimated whole weight. See <http://www.fao.org/docrep/006/Y5105B/y5105b0z.htm>.

The tuna fattening industry has developed without any conservation planning through a defined link to fisheries management policies. Today more than 60 tuna fattening farms are registered throughout the Mediterranean basin, particularly in Croatia, Cyprus, Spain, Greece, Italy, Malta, Tunisia, Turkey (Figure 4), with a potential combined capacity in 2007 over 55,000 t. This capacity exceeds the Total Allowable Catch (TAC) set for the years 2003-2006 at 32,000 t for the East Atlantic and Mediterranean by the International Commission for the Conservation of Atlantic Tunas (ICCAT) [Rec. 02-08]. The declared Mediterranean bluefin tuna catch in 2004 was 25,000 t (see <http://www.iccat.int/Documents/SCRS/ExecSum/BFT%20EN.pdf>).

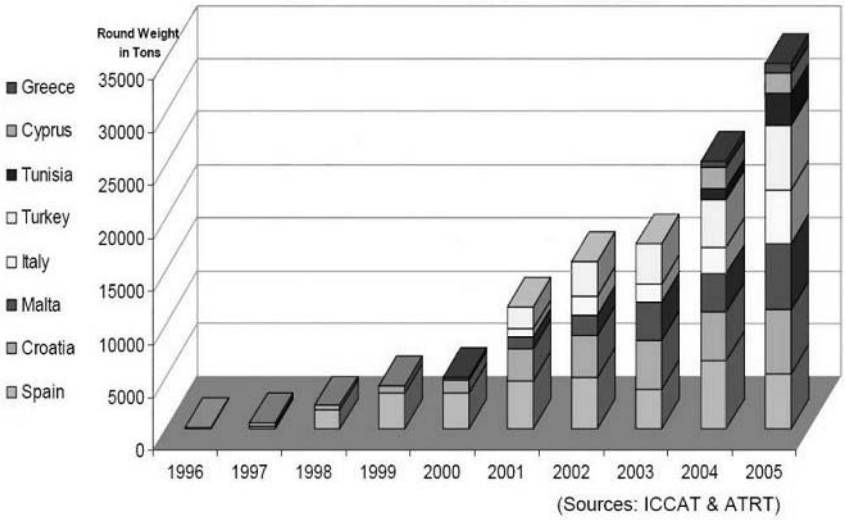



Fig. 4. Bluefin tuna fattening in Mediterranean countries (read graph in same vertical sequence of countries as listed in boxes).

However, available information clearly indicates that the actual Mediterranean catch of bluefin tuna exceeds the TAC and stands at 43,000 t, which indicates serious under-reporting. Such under-reporting is due to the fact that illegal fishing is partly driven by the profitable tuna fattening activities, seriously undermining the conservation of the bluefin tuna stock that is already overexploited. Closing the life cycle of tuna to produce juveniles for aquaculture has been successful in Japan (Sawada *et al*., 2005), but is far from an economic process at present.

Tuna farming has a plethora of direct or indirect effects on the environment. First, tuna farming enhances overfishing, both of tuna stocks (through illegal fishing, see above) and of other small pelagic fish stocks that are used to feed the tuna. The 'wild fish to aquaculture tuna production' ratio is usually less than 20:1 for the southern bluefin tuna typically ranched in Australia (Volpe, 2005). Mediterranean bluefin tuna are typically caught at much larger size for ranching (50-300 kg; Vita and Marin, 2007) (Figure 5) and therefore the ratio averaged across production in the Mediterranean may be as high as 40-45:1 (Borg and Schembri, 2006). The ratio is so high as biomass increase of tuna during the fattening process, which takes 3 – 6 months, rarely exceeds 10% (Aguado-Gimenez *et al*., 2006). The high ratio implies large quantities of wild fish used for food. Indeed, the Mediterranean tuna fattening industry, which is dominated by Spain, annually uses 225,000 t of mostly frozen and untreated fish from the North Atlantic, West Africa and South America (see <http://www.eurofish.dk/indexSub.php?id=3074&easysitestatid>). Tuna ranching may lead to an increased local fishing pressure on small pelagic species, to meet the requirements of local farms, thus putting local stocks in jeopardy.

The use of wild fish feed may cause mass mortalities through translocation of pathogens if wild fish feed is imported from non-local sources. To cite Gaughan (2002), "in 1995 and 1998/99 single species mass mortalities of sardine/pilchard *Sardinops sagax* spread rapidly throughout this species' range in Australia … dramatically decreasing the population size and representing the two most extensive mass mortalities recorded for marine organisms". The cause of the mass mortality was a herpes virus previously unknown in Australian waters. Southern bluefin tuna (*Thunnus maccoyii*) farms in the area were at the time importing the largest quantities of *S. sagax*

Fig. 5. A bluefin tuna *(Thunnus thynnus)* of approximately 250 kg harvested from a sea-cage ranch off the Spanish Mediterranean coast (left) and approximately 25 tons of frozen whole wild fish on the deck of a fish farm boat on route to feed caged tuna (right).

into Australia for use as tuna feed, providing qualitative evidence of a link between the two events and imported *S. sagax*.

In addition, the environmental effects of tuna farms are worse than in other types of fish farms (e.g. in terms of phosphorus inputs and sedimentation; Aguado-Gimenez *et al*., 2006; Vita and Marin, 2007). The accumulation of dead fish on the bottom under the tuna cages might cause a shift in the benthic community composition from omnivores and carnivores to scavengers and lead to an increase in the mean trophic level of local communities (lower-order predators eating fish; Borg and Schembri, 2006).

Finally, tuna fattening may also have socio-economic effects, because of the very low employment rate in tuna farms, compared to other farm activities. They also bring about competition for fish food with mariculture activities that have far lower 'wild fish to cultured fish' production ratios.

## 4. THE EFFECT OF SCALE

The realised and projected changes in the scale of aquaculture production are likely to result in difficult-to-predict environmental impacts. The increasing number of marine habitats affected, and particularly those of importance for the reproduction of important marine species, the potential for change of the trophic status of larger water bodies, the growing amount of escapees, the change in frequency of parasitic infestation and/or the emergence of parasites new to the area and the loss of local strains could all induce significant ecological changes in a relatively short period (10-15 years). The extent and severity of these changes can not be predicted by extrapolation based on the knowledge gained through the study of aquaculture-environment interactions at present and past levels of production. Although it is unlikely that those changes will cause a regime shift (i.e. catastrophes) at a basin scale, the risk that ecosystem goods and services could be degraded cannot be excluded. Coordinated long-term monitoring, experimental testing in high production areas should be used to obtain realistic or worst case scenarios for future changes.

## 5. LOCAL SCALE IMPACTS

### 5.1. Waste discharges

#### *5.1.1 Organic and inorganic wastes*

Mariculture farms produce wastes that are potentially harmful to the environment: unlike land-based installations, the untreated wastes are passively discharged directly into the surrounding sea. To date, these effluents are insignificant compared to other sources of marine pollution (Karakassis *et al*., 2005), but national and regional plans laid out for industry growth may change this in the near future. Mariculture farms often cluster to take advantage of favorable localities and benefit from economics of scale, raising the specter of cumulative impacts, especially in poorly flushed bays. The type and amount of wastes hinges on the species chosen, the culture methodology and practice (feed composition, conversion and wastage), and the hydrological and geomorphological characteristics of the site. Intensive finfish cage-farming – the sector

experiencing the highest growth rate in the Mediterranean – requires large amounts of feed and chemicals, resulting in copious discharges of dissolved inorganic nutrients, particulate organic wastes (undigested feed, feces, carcasses), as well as pharmaceuticals such as antibiotics, anti-parasites.

Producing a ton of gilthead sea bream releases 13.2 kg nitrogen that settles on the sediment plus 89.7 kg of soluble nitrogen (Lupatsch and Kissil, 1998). Simply put, the 2006 production of nearly 120,000 t of sea bream in the Mediterranean (Kirsch, 2006), released some 12,000 t of nitrogen. This may seem on balance a rather moderate input, except that most farms cluster in enclosed bays in a tideless sea. Karakassis *et al*. (2005) examined the finfish production and concluded that "the overall N & P waste from fish farms in the Mediterranean represents less than 5% of the total annual anthropogenic discharge, though … long term changes in nutrient concentration are likely to have severe effects on the biodiversity of the Mediterranean". The effects of organic wastes on the sediments and on the benthos beneath cages have been documented (Maldonado *et al*., 2005; Karakassis, this volume; Kuspilic *et al*., this volume; Mirto *et al*., this volume; and references therein), though the environmental impacts have been variously measured and interpreted. A number of EU funded projects strive to quantify the impacts, identify a useful biochemical signature of organic wastes.

Further the organic enrichment typical of sea-cage activity may selectively enhance invertebrate populations that may serve as vectors or intermediate hosts of certain parasite groups (e.g. gastropods and other molluscs (hosts for digeneans) and annelids (for Myxozoa)). This may increase or decrease the densities of particular parasite stages in the area.

### *5.1.2 Antibiotics and biocides*

Cage farming, where large numbers of fish are kept in close confinement, is conducive to disease outbreaks. Both prophylactic and therapeutic treatments utilize drug supplemented feeds to keep farmed fish free of disease and parasite. Antibiotics such as oxytetracycline [OTC] and quinolone drugs such as oxolinic acid [OA] are the most widely used in Mediterranean aquaculture in feed (Rigos and Troisi, 2005), while pesticides, for control of parasites, are poured *in situ* for "bathing" treatments. Both treatments, in effect, discharge drugs directly into the marine environment, where they are relatively resistant to biodegradation. Rigos *et al*. (2004) found that 60-73% of the OTC and 8-12% of the OA administered to farmed sea bream were excreted with the feces. It has been calculated that Greek sea bream farms alone discharge 1,900 kg unmetabolized OTC and 50 kg unmetabolized OA annually, in addition to unknown quantities "released via uneaten medicated feed, leached drugs and other routes of fish elimination (renal excretion, branchial secretions)" (Rigos *et al*., 2004). Antibacterial drugs have been shown to persist in the sea, including in the aquatic food chain (CIESM, 2004).

The development of antibiotic resistance is one of the dire consequences of drug pollution, yet only a single study has been conducted to date in the Mediterranean on the occurrence of resistant bacterial populations in the vicinity of fish farms. That study has shown that antibiotics, discharged through feces or undigested feed, contribute to high incidences of quinolone, tetracyline and penicillin-resistant benthic bacteria and cause a shift in the structure of the benthic microbial assemblage next to fish farms (Chelossi *et al*., 2003). Moreover, a considerable increase in resistance to several anti-microbial drugs has been discovered in some species of *Vibrio* and *Pseudomonas* recovered from diseased farmed sea bream off southwestern Spain (Zorrilla *et al*., 2003). Considering the high volume usage of anti-bacterial drugs in Mediterranean fish farming, there is an urgent need for monitoring drug contamination in water and sediments, and examining non-target species in the vicinity of fish farms for potential bioaccumulation.

### *5.1.3 Other potentially harmful materials*

Other potentially harmful mariculture-derived chemicals may originate from fish oils and meals added to the fish feed. The identities and the quantities of persistent and bioaccumulative organic chemicals, such as pesticides and polychlorinated biphenyls (PCBs), in the feed are variable and unknown. Yet, they have been found elsewhere in sediments near fish farms where they may become available to the resident biota (Hellou *et al*., 2005). Bio-accumulation of mercury in long-

lived, slow growing wild fish which are resident for long periods around cages can occur and has implications for human health if fisheries target such fish (deBruyn *et al*., 2006).

Metals are present in the feed either as part of constituents of the meal or as supplements for perceived nutritional requirements. Usually the feed is enriched with copper, zinc, iron, manganese, as well as cobalt, arsenic, cadmium, fluorine, lead, magnesium, selenium and mercury. Concentrations of copper in feeds produced for Atlantic salmon range from 3.5 to 25 mg Cu $kg^{-1}$, whereas its estimated dietary requirements are 5 to 10 mg Cu $kg^{-1}$
(see <www.scotland.gov.uk/cru/kd01/green/reia-04.asp>). The excess copper may accumulate in the sediment and the fauna, affecting ecosystem health. New technological developments can help sustain aquaculture production through DNA vaccination. However activation of other genes than those that are central in immune defence mechanisms, may occur and warrant further investigations (Myrh and Dalmo, 2005).

### 5.2 Spread of benthic pathogens

A relatively neglected aspect is the possibility that fish farms act as sources of bacteria and viruses potentially pathogenic to both fish and humans, through interactions between farms, the surrounding marine environment and wild fish. Environmental microbiological monitoring within areas influenced by aquaculture activities is important for assessing the potential of disease outbreaks for reared species, the potential risks for humans, and the fate of pathogenic microorganisms in surrounding coastal areas (Crawford, 2003).

Marine coastal sediments may contain concentrations of pathogens 100 to 1000 times higher than the overlying water (Grimes, 1975, 1980). The expansion of aquaculture may increase the risk related to the release and spread of pathogenic bacteria into coastal marine environments. Of major concern from both ecological and human health perspectives are several pathogenic bacterial strains in sediments near fish farms (La Rosa *et al*., 2001).

### 5.3 Decline of seagrass (*Posidonia oceanica)* meadows

The decline of seagrass meadows in the vicinity of fish farms is of major concern, since *P. oceanica* acts as an umbrella species, and is important for the structure and function of coastal ecosystems in large parts of the Mediterranean Sea. Pergent *et al*. (1999) reported significant changes in meadows off Corsica and Sardinia, from absence next to farms to a decline in density farther away, suggesting that "because of its ability to record environmental alterations caused by these facilities, the *Posidonia oceanica* meadow is a good bio-indicator for use in monitoring studies." Similarly, a study off Murcia, Spain, found that the *Posidonia* meadow affected by the farm was seven times as large as the facility itself, with a marked decrease in "shoot size, leaf growth rate and the number of leaves per shoot in plants close to the fish farm" (Ruiz *et al*., 2001). The effects of discharges from farms continue after cessation of operations: long term effects were recorded three years after closure of a farm off Minorca (Delgado *et al*., 1999): the site closest to the defunct facility showed "reduced shoot density, shoot size, underground biomass, sucrose concentration and photosynthetic capacities." The authors concluded that the decline was due to the persistence in the sediment of organic wastes from the fish farm.

Future moves to locate fish farms further offshore into deeper waters would reduce the impact on the depth-limited *Posidonia* meadows, but may imperil maerl beds, and the waste would be dispersed over a significantly larger area.

### 5.4 Introduction of alien species

#### *5.4.1 Intentional and unintentional introduction of alien species*

The environmental impacts of marine alien species are potentially severe, unpredictable, and often irreversible. The impacts may stem from purposeful introductions, or from the unintentional introduction of organisms associated with the intentionally-farmed species. The commercially-important shellfish, *Crassostrea gigas* and *Ruditapes philippinarum*, were intentionally introduced to the Mediterranean in the 1960s and 1970s, respectively. Transport and transplantation of alien molluscs resulted in numerous unintentional introductions and oyster farms served as effective gateways into Mediterranean coastal waters for alien macrophytes (Mineur, this volume). Yet, in complete disregard for their ecological impacts, a broader array of alien species are being

introduced for mariculture purposes. According to Marttin (2002), half of all introductions in the Mediterranean region are driven by aquaculture. Because of the high permeability of aquaculture facilities, all introductions should be regarded as probable releases into the wild, as reflected by two recent records. A single adult red drum *Sciaenops ocellatus* was collected from Hadera harbour on the Mediterranean coast of Israel in 1999, where it probably arrived from a nearby land-based fish farm (Golani and Mires, 2000; Marttin, 2002). *Pagrus major*, a eurythermal sparid fish, was imported from Japan to Croatia, and raised in cages off Pasman Island till 1999 (Marttin, 2002). In 2004, a mature individual was caught off Molat Island; it had survived in the eastern Adriatic for at least five years (Dulcic and Kraljevic, 2007). Hybridization between *P. major* and the native *Dentex dentex* was effected in captivity (Kraljevic and Dulcic, 1999). Among alien species considered as "candidates for farming" are the Erythrean alien *Siganus rivulatus*, the eastern Pacific striped jack *Carnax vinctus,* the western Pacific flatfish *Paralichtys olivaceus*, and *Solea senegalensis*. Meanwhile, some segments of the mariculture industry resort to unreported introductions: thus a bilaterally ablated female banana prawn, *Fenneropenaeus merguiensis*, was collected in the Bay of Iskenderun, in southeastern Turkey, in late 2006 (Ozcan *et al*., 2006). Since eyestalk ablation is commonly used in aquaculture for inducing maturation of gonads, there is no doubt that this ablated mature female specimen escaped or was released from a nearby aquaculture facility. However, neither the Turkish authorities or FAO had been notified of the importation of that species for mariculture in the Mediterranean.

### *5.4.2 Alien parasites and pathogens*

Alien species frequently arrive with their complement of parasites and pathogens, and the permeability of mariculture installations, in particular cage farms, facilitates the transmission of parasites and pathogens to wild populations (Diamant, this volume; Krkošek *et al*., 2006). Mariculture is a likely source of pathogens in wild populations and nutrient runoff serves as a synergistic stressor Harvell *et al*. (2004). Cage mariculture creates opportunities for transfer, such as the near-constant presence in the water column of feces and food particles, which expose surrounding wild fish (both demersal and water column living species) to increased risk of infection. Transfer of diseased spat between farms has long been the plea of the industry, and has occurred time and again with devastating regularity. Farm feed too may be implicated in alien pathogen transport: mass mortalities in wild Australian clupeids caused by an alien pathogen near tuna feedlots was blamed on imported infected frozen fish used for feed (Gaughan, 2002). Man-made risk factors responsible for introducing pathogens (e.g. stocking with infected fingerlings, contaminated well boats, nets, pipes and ballast waters, contaminated frozen fish used as food for BFT) need to be recognized and controlled. At the same time, biotic vectors and physico-chemical parameters (wild fish carrying clinical or latent infections, encrusting organisms that act as intermediate hosts, high water temperatures that drive infections, etc.) need to be identified and characterized. Welfare issues are of concern, as dense over-stocking of fish, or fish kept in farms subjected to compromised environments, will render the fish more susceptible to infections due to increased host stress and consequential reduced immunity. Clustering of cage farms in sheltered bays may create infection foci that can potentially endanger native fish communities.

## 5.5 Impacts of fish farms on cage-associated wild fish assemblages

Cage farming attracts large numbers of both pelagic and demersal wild fish as feed lost from the farm and profuse fouling increase the cages' draw as Fish Attraction Devices (Dempster *et al*., 2002; Tuya *et al*., 2006; Sanchez-Jerez *et al*., this volume; and references therein). Potential effects of wild fish feeding on anthropogenic feed include changes in feeding behaviour with corresponding physiological changes (Fernandez-Jover *et al*., 2007a) and bioaccumulation of potentially harmful chemicals such as heavy metals, pesticides and PCBs (see above). The close association between wild and farmed fish fosters mutual pathogen transfer. Further the high stocking densities practiced in cage-farming allow for amplification of wild parasites (such as cymothoid isopods) and pathogens, and their subsequent transmission from cultured to wild populations (Zlotkin *et al*., 1998; Papapanagiotou *et al*., 1999; Horton and Okamura, 2001; Papapanagiotou and Trilles, 2001). Cases of introduction and transmission of alien parasites and pathogens that effect complete host shift to native species are well documented (Diamant *et al*., 2004; Diamant *et al*., this volume; and references therein). Large aggregations of wild fish near

cages attract legal and illegal fishing and increase the vulnerability of already depleted populations (Dempster *et al*., 2006).

*A contrario* intensive aquaculture zones have been found to boost local fisheries by increasing the size and number of demersal fish up to a dozen km away from the farming zone (Machias *et al*., 2005). Management of such impacts can be undertaken through traditional fisheries management approaches.

### 5.6 Impacts of escapees

Escapes of juvenile and adult fish from sea bream and sea bass installations occur in the Mediterranean, but their extent and impact upon wild stocks are largely unknown (Dempster *et al*., this volume). 'Escape through spawning' by cultured fish held in cages also occurs, as documented for sea bream in Greece (Dimitriou *et al*., 2007). Negative hybridisation effects of escapees mixing with wild fish are possible (García-Vázquez *et al*., this volume), although their importance will vary, depending on the population size and structure and on the life-history strategy of each species, from virtually nothing to measurable fitness depression (Bonhomme, this volume).

Demographic effects leading to negative impacts on fisheries have also been suggested as an effect of escapees. Dimitriou *et al*. (2007) showed that a 5-fold increase in the numbers of wild sea bream in the Messolonghi Lagoon in Greece was correlated with the onset of intense sea-bream farming activities and possibly escapes through spawning within cages. However, the sea bream captured by the local fishery were far smaller after the onset of farming activities, suggesting that the higher numbers of fish placed density dependent limitations on growth rates. These small sized sea-bream fetched a lower price, so much so that the overall value to the fishery declined by 6% despite greater catches.

Better information to document the extent of escapes in the Mediterranean is required, such as mandatory reporting of escape incidents, while improved methods to trace escapees (Triantaphyllidis *et al*., this volume) and prevent escapes (Dempster *et al*., this volume) need development. As storm damage is one of the greatest causes of escapes, development of a technical standard specific for sea-cage installations in the Mediterranean Sea would help ensure that each farm is engineered to match the environmental loading (waves, currents) experienced at each specific site. As farms move to more exposed, offshore locations, the demand for such technical requirements will keep growing.

## 6. TOOLS FOR MANAGEMENT

### 6.1 Genetic tools for managing mariculture

An effective basis for managing wild species' fisheries is to know the stock structure along with the distinctive ecological characteristics of each stock. Advances in molecular biology and biochemistry have permitted the development and use of a variety of genetic markers to answer questions relevant to the management and conservation of commercially important marine species. Genetic data are valuable for both stock structure analysis and the conservation of genetic resources, where the extent of fisheries activities (e.g. harvesting, size-selective mortality) and genetic component of genetic differentiation are important management considerations (Carvalho and Hauser, 1994). In marine populations one finds in general less spatial variation in genetic structure than in freshwater populations. This has been attributed to a number of factors (see Graves, 1998): i) many marine organisms have exceptional dispersal and migratory capabilities, ii) species ranges can be vast and iii) their life history may include high fecundities and explosive reproductive potentials. This does not prevent that striking differences in genetic structure have been found in certain cases (e.g. Kotoulas *et al*., 1995; Magoulas *et al*., 1996; Nesbø *et al*., 2000).

These differences underscore the need for a thorough understanding of the genetic basis of population structure of marine species, especially at the local scale. The possibility to detect escapees of aquaculture origin will be enhanced if there is a detailed knowledge of population structure of wild as well as farmed populations. The existence of baseline data to assign the origin of individuals being tested should be a prerequisite, since markers diagnostic for farmed individuals is lacking in most cases. As information on population structuring is limited or lacking for most

of the species of interest, research on this aspect should be a major priority in aquaculture areas and in situations where stocking is undertaken.

Research should include extended spatial and temporal monitoring of populations and not rely on a single set of samples from a single time period. Even in cases were data have been collected on molecular markers, comparative studies cannot be easily exploited due to lack of standardization among studies and marker characterization (sampling design, appropriate use of controls, replicate screening within and between laboratories). Intercalibration of the results of different laboratories is still minimal. Databases of produced genotypes and of the genetic material of control individuals are much needed so as to take advantage of the previous efforts invested on the genetic analyses of species of aquaculture interest. Knowledge is also much needed on the impact (quantitative and qualitative) of escapees on local populations. This would include data on the survival and reproductive success of escapees and on the fitness of hybrids between wild and hatchery reared individuals.

### 6.2 Siting and species selection

Mediterranean areas critical for the survival of species, such as SPAs, MPAs, or nursery areas, should be well distanced from mariculture farms. To reduce and limit the ecological impacts of parasites and diseases, siting of farms should avoid areas where transmission is likely.

The main ecological criteria for future siting of farms should be to reduce environmental impacts, in particular the physico-chemical damage (e.g. from effluents) and the impacts on nearby habitats and species. Gaps in specific knowledge render the application of such criteria difficult in many cases. There are gaps in marine habitats mapping and consequently on which habitats should be protected. At present, there are no common standards for siting. Obviously the standard values that exist should be better defined, e.g. for effluents, and. the future reference values defined by the Water Framework Directive will be welcome. In view of the current trend to move farms offshore (see below), new criteria for offshore locations will need to be developed and strictly followed.

A key question is whether mariculture should be highly concentrated in a few sites or spread across a greater number of locations to reduce the risk of disease transmission. This is a difficult issue to handle and generalize, since it depends on specific habitats and on the species cultured: for example in Japan aggregating farms seems to have little impact on disease transmission.

### 6.3 Inshore or offshore?

Land-based farms are easier to control but their impact depends on the sites. Generally speaking, the logistics of sheltered nearshore cage farming is less ”complicated“ than in the open sea or in land-based pond systems. Although some participants felt that in the Mediterranean land-based farms are not a good investment due to the generally high cost and demand for coastal land, it was recognized that there will be a general trend of moving fish farms either offshore into deeper waters (e.g. the Turkish government mandated that all marine fish farms must move offshore) or to retreat inland, depending on the area. Seawater pond systems currently envisioned for the near future are either semi-intensive, integrated polyculture systems or highly-intensive, recirculated pond systems. The former enable production of a higher diversity of species but require more pond surface area per kg of fish produced, while the latter are much less forgiving and highly susceptible to even short system breakdowns, due to the high density of fish standing crop.

### 6.4 Integrated or recirculated systems

Recent studies call attention to the potential of integrated aquaculture techniques (polyculture of species with different trophic levels, e.g. fish, shellfish and algae cultured together, with the algae and filter-feeding shellfish removing nutrients and particles) for capturing and capitalising on the flux of particulate and dissolved nutrients from land-based cultures or fish-cages (Chopin *et al*., 2001; Hussenot, 2003; Troell *et al*., 2003; Angel, 2004). Variations on this theme have been in use for many years in Asia, notably in China and Vietnam where polyculture aquaculture is a traditional practice (Alongi *et al*., 2000).

The potential for such systems to reduce the levels of nutrients introduced into marine waters and produce additional crops of value is of great interest and the process is currently tested in several

countries, such as Australia, Canada, Chile, Israel, South Africa, Spain and the U.K. Yet, much work remains to fully develop mariculture systems combining different species in a single production unit. Although the question of economic viability is unclear for certain species and locations, it could be the method of choice under certain conditions (Piedrahita, 2003; Hussenot, 2003; Neori *et al*., 2004). Few successful examples exist in the Mediterranean where the monoculture of sea bream, sea bass and tuna remains the norm.

### 6.5 Criteria for selection of future species?

The rapid growth of mariculture in the Mediterranean was followed by species diversification due to market saturation, high competition and plummeting profits. Alien species are among the species considered for introduction, or those already reared on a trial basis (see 5.4.1). The workshop participants debated the use of native/local species vs. introduced alien species, weighting ecological responsibilities against sustainability (e.g. low trophic level species), given that alien species may out-compete or interact negatively with local species when they escape. Some participants favored the use of an 'ecologically friendly' West African *Tilapia* species, while others suggested that local *Tilapia* spp. may be used for farming. In was pointed out that *Tilapia* is more suited generally to land-based systems, as it hybridizes easily. Some participants defended the precautionary principle that aquaculture should be limited to native species, unless it can be demonstrated that risk is negligible.

## 7. MAIN RECOMMENDATIONS FOR A SUSTAINABLE MARICULTURE

***Restrict fishing in the vicinity of mariculture farms***

Given the enhancing effects of aquaculture on the density of fish feeding around cages, the concept of setting aside mariculture areas as a no-fishing zones should be given serious consideration. Both demersal and pelagic fish aggregate at farms and they tend to be large adults (Dempster *et al*., 2002; Boyra *et al*., 2004). A no-fishing zone could both protect a proportion of the spawning stock (Dempster *et al*., 2006) and allow these fish to provide the farm with an 'ecosystem service' as they eat the waste feed from the farm and lessen the intensity of the benthic impact (Vita *et al*., 2004b). Regulations for fishing in the vicinity of fish farms should be defined and implemented. To begin with, no-fishing zones would have to be sufficiently large to have a protective effect. For full effect, management and enforcement of such no-fishing zones should be included within the responsibilities of regional MPA networks and should not be the responsibility of farmers. Feeding around the farms may change the condition and reproductive output of some species (Fernandez-Jover *et al*., 2007a) and thus change the local fish community structures. However, the alternative, to allow open access fishing in the close vicinity of fish farming sites, seems less desirable.

***Impose common regulations to control the spread of diseases***

It is currently impossible to impose uniform regulatory measures on all fish farms around the Mediterranean. In the case of farms in EU countries already possessing a history of specific diseases, current regulations and directives are in effect. In other countries, the sanitary supervision of farms, the communication between authorities and farms, health certification and the information-transfer between growers (usually due to competition) is often less than perfect. It would be preferable if the guideline standards developed by the EU Commission were adopted and implemented by the full range of countries surrounding the Mediterranean, although this is currently difficult to envisage.

***Improve consumer information on mariculture products***

We should take action to change public perception of aquaculture products so that consumers can make informed choices. We should inform the public on the value of eating low trophic level aquaculture products to encourage more environmentally sustainable production. In this respect, the creation of a 'green' label for low trophic level products and responsibly reared species, such as mussels or tilapias, may be an appropriate measure. There is a database on nutritional values for fish that could be used to increase awareness and encourage the use of different species. Finally, the establishment of regulations so that environmentally sustainable species are preferred over species reared in fish farms without any guaranty of good farming practice is recommended.

***Use large scale indicators of fish farming impact***

We still have very little understanding of the environmental impacts of mariculture operations at the ecosystem level. In addition robust operational protocols and management procedures to support policy making are lacking on a European level.

Environmental impact studies and monitoring strategies are largely based on water quality parameters but little attention has been offered to descriptors of key ecological processes (e.g. primary production rates, phytoplankton responses to nutrients or shifts in zooplankton and grazing patterns) (Buford *et al*., 2003).

We need to develop an ecosystem-based approach to aquaculture by identifying the indicators and thresholds for environmental sustainability, using a suite of indicators representative of ecosystem structure and function (Dale and Beyler, 2001).

As stronger environmental regulations are implemented, larger companies with global reach may be tempted to move to areas with lesser regulations, thereby shifting the environmental impact elsewhere, hence the need to strengthen dialogue and communications between EU and non-EU Mediterranean countries in this regard.

***Investigate overall mariculture impact in relation to other stressors***

Mariculture must be part of Integrated Coastal Zone Management, taking into account both environmental and aesthetic considerations. There are multiple "users" of the coastal zone, but aquaculture is one of the activities impacting the environment the most, adding its impacts to other stressors such as maritime traffic, sewage pollution, etc. There is a gap of knowledge concerning those cumulative impacts.

Until proper regulations are developed, aquaculture will remain focused on production for economic benefit alone, and will fall short of its goals of increasing fish supplies.

The scientific community should promote the growth of sustainable aquaculture. Further research related to 'foot-printing' the overall effect of mariculture will decisively help in this effort.

# II - Workshop Communications

# Ecological assessment of aquaculture impact in the Mediterranean Sea

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## Abstract

The exponential growth of off-shore mariculture that has occurred worldwide over the last 10 years has raised concern about the impact of the waste produced by this industry on the ecological integrity of the sea bottom. Investigations into this potential source of impact on the biochemistry of the sea floor have provided contrasting results, and no compelling explanations for these discrepancies have been provided to date. To quantify the impact of fish-farm activities on the biochemistry of sediments, we have investigated the quantity and biochemical composition of sediment organic matter in four different regions of the temperate-warm Mediterranean Sea: Akrotiri Bay (Cyprus), Sounion Bay (Greece), Pachino Bay (Italy) and Gulf of Alicante (Spain). In these four study areas, the concentrations of biopolymeric C in the sediments were measured, comparing locations receiving wastes from fish farms to control locations in two different habitats: seagrass beds (*Posidonia oceanica*) and soft, non-vegetated bottoms. The biopolymeric C concentrations in the sediment suggest idiosyncratic effects of fish-farm waste on the biochemistry of sediments. These are possibly related to differences in the local physico-chemical variables that could explain a significant proportion of the differences observed between the control and fish-farm locations. Results indicate that quantitative and qualitative changes in the organic loads of the sediments that arise from intensive aquaculture are dependent upon the ecological context and are not predictable only on the basis of fish-farm attributes and hydrodynamic regimes. Therefore, the siting of fish farms should only be allowed after a case-by-case assessment of the ecological context of the region, especially in terms of the organic matter load and its biochemical composition.

## Introduction

Overfishing, aquaculture industry, maritime transportation, tourism and recreational activities in coastal areas have reached a global scale, determining significant changes in marine ecosystems worldwide (Schiermeier, 2002). These, in turn, are expected to reduce the value of the natural capital and the benefits that humans get from the exploitation of coastal resources (Cloern, 2001).

Coastal eutrophication is recognized as one of the most important emerging problems and, during the past four decades, has exponentially increased in intensity, geographic extension and

environmental consequences. Eutrophication is typically related to the increase of nutrient and organic matter loads, which cause a progressive decrease of dissolved oxygen concentrations (Cloern, 2001). Therefore, changes in the trophic state of a coastal system are expected to induce significant changes in the benthic compartment (Karakassis *et al*., 2000).

Over the last decade, mariculture has experienced an almost exponential development worldwide. For instance, in the most oligotrophic regions of the Mediterranean Sea, mariculture alone is responsible for up to 7% and 10% of the nitrogen (N) and phosphorous (P) loads, respectively (Pitta *et al*., 1999). As with other farming activities, the environmental effects of this emerging industry have prompted widespread criticism and have initiated a global effort to develop more sustainable farming techniques (Troell *et al*., 2003).

Mediterranean coastlines are subject to increasing eutrophication (Danovaro, 2003), which is causing serious concern (De la Mare, 2005). Changes induced by fish farming in the Mediterranean region generate serious conflicts between aquaculture practices and the conservation of marine habitats, including the protection of benthic primary producers, such the seagrass *Posidonia oceanica*, which plays a key role as a refuge for the juvenile stages of many organisms and contributes to preserve the biodiversity and physical integrity of the Mediterranean coastal ecosystems (Hemminga and Duarte, 2000).

The potential adverse effects of aquaculture discharges are widely reported, but poorly documented in rigorous scientific studies (Burford *et al*., 2003). Moreover, most of the descriptors and procedures used to assess environmental impact from aquaculture have rarely followed proper scientific standards, and only local views of the responses of the ecosystem to disturbances are available (Mirto *et al*., 2002; Karakassis *et al*., 2000).

Intensive fish farming results in the release of large amounts of dissolved and particulate nutrients to the surrounding environment (Holmer and Kristensen, 1992; Pitta *et al*., 1999). Change induced by fish farming can be more relevant in oligotrophic than in eutrophic areas, inducing significant changes in several ecosystem compartments.

Several studies have demonstrated that the most evident consequences of fish farming on the benthic environment are an increase in total organic carbon accumulation in the sediment and a decrease in oxygen availability for the benthos (Holmer and Kristensen, 1992; Karakassis *et al*., 1998). These changes, in turn, have a significant impact on the abundance and diversity of micro, meio- and macrobenthic organisms (La Rosa *et al*., 2004; Mirto *et al*., 2002; Karakassis *et al*., 2000).

More recent studies have demonstrated that fish farming effluents have effects also on the biochemical composition of the organic matter in the sediment. Fish-farm sediments are sometimes enriched in lipid content due to the accumulation of uneaten fish-food pellets on the seafloor (Mirto *et al*., 2002), and are characterised by increased microbenthic algal biomass in response to the increased availability of nutrients below the cages (La Rosa *et al*., 2001). However, the results of these studies are not always consistent across different ecological contexts (Mirto *et al*., 2002) and have been generally obtained from investigations conducted on small spatial scales (hundreds to thousands of meters; Danovaro *et al*., 2003).

Also the seagrass *P. oceanica* is severely impacted by intensive aquaculture activities, as the light levels are typically reduced around the fish cages and the enhanced organic inputs to the sea bottom deteriorate the sediment properties essential for seagrass growth (Holmer *et al*., 2003).

## Problem approach

To evaluate changes driven by aquaculture activities in the Mediterranean Sea, it is of paramount importance to assess whether the effects of organic enrichment caused by fish farms result in changes in the sediment biochemistry and in the associated benthic fauna (i.e. meiofauna) along different environmental gradients affected by this source of disturbance.

Eutrophication is generally assessed through chemical measurements (e.g. inorganic N and P) and/or assessments of algal biomass in the water column (Stefanou *et al*., 1999). These proxies, however, may fail to detect the consequences of increased nutrient loads on benthic systems

(Cloern, 2001). On the other hand, previous studies have demonstrated that changes in the concentrations and relative importance of the different classes of organic compounds reflect relevant changes in the trophic state of the sediment (Dell'Anno *et al*., 2003; Pusceddu *et al*., 2007a). For instance, systems poor in organic C concentrations (namely, oligotrophic) are generally characterized by a larger carbohydrate fraction, whereas systems with higher organic C concentrations are characterized by a protein dominance, so that increasing protein concentrations in the sediment are typically associated with meso- to eutrophic conditions (Pusceddu *et al*., 2007a).

We report here the results of a large-scale investigation covering the entire Mediterranean basin, with a consistent sampling strategy. The comparison of four different regions was designed to reveal eventual generalities and/or idiosyncratic responses.

To achieve this objective, we investigated the quantity and biochemical composition of sediment organic matter and meiofaunal assemblages in four regions of the temperate-warm Mediterranean Sea: Akrotiri Bay in Cyprus; Sounion Bay in Greece; Pachino Bay in Italy and the Gulf of Alicante in Spain (Figure 1). In these four regions, locations with organic matter deposited from fish farms were compared with putatively pristine locations as controls. The potential impact of organic enrichment induced by fish farming was quantified in two different habitats that have often been selected for the siting of aquaculture industries: a) vegetated substrates, characterised by the presence of meadows of the seagrass *P. oceanica*; and b) non-vegetated soft sediments, characterized by different structural and functional properties.

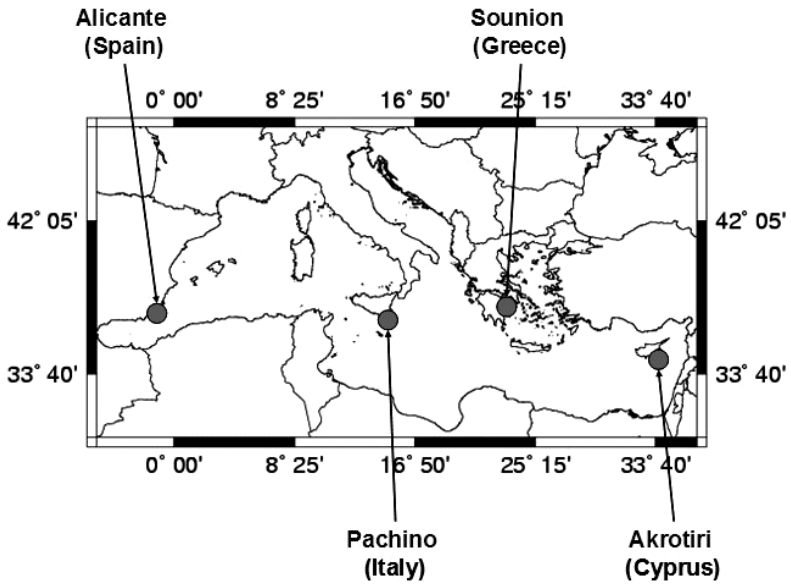


Fig. 1. Localization of the four study regions in the Mediterranean Sea.

Sampling was carried out along an East-to-West longitudinal transect (ca. 3,500 km wide). The sampling areas, located at similar latitudes and depths (between 16 m and 39 m) were selected on the basis of the presence of fish farms, previously characterized in terms of their main environmental features (e.g. start of activities, distance from the shore, species reared, annual production and food input; Table 1).

In each habitat, the impact was quantified by comparing the fish-farm locations with control locations. The control locations were situated upstream of the main currents, and at least 1,000 m from the fish farms. They were characterized by relatively pristine conditions and by environmental features comparable to those found beneath the cages. Replicates were selected randomly from the central area of each fish-farm location (i.e. beneath the cages) and in each control location.

Table 1. Characteristics of the fish farms in the four regions in the Mediterranean Sea.

| Area | | Bottom temperature | Bottom current | Sediment type | In activity since | Distance from the shore | Reared species | Annual production | Food input |
|---|---|---|---|---|---|---|---|---|---|
| | | °C | cm sec$^{-1}$ | | | m | | tonnes | tonnes y$^{-1}$ |
| Cyprus | Akrotiri Bay | 17-18 | 20-40 | carbonate mud | 1988 | 1050 | Sea bream *(Sparus aurata)*<br>Sea bass *(Dicentrarchus labrax)* | 300 | 660 |
| Italy | Pachino Bay | 17-18 | 20 | carbonate sand | 1992 | 1000 | Sea bream *(Sparus aurata)*<br>Sea bass *(Dicentrarchus labrax)*<br>Sharpsnout sea bream *(Diplodus puntazzo)* | 1150 | 2749 |
| Greece | Sounion | 17-18 | 6.3 | carbonate sand | 1996 | 500 | Sea bream *(Sparus aurata)*<br>Sea bass *(Dicentrarchus labrax)* | 400 | 640 |
| Spain | Alicante | 17-18 | 4.7 | carbonate fine sand | 1996 | 2600 | Sea bream *(Sparus aurata)*<br>Sea bass *(Dicentrarchus labrax)* | 260 | 520 |

## BIOCHEMICAL SIGNATURES OF FISH-FARMING IMPACT

Recent studies have indicated that the concentration of biopolymeric C in marine sediments is a good proxy for the benthic trophic state, with values typically increasing from oligo- to meso- and hypertrophic conditions (Pusceddu *et al*., 2007a).

We demonstrated that the effects of fish farming on the quantity and biochemical composition of sediment organic matter varied across habitats and regions, indicating that the response of the benthic trophic state to intensive aquaculture is idiosyncratic, and that actual predictions on the potential impact of this industry on the organic loads in the sediments are difficult to make (Pusceddu *et al*., 2007b). However, it appears that a significant increase in the organic load can be detected only in those control regions characterised by biopolymeric C contents typically <2.0 mg C g$^{-1}$, such as in Greece (both habitats), in non-vegetated sediments in Italy, and in seagrass sediments in Cyprus (Figure 2). Conversely, when biopolymeric C concentrations exceed 2 mg C g$^{-1}$ (as in the case of non-vegetated sediments in Cyprus, and both habitats in Spain), no clear differences in organic C loads emerge between control and fish-farm locations.

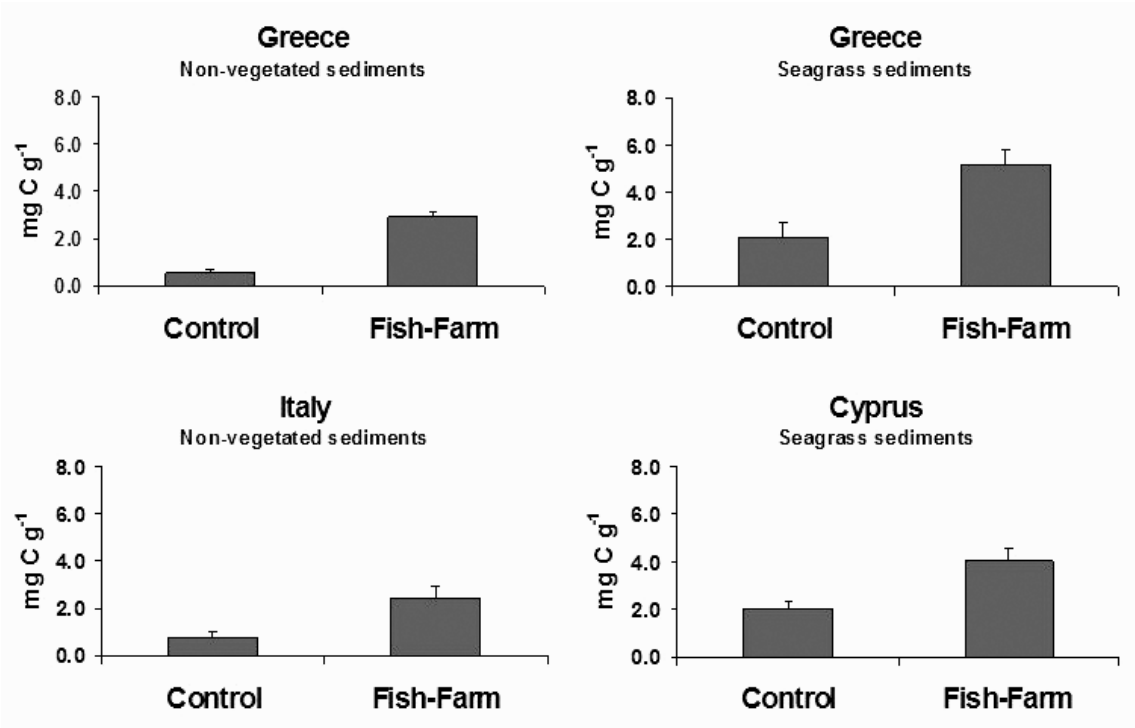


Fig. 2. Biopolymeric C levels in control and fish-farm locations (for systems characterised by control biopolymeric C levels <2.5 mg C g$^{-1}$).

Sediments characterized by different levels of organic enrichment can have clear differences in the biochemical composition of their sediment organic matter. Generally, systems poor in biopolymeric C are characterized by a larger carbohydrate fraction, whereas systems with higher biopolymeric C concentrations are characterized by the dominance of proteins (Pusceddu *et al*., 2007b). This can be explained by the nutrient limitation of oligotrophic systems, where organic N- and P-rich compounds are rapidly degraded and recycled (Danovaro *et al*., 1999). In contrast, systems receiving huge inputs of biopolymeric C, such as sediments affected by fish-farm wastes, tend to accumulate N-rich compounds. This trend is generally seen in terms of increasing values of the protein to carbohydrate ratio in organically enriched sediments (such as harbour sediments, coastal lagoons or eutrophicated coastal regions; Pusceddu *et al*., 2007a).

Accordingly, in the present study, the waste released from fish farms altered the biochemical composition of sediment organic matter, but, again, the effects were different across regions and habitats. For instance, sediments of fish farms in regions featured by background biopolymeric C concentrations >2.5 mg $Cg^{-1}$ (e.g. seagrass sediments in Italy, and non-vegetated sediments in Spain and Cyprus) had significantly higher protein to carbohydrate ratios than their relative control sediments (Figure 3). The increase in the relative importance of proteinaceous material in fish-farm sediments is likely to be related to the composition of the food pellets provided to the fish being reared. Indeed, for all four fish farms, although provided by different manufacturers, the food pellets were typically composed of 48% to 52% protein, which when not consumed by the fish, accumulates in the surface sediments beneath the cages. On the other hand, with fish-farm sediments in both habitats in Greece and in seagrass sediments in Cyprus, which were characterised by lower biopolymeric C concentrations in control locations, the protein to carbohydrate ratios did not differ between control and fish-farm locations. This indicates that the response of the sediment biochemistry to the fish-farm impact appears idiosyncratic, and that no predictions as to these effects can be made here on the basis of the separate determination of organic loads and their biochemical composition.

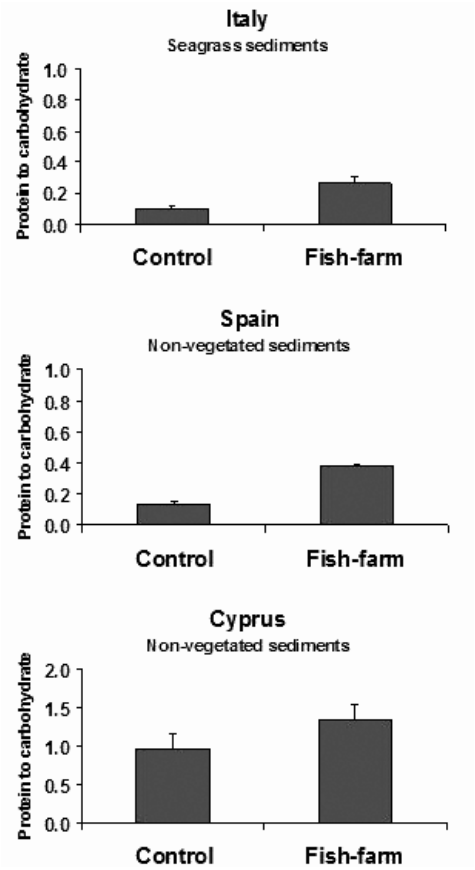


Fig. 3. Protein to carbohydrate ratios in control and fish-farm locations (for systems characterised by control biopolymeric C levels >2.5 mg C $g^{-1}$).

## MEIOFAUNA RESPONSE TO FISH-FARM BIODEPOSITION

Meiofauna, for their ecological importance and the lack of larval dispersion, is becoming a popular tool for investigating structural and functional changes of natural and anthropogenically-impacted ecosystems.

Using hierarchical sampling strategy, we tested the null hypothesis that changes induced by fish-farm on the quantity and biochemical composition of farm sediments do not affect the abundance, assemblage structure, taxon richness and patterns of distribution of meiofauna at different spatial scales.

Meiofaunal abundance in non-vegetated and vegetated sediments at all investigated fish farms is reported in Figure 4 (a and b). Meiofaunal assemblages in all investigated fish farms displayed a common and consistent response to fish-farm biodeposition. Meiofaunal abundance increased in impacted sediments beneath the cages in both non-vegetated and vegetated sediments. These results are generally in contrast with previous studies that reported a decrease of meiofaunal abundance in systems subjected to high organic load (Mirto *et al*., 2002) but are in good agreement with results reported in studies on mussel farm biodeposition (Danovaro *et al*., 2004). Our results suggest that meiofauna assemblages under the cages, in both non-vegetated and vegetated sediments, tend to respond positively to the biodeposition.

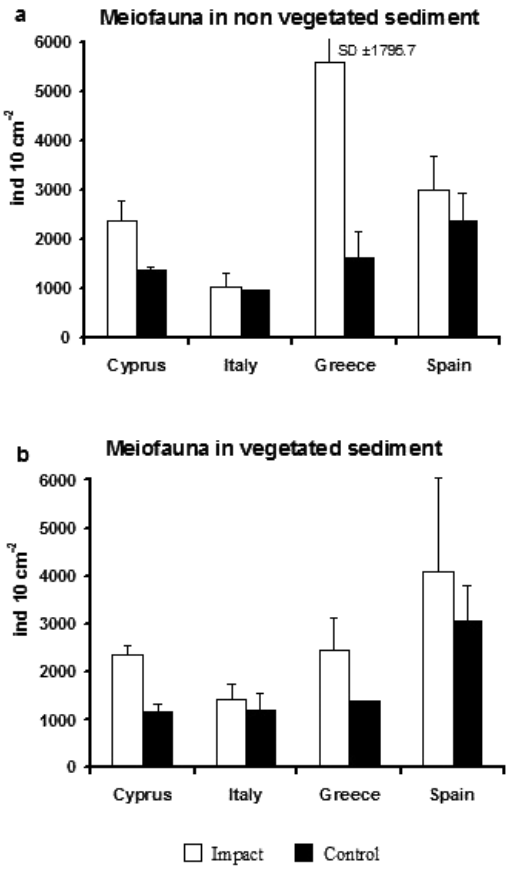


Fig. 4. Total meiofaunal abundance in Cyprus, Italy, Greece and Spain in unvegetated (a) and vegetated (b) locations.

Such effect can be related to the fact that the organic enrichment in the sediments beneath the cages was limited in all investigated sites. In fact, almost all the sedimentary variables beneath the cages were only slightly higher than in control locations. It is also worth noting that, for four locations across the Mediterranean basin, the impact on the meiofaunal assemblages is consistent and independent from the overall background trophic state of the system.

Biodeposition influenced also the meiofaunal community structure. Taxa showed a different sensitivity to the presence of fish-farms (Figure 5). Nematodes, as previously reported (Mirto *et al*., 2000), confirmed their ability to benefit from the biodeposition, generally increasing in abundance and dominance in the impacted locations. Conversely, copepods displayed a negative

response to the biodeposition, generally decreasing beneath the cages together with amphipods and kinorhynchs.

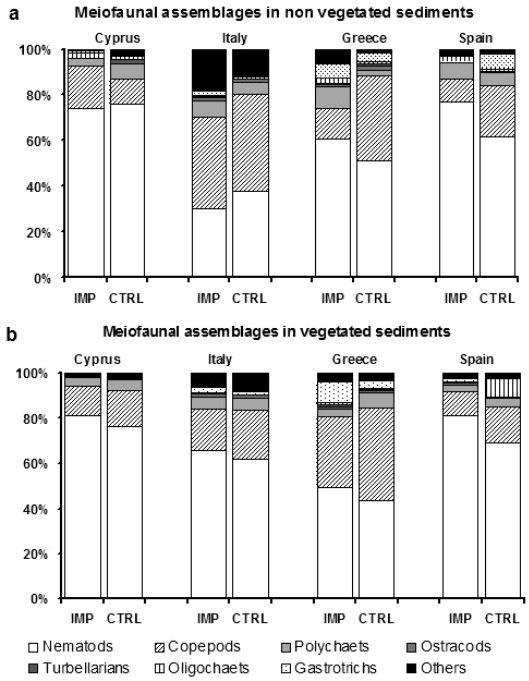


Fig. 5. Meiofaunal assemblages structure in Cyprus, Greece, Italy and Spain in unvegetated (a) and vegetated (b) locations.

At all investigated farms the meiofaunal taxon richness significantly decreased beneath the cages, due to the disappearance of the more sensitive taxa (Figure 6). Previous studies reported that kynorhynchs are among the most sensitive taxa to organic enrichment (Mirto *et al*., 2000), but this was supported only in one of the four investigated farms (in Italy). The taxa that disappeared beneath the cages were different in the three other farms. This suggests that meiofaunal response to biodeposition in terms of taxonomic composition is site-specific, and might depend on the local environmental conditions and the composition of the meiofaunal assemblage.

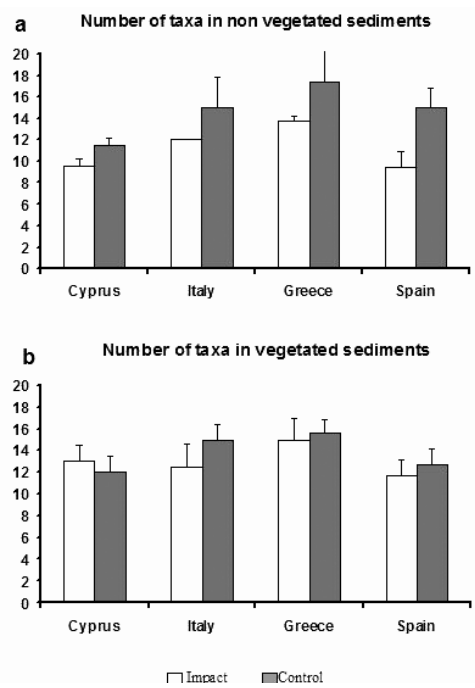


Fig. 6. Total meiofaunal taxa in Cyprus, Greece, Italy and Spain in unvegetated (a) and vegetated (b) locations.

## CONCLUSIONS

The results of our investigations highlight that changes in the quantity and biochemical composition of sediment organic matter caused by intensive aquaculture are critical for assessing the presence and levels of impact induced by fish-farm activities. Our results suggested that the impact of aquaculture on the benthic compartment is site-specific, thus confirming the crucial role of local ecological settings on the possible response to the presence of a fish farm. Therefore, in the perspective of a more environmentally sustainable allocation of marine landscapes to new fish-farming industries, we stress the need to extend the *a priori* environmental impact assessment procedures also to the sedimentary organic load, its biochemical composition and the main physico-chemical characteristics of the region of interest. Since the background ecological features on a local scale appeared to have a major role in determining the effects of fish-farm-induced eutrophication, the future siting of fish farms should be preceded by well designed *a priori* monitoring programs that are able to describe the whole ecological setting and should be tailored to the basis of the local ecological context.

Moreover, the analysis of the structural and functional properties of the meiofaunal community suggests that even classes or phyla of meiofauna can disappear in areas subject to fish farm biodeposition. The presence of a relevant biodeposition changed the relative importance of the main taxa (specifically nematodes and copepods), thus presumably having profound implications on the benthic ecosystem functioning, the functional role of species production and their energy transfer to higher trophic levels.

Therefore, we recommend a precautionary approach for the siting and management of intensive aquaculture plant (fish farming), as their potential impact on the environment and benthic biota might become crucial for the sustainability of this industry.

**Acknowledgements**

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# Impact of fish farming on marine ecosystems Croatian experiences

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## Abstract

Mariculture in Croatia is an important economic sector and has expanded significantly during the last decade. To minimize negative ecological impacts of mariculture on the marine environment, certain legislative regulations have been enforced including obligatory periodical monitoring of physico-chemical parameters and of benthic communities at farming sites. Some of the monitoring results presented in this paper have led to further legislative improvements introducing minimum site depth and distance to coast as important parameters for location permission.

## Introduction

Mariculture production in Croatia, like in other Mediterranean countries, has expanded significantly during the last years. Production growth was especially significant in seabream, seabass (SB&SB) and bluefin tuna (BFT) farming, which increased from < 1500 t/year in 1997 to > 7,000 t/year in 2006. This "boom" enhanced the interest of the public and scientific community in Croatia to this economical sector, especially to its ecological impacts.

Degradation of the seabed beneath and around the fish cages due to inputs of uneaten fish food and excretory metabolic products is the most widely documented effect of fish farming (Gowen *et al*., 1991; Wu, 1995; Fernandes *et al*., 2001). The effects have been demonstrated through different parameters in sediment in numerous publications as: negative sediment redox potential (Hargrave *et al*., 1993; Pawar *et al*., 2001), organic carbon accumulation, accumulation of phosphorus (Matijević *et al*., 2006) and nitrogen compounds (Hall *et al*., 1990; Hargrave *et al*., 1997; Holby *et al*., 1997; Porello *et al*., 2005) and consequently changed or reduced benthic communities (Mazzola *et al*., 1999, Kovač *et al*., 2001, 2004; Karakassis *et al*., 1999, 2000, 2002; La Rosa *et al*., 2001; Mirto *et al*., 2002; Boyra *et al*., 2004b). The effects of mariculture on different groups of vertebrates in marine ecosystem have been considered to a lesser extent (Machias *et al*., 2004, 2005; Vita *et al*., 2004a), as well as socio-economic issues related to fishery and mariculture economic sectors.

## Observed effects

### Nutrients

Fish farms generate a wide range of dissolved and particulate matter. Considering the inability of direct quantification of nutrient discharge from BFT and SB&SB farms, we used the models TunaMod and AquaKult developed by one of us (M. Tudor) for calculation of nitrogen and

phosphorus discharge in dissolved and particulate forms. The models take in account initial fish size, total fish mass, fish growth rate and annual temperature cycle, but nutrient inputs from uneaten food are not considered.

Nutrient outputs for annual production of 3,000 tons of SB&SB and 4,000 tons of BFT are given in Table 1.

Table 1. Discharge of nitrogen and phosphorus (tons/year) from fish farms to the Croatian part of Adriatic.

| Parameter | SB&SB | BFT | *Total* |
|---|---|---|---|
| Dissolved nitrogen | 364 | 975 | 1339 |
| Particulate nitrogen | 28.65 | 27 | 55.65 |
| Total nitrogen | 392.65 | 1002 | 1394.65 |
| Dissolved phosphorus | 35.7 | 20 | 55.7 |
| Particulate phosphorus | 15 | 1.1 | 16.1 |
| Total phosphorus | 50.7 | 21.1 | 71.8 |

On the basis of calculated nutrient quantities from other sources like municipal and industrial sewage, river and groundwater discharge (Kušpilić, 2005), the annual discharge of nitrogen and phosphorus from fish farms contribute with 9% (N) and 3% (P) to the total discharge of these elements on the Croatian coast of the Adriatic (Figure 1). These percentages are in good agreement with Pitta *et al*. (1999) who reported nutrient loads from mariculture up to 7% and 10% for nitrogen and phosphorus, respectively.

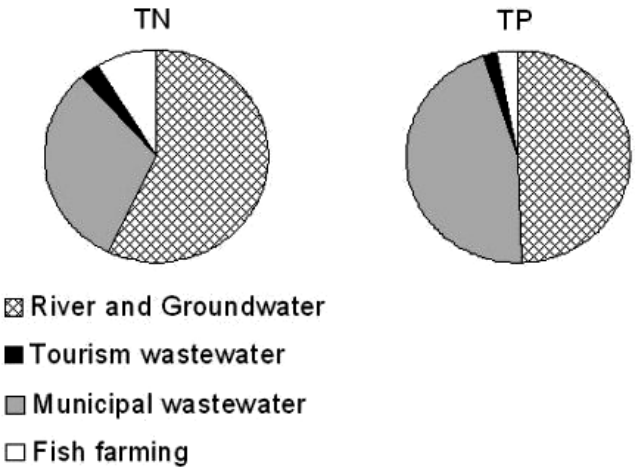


Fig. 1. Portion of annual nitrogen and phosphorus discharge through river and groundwater, municipal wastewater, tourist wastewater and fish farms.

Results of our fish farm monitoring programmes have shown that concentrations of dissolved nitrogen and phosphorus in the water column at most farming sites were enhanced, on occasions, leading to higher chl *a* concentrations and primary production between June and November (Figure 2).

Measurements of physico-chemical parameters in sediment beneath fish farms (organic carbon, total nitrogen content, phosphorus content, redox potential), showed disturbances of some parameters in relation to the natural state of the environment, especially redox-potential and phosphorus content. Negative redox potential, established at most farms, indicate transition of natural, aerobic state of sediment to anaerobic, with consequences for the organic matter degradation (Westrich and Berner, 1984), adsorption properties of sediment, phosphorus buffering capacity (Sundby *et al*., 1992) and benthic fluxes. Phosphorus in sediment was found to be a very sensitive parameter of fish farming impact, while results for total nitrogen should be used with caution due to partial loss of nitrogen in the denitrification and anammox processes (Risgaard-Petersen *et al*., 2003).

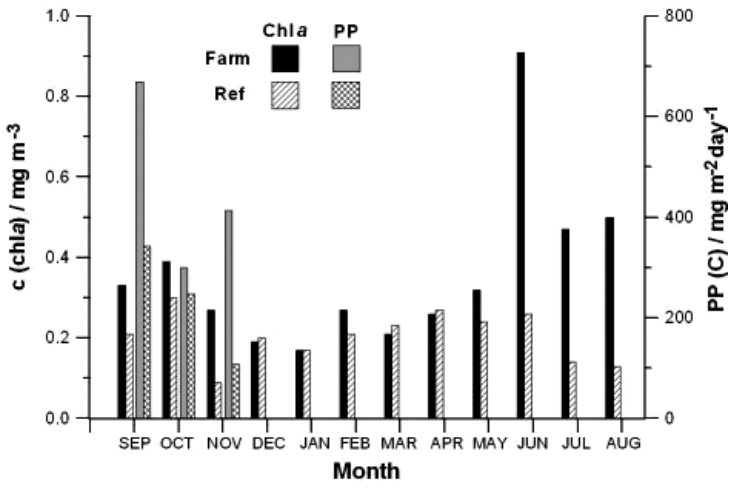


Fig. 2. Biomass (Chl *a*) and primary production (PP) at seabream and seabass fish farm and reference station for the period September 2005- August 2006.

Phosphorus in sediment as an indicator of environmental fish farming influence have been used in studies across the world (Hall *et al*., 1990; Hargrave *et al*., 1997; Holby *et al*., 1991; Karakassis *et al*., 1999; Cancemi *et al*., 2003; Soto and Norambuena, 2004; Porello *et al*., 2005; Kassila *et al*., 2000; Kalantzi and Karakassis, 2006). Soto and Norambuena (2004) selected and proposed phosphorus as the most useful parameter indicative of the impact of fish farming because it shows the lowest natural variability and stable concentrations at control sites and can be easily related to P content in fish food and products of its degradation, as well as with production in the water column.

Our phosphorus analysis of fish farm sediment was based on the combination of different sequential extraction (SEDEX) techniques (Ruttenberg, 1992; Anshutz *et al*., 1998; Schenau and De Lange, 2000). Proposed SEDEX method enables distinction between biogenic and authigenic apatite phosphorus in sediment introducing the FDP "fish debris" fraction – originated from hard parts of fish material (fish bones and tooth) and very small amounts of P loosely adsorbed onto mineral surfaces and carbonates (Schenau and De Lange, 2001) (Figure 3).

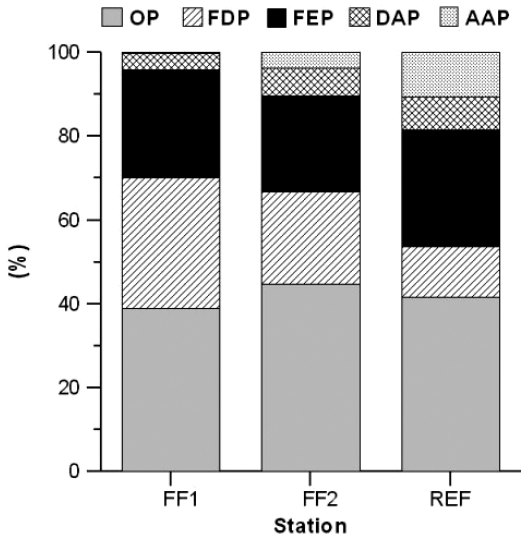


Fig. 3. Portion of sediment phosphorus species in total sediment phosphorus (organic phosphorus-OP, fish debris phosphorus-FDP, phosphorus adsorbed on iron oxyhidroxides-FEP, detrital apatite phosphorus-DAP, authigenic apatite phosphorus-AAP) at two tuna farms (FF) and reference station (REF).

## Plankton communities

The effects of fish farming on phytoplankton communities in the Mediterranean have been investigated by Pitta *et al*. (1999), Diaz *et al*. (2001), Karakassis *et al*. (2001), La Rosa *et al*. (2002), who pointed out no significant differences in species composition between farming and control sites. Our preliminary results regarding phytoplankton composition from SB&SB farm (Figure 4) show likewise no major differences in community structure between the farm site and the natural environment: both sites were dominated by nanoflagellates (> 80%) and diatoms. Preliminary results for the whole research period indicate that phytoplankton community structure was determined by seasonal changes rather than effects of fish farm.

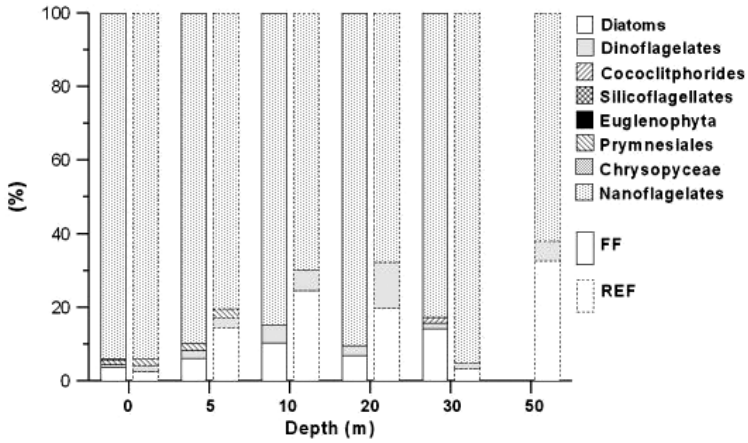


Fig. 4. Phytoplankton composition in the water column on seabream and seabass fish farm (FF) and reference station (REF) for September 2005.

## Benthic communities

Seabream, seabass and bluefin tuna farming change many ecological factors that impact on the composition and distribution of benthic communities in the vicinity of rearing cages. Increased sedimentation of fine particles may change texture of the sea bottom (eventual mudding) as well as composition of existing natural communities. Fine particles, which are settled on the sediment and on existing organisms, may have a different impact such as reduction or elimination of existing benthic communities. Finally, increased sedimentation of organic matter creates conditions of hypoxia/anoxia (development of bacteria *Beggiatoa*) that destroys existing communities. One observes higher abundance of herbivorous species (fish, snails, sea-urchins) near farming cages (Figure 5), which could reduce benthic algae and seagrass. The number of sea-urchins *Paracentrotus lividus* and *Arbacia lixula* near seabass and seabream cages could enhance and reach depths unusual for these organisms (25 m) on the Croatian coast of the Adriatic (Grubelić *et al*., 2000). Furthermore, the increase of grazing damages or even destroys the seagrass of *Posidonia oceanica* (Figure 6). This will limit the shelter and breeding area of many sessile and vagile organisms. The disappearance of *Posidonia oceanica* drastically changes ecological, biological and environmental biodiversity at the fish farm area (Karakassis, this volume). For example on cages with accompanying floating objects one finds the nitrophilic algae *Enteromorpha*, *Ulva* and *Cladophora* that tolerate high nutrient concentrations (Katavić and Antolić, 1999). The main elements of existing stratified algal layers (genus *Cystoseira*) on the rocky bottom under and near farm cages could disappear while low unstratified algae cover remains.

## Effects of aquaculture on wild fish communities and on humans

There is no evidence that mariculture practices have an adverse effect on wild fish assemblages. Mariculture facilities generate a "fish aggregating device" (FAD) effect by offering additional food source for wild fish in the vicinity of the cages (Sánchez Jerez, this volume). Studying changes in wild fish assemblages after establishment of a fish farming zone in an oligotrophic marine ecosystem, Machias *et al*. (2004) concluded that the release of nutrients from fish farming facilities can have a positive effect on local fish populations resulting in higher fishery production, with no

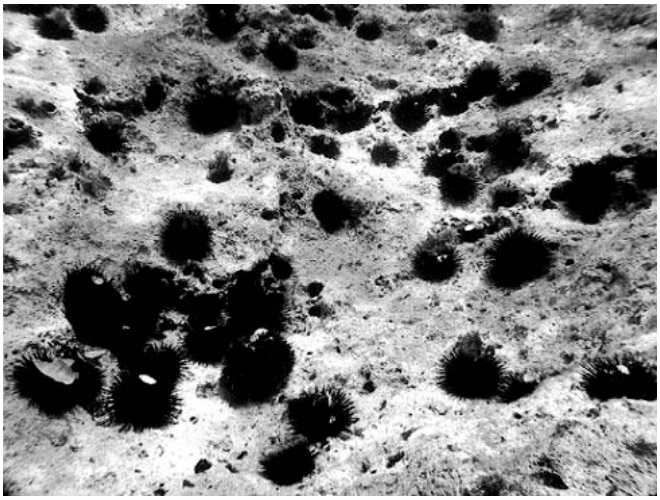

Fig. 5. Colonization of sea urchins at fish farm site.

Fig. 6. Completely destroyed *Posidonia oceanica* bed in the vicinity of a fish farming cage.

visible negative changes in species composition or biodiversity. Furthermore, it was found that the wild fish aggregated around the cages reduce adverse effects on the benthos, acting as removers of feed wastes (Vita *et al*., 2004b). The model developed by Sánchez Jerez *et al*. (this volume) also showed a great reduction of the impact of the feed waste on the benthos around fish farms.

No fishing is permitted within concession zones with rearing cages installations. According to Dempster *et al*. (2002), wild fish that gather within these zones tend to be large adults in good conditions, due to food supply from the cages, which eventually enable good spawning success of these fish. The same authors suggested that fish farms might act as small marine protected areas.

In the case of BFT aquaculture a number of fishing vessels have switched their fishing activities from bottom trawling to aquaculture related activities (Katavić *et al*., 2003), consequently decreasing fishing pressure on heavily exploited demersal fish stocks. According to Katavić and Tičina (2005), about 30 bottom trawlers in Croatia found interest to be fully integrated into tuna farming operations, thus reducing fishing mortality of already heavily exploited demersal fish stocks. Therefore, it seems that BFT aquaculture may have indirect positive effects on local fish communities, but more research efforts are needed to properly address this issue.

In the case of BFT, it is actually capture-based aquaculture, and demand for the live tuna to be stocked in the cages has become an important driving force in the BFT fishery. Furthermore, BFT

aquaculture in the Mediterranean Sea relies on small pelagic fish (from the Mediterranean and from the Atlantic) that are used as feed for tunas in the cages. There is an increasing need for sustainable and efficient management of fishing activities related to live tuna collection, as well as of fisheries targeted to the fish populations used as a feed.

From an economic perspective, BFT aquaculture can be considered as an additional market, not for live BFT, but also for small pelagic fish used as a feed. In the case of Croatia, where economic re-structuring processes forced the closing of most fish processing plants (i.e. the main market for small pelagic fish), this "new" market for small pelagic fish saved the livelihood of many fishermen families in Croatia. In addition, BFT aquaculture activities created a lot of new jobs, thus having a positive effect on employment in local fishing communities. Also, due to its FAD effect (Sánchez Jerez, this volume), fishing grounds around concession zones with fish cages are among the most favorable for artisanal small-scale fishing and sport fishing.

However, beside these positive socio-economic effects for consumers and fishing communities, BFT aquaculture may cause conflicts with other ecosystem users, particularly if facilities are not suitably located. The right choice of location made in accordance with integrated coastal zone management (ICZM) plan is necessary to prevent such conflicts in the future.

**Effects of BFT aquaculture on threatened species**

***a) Sharks***

The Mediterranean Sea, hosts a number of large pelagic shark species. Many of them are listed in the Red Book of Threatened Species (IUCN, 2006) such as thresher shark, great white shark, basking shark, blue shark, hammerhead sharks, porbeagle, etc. These species are (as by-catch) usually the most affected by fishing gears, such as large pelagic driftnets and pelagic longlines, that target various tuna species and swordfish, but very rarely appear as by-catch in purse-seine fishery. It is very likely that BFT aquaculture practices, acting as a new market for live tuna, will discourage the use of pelagic longline fishery and driftnet fishery in favor of purse seine fishery, thus having an indirect positive effect on the protection of threatened large pelagic shark species.

***b) Turtles***

There are three turtle species in the Mediterranean Sea (the loggerhead turtle *Caretta caretta*; the green turtle *Chelonia mydas*; the leathery turtle *Dermochelys coriacea*) that are considered as threatened species (IUCN, 2006). In the same manner as large pelagic shark species, they are affected by fishing gears, such as pelagic driftnets and pelagic longlines, target tuna. According to Lee and Poland (1998) Mediterranean fisheries have a serious impact on the local turtle stock, and more than 60,000 turtles are caught annually as a result of fishing practices, with mortality rates ranging from 10% to 50% of individuals caught. Turtles caught as by-catch by pelagic driftnets and longlines are usually exposed to prolonged immersions that usually cause anoxic brain damage and then death (Lee and Poland, 1998). On the other hand, rare occasional catches of turtles by the purse seining fishing practice do not seem to have a serious adverse impact on turtles, since by-catches of turtles are discarded alive. Therefore, the changes in fishing practices (i.e. switch from pelagic longline and driftnet fishery to purse seine fishery) driven by BFT aquaculture demand for live tuna, probably have an indirect positive effect on conservation of threatened turtle species and therefore on conservation of ecosystem biodiversity.

## CONCLUSIONS

Monitoring seabream, seabass and bluefin tuna farms in Croatia indicates a moderate impact on parameters in water column. Impact on benthic communities and physico-chemical properties of sediment were more pronounced, but still limited to a relatively small area around farming sites. Offering an alternative use of bottom trawling fishing vessels, BFT aquaculture likely causes a decrease in fishing pressure on already heavily exploited demersal fish stocks. In this case, changes in fishing practices (i.e. switch from pelagic longline and driftnet fishery to purse seine fishery) driven by demand for live tuna, probably have an indirect positive effect on the conservation of threatened turtle and shark species. Mariculture activities create new jobs and therefore represent an important socio-economic factor for the population on the islands and along the coast of Croatia.

# Effects of aquaculture on Mediterranean marine ecosystems: findings of recent EU-funded projects and ongoing research activities

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## Abstract

A number of projects have recently addressed complementary aspects of the aquaculture-environment interactions, ranging from local effects such as benthic enrichment in the vicinity of fish farms, to mesoscale and large scale effects such as the impact on sea grass meadows and wild fish. Similarities and differences among regions, types of mariculture and affected ecosystem processes should be taken into account in the context of environmental impact assessment. Ecosystem level approach should consider impacts of and on aquaculture, which is now an integral part of many marine ecosystems and local economies.

## Introduction

Expansion of aquaculture during the last two decades has given rise to concerns on environmental integrity of coastal areas, attracting more and more negative publicity, which is often fueled by conflicts with other users of the coastal zone. Aquaculture, and particularly fish farming, releases a variety of wastes into the marine environment including nutrients such as nitrogen and phosphorus, organic material, and a number of associated by-products such as pharmaceuticals and pesticides, which can have undesirable impacts on the environment (Fernandes *et al*., 2001). Furthermore, aquaculture interferes directly and indirectly with different biogeochemical processes in the marine environment (Karakassis, 1998), it takes place mainly in the coastal zone where biodiversity is high and human pressures are increasing and it involves impacts at varying spatial and temporal scales (Silvert, 1992). An extensive list of potential impacts of aquaculture on various biotic communities (Table 1) has been compiled by Milewski (2001), including effects related to the physical presence of floating structures and nets, to management issues such as the use of anti-predator practices, to the use of chemicals for various purposes as well as to normally discharged particulate and dissolved wastes.

Some of the impacts shown in Table 1 are extensively studied and well documented but the majority is not. The spatial scales affected depend on the topographic and hydrographic characteristics of the site, the environmental behaviour and dispersion ability of the associated pollutants and the mobility of the affected communities. Recovery of marine communities in general and particularly in relation to aquaculture effects is poorly studied. Most of the impacts are expected to be negative although the severity of the impact can vary between sites and individual farms.

Table 1. Effects of aquaculture on marine biotic communities (modified after Milewski, 2001).

| Source of pressure | Potential effect on biota | Level of scientific documentation | Communities affected | Relevant/expected spatial scale | type of impact | Estimated recovery of the community |
|---|---|---|---|---|---|---|
| physical structure | Direct mortality through entanglement | poor | Vertebrates | local | - | medium |
| | Behavioral changes in coastal pelagic fish | medium | Vertebrates (Fish) | local | ? | unidentified |
| | Behavioral changes in coastal birds and marine mammals (e.g., avoidance) | poor | Vertebrates | local-meso | - | unidentified |
| predator control systems | Direct mortality | poor | Vertebrates | local-meso | - | unidentified |
| | Behavioral changes of wild fauna | medium | Vertebrates | local-meso | - | unidentified |
| fish escapement | Disease transmission to other species | poor | various (probably fish) | meso-large | - | unidentified |
| | Genetic interactions with wild fish | High | Vertebrates (Fish) | meso-large | - | slow |
| | Displacement of wild fish from natural habitat (e.g., through competition, predation) | poor | Vertebrates (Fish) | meso-large | - | unidentified |
| release of uneaten food | Suffocation and displacement of benthic organisms | High | Macrofauna | local | - | slow |
| | Loss of foraging, spawning and/or nursery habitat for wild species | High | various | local | - | slow |
| | Loss of biodiversity | High | Macrofauna | local | - | slow |
| | Fragmentation of benthic habitat | poor | various | local-meso | - | slow |
| release of nutrients | Change in water quality | poor | various | local-meso | - /+ | rapid |
| | Mortality of plankton (including fish and invertebrate egg and larvae) | poor | various | local | - | rapid |
| | Increased primary productivity | poor | various | local-meso | - /+ | rapid |
| | Shift in plankton community composition | poor | Phytoplankton | local-meso | ? | rapid |
| | Increase in harmful algal blooms | poor | various | local-meso | - | rapid |
| | Decline of seagrass meadows | poor-medium | marine plants & various indirectly | local-meso | - | slow |
| antibiotics | Tainting of wild species | poor | various | local | - | rapid |
| | Changes in benthic bacterial community | poor | microbes | local | - | unidentified |
| | Resistant microbial strains | poor | various indirectly | unknown | - | unidentified |
| pesticides | Direct mortality and sublethal effects | poor | invertebrates | local | - | unidentified |
| | Tainting of wild species | poor | various | local | - | unidentified |
| disinfectants and antifoulants | Direct mortality and sublethal effects | poor | invertebrates | local | - | unidentified |
| | Tainting of wild species | poor | invertebrates | local-meso | - | unidentified |
| | Changes in physiology | poor | invertebrates | local-meso | - | unidentified |

Some of these impacts are related to inefficient management and can be avoided or considerably reduced by adopting alternative management options, improved technologies or other mitigation measures (e.g. use of more efficient containment methods to avoid escaping of fish, use of vaccines to replace antibiotics, minimisation of food wastage, etc.). On the other hand, there are impacts such as the release of nutrients and faeces which are difficult to avoid since they are part of the physiology of the farmed species.

A series of projects have addressed complementary aspects of the aquaculture-environment interactions issue through surveys carried out partly or entirely in the Mediterranean, i.e.:

– AQCESS: Aquaculture and Coastal, Economic and Social Sustainability.

– BIOFAQs: Biofiltration and Aquaculture: an evaluation of hard substrate deployment performance within Mariculture developments.

– MERAMED: Development of monitoring guidelines and modelling tools for environmental effects from Mediterranean aquaculture.

– MedVeg: Effects of nutrient release from Mediterranean fish farms on benthic vegetation in coastal ecosystem.

– ECASA: Ecosystem Approach for Sustainable Aquaculture.

– SAMI: Synthesis of Aquaculture and Marine Ecosystems Interactions

## Effects on nutrients and plankton communities

Fish farming releases considerable amount of nutrients in the water column. A series of studies have shown that only a small part (less than 20%) of the N and P supplied to the farmed fish is recovered through harvesting whereas large fractions are lost as dissolved nutrients in the water column. These nutrients could be expected to cause considerable deterioration in the water quality. Karakassis *et al*. (2005) have shown that there is little risk of hypernutrification for large spatial scales in the Mediterranean and concluded that changes in water quality are likely to occur at short spatial scales. The effect of fish farming at small spatial scales, i.e. in the immediate vicinity of fish cages of individual farms has been addressed by various authors (Pitta *et al*., 1999, 2006; La Rosa *et al*., 2002; Belias *et al*., 2003; Soto and Norambuena, 2004). In most cases, very little change in nutrient concentrations has been found around fish farms and even less so in the case of

eutrophication-related variables such as chlorophyll *a* or POC in the water column. Recent studies on mesoscale effects of aquaculture (Pitta *et al*., 2005) have shown that most of the significant changes in nutrients, chlorophyll *a*, or PON were found in the deepest layer of the water column below the thermocline, indicating that it is related to the remineralization of benthic organic material. Dalsgaard and Krause-Jensen (2006) used dialysis bags and Ulva bioassays and found increased primary production in the vicinity of fish farms in the Mediterranean. We have repeated the experiment with dialysis bags, using filtered and unfiltered seawater and found that grazing played an important role in the regulation of phytoplankton communities.

### Organic enrichment of the seabed

The most widely known effect of fish farming is on the benthic enrichment beneath the fish farms. Several authors have reported the presence of a loose and flocculent black sediment under fish cages commonly named "fish farm sediment" (Holmer, 1992). This sediment is characterized by low values of redox potential, high content of organic material and accumulation of nitrogenous and phosphorous compounds (Hall *et al*., 1992). The severity of the impact on the seabed depends largely on the sediment type. In coarse sediments, impacts are in general low whereas at muddy or silty sites the effects are more pronounced. The results of a study in the Mediterranean (Karakassis *et al*., 2000) showed that the organic carbon and nitrogen content of the sediment near the cages increased by 1.5-5 times and ATP content by 4-28 times.

Similar effects were found in studies addressing the impacts of salmon cage farming in Scotland (Brown *et al*., 1987) and the East coast of Canada (Hargrave *et al*., 1993) as well as in a sandy bottom farm in Puget Bay in N.E. Pacific (Weston, 1990). Levels of increase in sediment concentration of organic material by a factor of 2 were reported for silty seabed by Brown *et al*. (1987) and Hargrave *et al*. (1993) as well as by Holmer and Kristensen (1992) in non-specified sediment types. Considerably higher levels of increase (by a factor of four) were reported for a salmon farm located over a sandy seabed by Weston (1990).

Sediment anoxia, patches of Beggiatoa and absence of macrofauna have been reported in relation to salmon farming in the North Atlantic (Rosenthal and Rangeley, 1988; Hansen *et al*., 1991) and the Baltic Sea (Holmer and Kristensen, 1992). Despite the microtidal regime of the Mediterranean, results from the study in Greek coastal waters (Karakassis *et al*., 2000) showed that even in the sampling stations located right under the cages there was no extensive "azoic" zone as defined by Pearson and Rosenberg (1978).

### Effects on biodiversity

Although species diversity beneath the cages is generally reduced, it is not certain that biodiversity is threatened by fish farming. Following the clear distinction (Margalef, 1997) between biodiversity (i.e. the total number of available species or genotypes in an area) and eco-diversity which can be inferred by sampling local biotic communities, the local changes in community structure, affecting a small patch of the seabed or a few cubic meters of seawater cannot be considered as a decline in biodiversity. By contrast, risks for biodiversity arise when a specific type of habitat (usually rare or supporting an endangered species) or a key-habitat (supporting life of the wider area) are severely degraded or when populations of species (with large size and low reproduction rates) are reduced to unsustainable sizes. At present most of the scientifically documented effects are those on macrofaunal invertebrates at a zone beneath and close to the farm cages. These organisms are ecologically important but it is very unlikely that they will become extinct or that their population at larger spatial scales will be significantly affected.

In this context the potential problems affecting biodiversity in relation to aquaculture are the mortality of large fauna, the effects on sea-grass meadows, the introduction of alien species, the changes in the trophic status of large water bodies and the loss of genetic information from populations of farmed species.

### Effects on wild fish communities and fisheries

During the last years there has been considerable progress on the study of mariculture-wild fish interactions. Besides the studies on farmed and wild salmon interactions, there was a focus on the

assemblages aggregating beneath the farm cages (Dempster *et al*., 2002, 2005; Fernandez-Jover *et al*., 2007b). In the Eastern Mediterranean, studies focused on larger spatial scales have shown that fish farming has a "positive" effect on fisheries with no significant effects on biodiversity metrics (Machias *et al*., 2004, 2005, 2006; Giannoulaki *et al*., 2005). This effect was attributed to the rapid transfer of nutrients up the food web which is compatible with the findings of the above mentioned surveys on water column variables.

### Effects on sea grass meadows

Results from previous studies (Delgado *et al*., 1999) as well as those from the MedVeg project (Holmer *et al*., 2007) showed that there was a significant degradation of the *Posidonia oceanica* meadows in the vicinity of fish farms. Yet this degradation was not readily detectable through standard macrofaunal analysis implying that the usual monitoring strategies are not suitable for all types of ecosystem effects . The conclusion of the MedVeg project was that site selection for fish farms should ensure that they are not placed closer than 800m from important *Posidonia* meadows.

### Effects of the environment on aquaculture

Although aquaculture is a source of risks for environmental degradation it is also among the human activities of the coastal zone most sensitive to environmental degradation. Unlike other uses of the coastal zone (such as sewage discharge, mineral extraction, transport, etc.) which tend to be unaffected by the environmental changes they induce, aquaculture depends on high water quality. In the framework of the ECASA project we are analysing the interactions among different coastal uses in order to identify criteria for mutual exclusion of activities and to provide indicators for site-selection in the coastal zone.

# Oyster transfers as a vector for marine species introductions: a realistic approach based on the macrophytes

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## Abstract

Transfer of livestock is a common practice in shellfish aquaculture. As part of the EU Program ALIENS 'Algal Introductions to European Shores' and the Programme National sur l'Environnement Côtier (PNEC) "Lagunes Méditerranéennes", an assessment of the efficiency of oyster transfers as vector of unintentional species introduction was carried out, focused on the marine macrophytes. This investigation included a field study of the exotic flora of two major French aquaculture sites: the Thau Lagoon (Mediterranean) (58 exotic species identified) and the Arcachon Basin (NE Atlantic) (21 exotic species identified), a bibliographical analysis of the exotic marine flora of 34 Mediterranean coastal lagoons (68 exotic species listed) and finally an experimental study of the vector efficiency by simulation of oyster transfers. The results confirmed the high degree of efficiency of the importation, transfer and farming of non-indigenous and native commercial shellfish especially oysters, as a vector of primary introduction and secondary dispersal of marine macrophytes. The importation of non-indigenous oysters, in particular the Japanese oyster *Crassostrea gigas,* involved massive quantities between 1964 and about 1980, and the regular transfers between aquaculture sites have been responsible for the introduction and the dispersal of several dozens of exotic macrophytes. When compared to the other major vectors of introduction (hull fouling, ballast waters, Suez Canal), the shellfish trade is by far the main vector of introduction of exotic macrophytes into the Mediterranean and the NE Atlantic. These results are discussed and recommendations for action are proposed.

## Introduction

Unintentional introductions of non-indigenous species are a growing concern in environmental management, especially for marine ecosystems. Each introduction involves at least one vector of transfer. Major vectors include shipping (fouling on hulls, ballast water), trans-oceanic canals and aquaculture activities. As far as living resources are concerned, a great number – in terms of species and individuals – of living organisms are deliberately transported around the world for direct consumption, aquaculture purposes or "freshening" in the marine environment (Carlton, 2001; Wolff and Reise, 2002). The movement of live marine organisms by mechanisms other than shipping has increased dramatically in recent decades, and the trend will likely continue (Ribera and Boudouresque, 1995; Weigle *et al*., 2005). Should an important aquaculture activity suffer a decline following a serious disease or parasite outbreak, a separate exotic strain or species will be imported in large number to rapidly replace this decline in production. Such direct transplants of

stock almost inevitably lead to the presence of escapees in the wild (Volpe *et al*., 1999) or the introduction of unwanted species (Minchin and Gollasch, 2002). Likewise certain environmental crisis can induce collapse of the shellfish livestock, which consequently need to be renewed by massive imports. For example, the Thau Lagoon is regularly subject to a severe summer anoxic crisis, called *"malaigue"*, that can destroy large quantities of shellfish. For example, in 2006, the losses reached 3,455 metric tons of oysters and 4,000 metric tons of mussels on a livestock of 20,000 – 25,000 and 4,000 – 6,000 metric tons, respectively. In addition, because of the ease of transplanting livestock using modern transport, unauthorised movements may regularly occur. Occasionally such illegal shellfish movements are intercepted with a great number of pests associated (Minchin and Rosenthal, 2002). Consequently, aquaculture has become a leading vector of aquatic invasive species worldwide and international and inter-regional transfers of livestock for aquaculture pose high ecological risks given the absence of strong policies in most countries (Wasson *et al*., 2001).

Among the marine organisms involved in aquaculture transfers, shellfish (especially oysters) have long been *a posteriori* associated with the introduction of marine organisms (Druehl, 1973; Gruet, 1976; Grizel and Héral, 1991; Zibrowius, 1994; Ribera and Boudouresque, 1995; Barber, 1997; Verlaque, 2001; Goulletquer *et al*., 2002; Minchin and Gollasch, 2002; Ribera-Siguan, 2002; Wolff and Reise, 2002; Weigle *et al*., 2005). Transport and transplantation of commercially important exotic oysters have resulted in numerous unintentional introductions of pathogens, parasites and pest species either carried in the packing materials, attached to shells or as parasites and disease agents in the living oyster tissues (Carlton, 1992; Sindermann, 1992; Minchin, 1996; Galil and Zenetos, 2002; Minchin and Eno, 2002; Minchin and Gollasch, 2002).

A majority of exotic marine species were discovered in, or close to, shellfish aquaculture areas (Cabioch and Magne, 1987; Rismondo *et al*., 1993; Curiel *et al*., 1995, 1999a and b; Cabioch *et al*., 1997; Farnham, 1997; Stegenga, 1997; Maggs and Stegenga, 1999; Reise *et al*., 1999; De Montaudoin and Sauriau, 2000; Wolff, 2005). Along the French Atlantic coast, the main area of species introduction (88 % of the primary introductions, 84 % of the secondary introductions) extends from Normandy to the Basin of Arcachon, i.e. in the areas with extensive oyster farming. There 28 % of the introduced species are presumed to have been brought in association with oyster shipments, and mainly *Crassostrea gigas* in the 1970s, (Goulletquer *et al*., 2002). In the USA, many species of polychaetes were probably imported with the oyster seed stocks (Blake, 1999). According to Ruesink *et al*. (2005), 46 % of the introduced marine species in northern Europe and 20 % in Australia likely entered with oyster aquaculture. In the USA, the percentage varies by region: 10 % on the Gulf Coast, 20 % on the East Coast and 49 % on the West Coast: the regions where a wider variety of oyster species have been cultured tend to have a greater number and percentage of "hitchhiking" non-native species.

According to Elton (1958): 'The greatest agency of all that spreads marine animals to new quarters of the world must be the business of oyster culture'. Into the North Sea area, the introductions due to the oyster imports would be slightly more important than those due to the transport on ship hulls, and clearly more important than the introductions through ballast waters (Reise *et al*., 2002). For others (Grizel and Héral, 1991; Grizel, 1994; Goulletquer *et al*., 2002; Wolff, 2005), shellfish transfers arrive in second position right after shipping activities.

As far as macrophytes are concerned, shellfish transfer is considered to be the most important vector of introduction (Eno *et al*., 1997; Maggs and Stegenga, 1999; Reise *et al*., 1999; Verlaque, 2001; Ribera Siguan, 2002, 2003). According to Wallentinus (2002), the transfers of oysters and other molluscs may be responsible for 44 % of the introductions of macrophytes, both intercontinentally and within Europe, with the northwest Pacific as the major donor area. However, a direct assessment of the efficiency of oyster transfers as vector of species introductions is lacking. As part of a Fifth Framework Program of the EU (ALIENS: 'Algal Introductions to European Shores') and the Programme National sur l'Environnement Côtier (PNEC) "Lagunes Méditerranéennes", this vector was analysed with a focus on marine macrophytes. The programmes encompassed: (i) a field study of the exotic flora of two major French aquaculture sites: the Thau Lagoon (Mediterranean) and the Arcachon Basin (NE Atlantic); (ii) a

bibliographical analysis of the exotic marine flora of 35 Mediterranean coastal lagoons; and (iii) an experimental study of the vector efficiency by simulation of oyster transfers.

## Shellfish aquaculture practices

Shellfish aquaculture (mussels, oysters and clams) constantly involves transport of livestock. Transfers of oysters date back to the Roman period (Héral, 1990). The modern European oyster industry depended for decades on the native oyster *Ostrea edulis* Linnaeus and a strain of *Crassostrea gigas* (Thunberg), called the "Portuguese oyster", which was probably introduced from Taiwan in the 16th century. In 1970, oyster farming in Europe faced a collapse due to disease, and required massive imports of *C. gigas* from the Pacific. To sustain future production, both adult oysters and spat were imported from British Columbia and Japan respectively. Around 10,000 metric tons (i.e. more than 5 billion small oysters) were imported between 1971 and 1977. Nowadays, such imports have been considerably reduced as a consequence of the self-sustaining spat production of *C. gigas* in Europe (see the review by Wolff and Reise, 2002) (Table 1). The European Union now restricts imports of oysters to those from a few countries around the Mediterranean (Croatia, Morocco, Tunisia, Turkey) and from the USA, Canada and New Zealand (EU 2003, 2004).

Table 1. European oysters aquaculture: History (based on Héral, 1990; Grizel and Héral, 1991; Wolff and Reise, 2002).

→ **19th century and early 20th century**:

- First attempts to restore natural beds and beginning of oyster farming.
- Accidental introduction of *Crassostrea angulata* into France.
- Regular imports of *Crassostrea virginica* (Britain).
- First trial of intentional introduction of *Crassostrea gigas* into Europe.

→ **1960s and 1970s**:

- Epidemics on *Crassostrea angulata.*
- Massive imports of *Crassostrea gigas* from Japan and British Columbia ("Résur" operation).

→ **During the 1970s**:

- French production relied mainly on Japanese spat.
- From 1971 to 1977, 10,000 metric tons of spat were imported (i.e. more than 5 billion small oysters) from Japan by air.
- Shipments were inspected and immersed in freshwater in order to avoid the introduction of exotic organisms.

→ **From 1977 to present:**

- *Crassostrea gigas* is the main oyster cultured in Europe.
- Imports from Japan have officially stopped.
- Transfers occur inside Europe and abroad.
- In the Mediterranean Sea, production is wholly dependent on the importation of spat or adults. The only *C. gigas* officially authorized in the French lagoons are that produced in the Atlantic.

In the Mediterranean, the European *C. gigas* production is wholly dependant on the importation of spat or adults. In France, only the spat produced in the NE Atlantic is authorized in the Mediterranean.

In France, oyster-farming areas are allocated to different activities, for example:
- spat production (Arcachon, Marennes-Oléron);
- growth (Brittany, Normandy, Thau Lagoon);
- "greening" (Marennes-Oléron).

Continuous transfers of livestock between areas occur to ensure optimal growth conditions for each part of the rearing cycle (Goulletquer and Le Moine, 2002; Girard *et al.*, 2005). Furthermore, additional transfers can occur between areas dedicated to the same activity, or other European areas.

## The Thau Lagoon (Mediterranean Sea)

With 2500 farming tables, more than 3.5 million ropes, a standing stock reaching 25,000 metric tons and an annual production up to 12,000 – 13,000 metric tons of *C. gigas,* respectively, the Thau Lagoon is by far the leading site of oyster farming in the Mediterranean sea (Verlaque, 2001).

Massive importations of *Crassostrea gigas* occurred from 1971 to 1977 ("Résur" operation; Grizel and Héral, 1991). Since 1977, the only spat officially authorized to enter Thau Lagoon is that produced in the Atlantic. As a result of the failure in decontamination processes and/or quarantine of these imports, an increasing number of species introductions have occurred in Thau Lagoon. When compared to the previous checklist (Verlaque, 2001), the exotic flora of the Thau Lagoon saw the recent addition of 13 taxa, giving a total of 58 exotic macrophytes (i.e. 25 % of the total flora) (Verlaque, 2005, amended) (Table 2).

Table 2. Exotic taxa recorded in Thau (*) and in other Mediterranean coastal lagoons. All the suspected introductions have been considered. For each taxon, the class, possible origin and vector of primary introduction and secondary dispersal are mentioned. Phyla: **R** = Rhodophyta; **O** = Ochrophyta; **C** = Chlorophyta. Origin: **A** = Atlantic; **C** = cosmopolite; **I** = Indian Ocean; **P** = Pacific; **T** = Tropical seas. Vectors: **C** = Antic and Suez Canals; **FB** = fishing baits; **Shell** = shellfish transfer; **Ship** = shipping (hull fouling, ballast waters). An exotic macrophyte can have several possible origins and vectors (from Verlaque, 2005, amended).

| Species | Phyla | Origin | Vector |
|---|---|---|---|
| *Acanthophora nayadiformis* (Delile) Papenfuss | R | I | C – Ship |
| * *Agardhiella subulata* (C. Agardh) Kraft & M.J. Wynne | R | A – P (?) | Ship - Shell |
| * *Ahnfeltiopsis flabelliformis* (Harvey) Masuda | R | P | Shell |
| * *Antithamnion nipponicum* Yamada *et* Inagaki | R | P | Shell |
| *Antithamnionella elegans* (Berthold) J.H. Price *et* D.M. John | R | P | Ship - Shell |
| * *Antithamnionella spirographidis* (Schiffner) E.M. Wollaston | R | P | Ship - Shell |
| * *Asparagopsis armata* Harvey, as "*Falkenbergia*" life history phase | R | P | Ship - Shell |
| * *Ceramium* sp. | R | P | Shell |
| * *Chondria coerulescens* (J. Agardh) Falkenberg | R | A | Shell |
| * *Chondrus giganteus* Yendo f. *flabellatus* Mikami | R | P | Shell |
| * *Chrysymenia wrightii* (Harvey) Yamada | R | P | Shell |
| * *Dasya sessilis* Yamada | R | P | Shell |
| * *Grateloupia asiatica* Kawaguchi *et* Wang | R | P | Shell |
| * *Grateloupia lanceolata* (Okamura) Kawaguchi | R | P | Shell |
| * *Grateloupia minima* P.L. Crouan & H.M. Crouan | R | A | Shell |
| * *Grateloupia subpectinata* Holmes | R | P | Shell |
| * *Grateloupia patens* (Okamura) Kawaguchi & Wang | R | P | Shell |
| * *Grateloupia turuturu* Yamada | R | P | Shell |
| * *Griffithsia corallinoides* (Linnaeus) Batters | R | A - P | Shell |
| * *Herposiphonia parca* Setchell | R | P | Shell |
| * *Heterosiphonia japonica* Yendo | R | P | Shell |
| *Hypnea cornuta* (Kützing) J. Agardh | R | I | C – Ship - Shell |
| *Hypnea spinella* (C. Agardh) Kützing | R | T | Ship - Shell |
| * *Hypnea valentiae* (Turner) Montagne | R | P | Shell |
| * *Laurencia okamurae* Yamada | R | P | Shell |
| * *Lithophyllum yessoense* Foslie | R | P | Shell |
| * *Lomentaria flaccida* Tanaka | R | P | Shell |
| * *Lomentaria hakodatensis* Yendo | R | P | Shell |
| * *Nemalion vermiculare* Suringar | R | P | Shell |
| * *Neosiphonia harveyi* (Bailey) M.-S. Kim, H.-G. Choi, Guiry & G.W. Saunders | R | P | Ship - Shell |
| * *Nitophyllum stellato-corticatum* Okamura | R | P | Shell |
| * *Polysiphonia atlantica* Kapraun *et* J. Norris | R | A - P | Ship - Shell |
| * *Polysiphonia fucoides* (Hudson) Greville | R | A | FB - Shell |
| * *Polysiphonia morrowii* Harvey | R | P | Shell |
| * *Polysiphonia paniculata* Montagne | R | P | Ship |
| * *Polysiphonia stricta* (Dillwyn) Greville | R | A | Ship - Shell |
| * *Porphyra yezoensis* Ueda | R | P | Shell |
| * *Pterosiphonia tanakae* Uwai *et* Masuda | R | P | Shell |
| * *Rhodophysema georgii* Batters | R | A - P | Shell |
| * *Rhodothamniella codicola* (Børgesen) Bidoux *et* F. Magne | R | P | Ship - Shell |
| *Solieria filiformis* (Kützing) Gabrielson | R | A | Ship - Shell |
| *Bonnemaisonia hamifera* Hariot | R | P | Ship - Shell |
| * *Acrothrix gracilis* Kylin | O | A - P | Shell |
| *Botrytella parva* (Takamatsu) Kim ? | O | P | Shell |
| * *Chorda filum* (Linnaeus) Stackhouse | O | A - P | Shell |
| * *Cladosiphon zosterae* (J. Agardh) Kylin | O | A | Shell |
| * *Colpomenia peregrina* (Sauvageau) Hamel | O | P | Ship - Shell |
| * *Desmarestia viridis* O.F. Müller | O | A - P | Shell |
| **Dictyota okamurae* (E.Y. Dawson) I. Hörnig, R. Schnetter *et* W.F. Prud'homme van Reine | O | P | Shell |
| *Ectocarpus silicosus* var. *hiemalis* (Crouan *frat. ex* Kjellman) Kjellman | O | A | Ship - Shell |
| *Fucus spiralis* L. | O | A | FB |
| * *Halothrix lumbricalis* (Kützing) Reinke | O | A - P | Shell |
| * *Laminaria japonica* Areschoug | O | P | Shell |
| * *Leathesia difformis* (Linnaeus) Areschoug | O | C | Shell |
| * *Microspongium tenuissimum* (Hauck) A.F. Peters | O | A | Shell |
| * *Pilayella littoralis* (Linnaeus) Kjellman | O | A - P | Shell |
| * *Punctaria tenuissima* (C. Agardh) Greville | O | A | Shell |
| * *Sargassum muticum* (Yendo) Fensholt | O | P | Shell |
| * *Scytosiphon dotyi* Wynne | O | P | Ship - Shell |
| * *Sphaerotrichia firma* (E. Gepp) Zinova | O | P | Shell |
| * *Undaria pinnatifida* (Harvey) Suringar | O | P | Shell |
| *Caulerpa racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman *et* Boudouresque | C | I | ? - Ship |
| * *Cladophora hutchinsioides* Hoek *et* Womersley | C | P | Shell |
| * *Codium fragile* (Suringar) Hariot | C | P | Ship - Shell |
| * *Derbesia rhizophora* Yamada | C | P | Shell |
| * *Monostroma obscurum* (Kützing) J. Agardh | C | P | Ship - Shell |
| * *Ulva fasciata* Delile | C | P | Ship - Shell |
| * *Ulva pertusa* Kjellman | C | P | Shell |

The majority of these taxa may originate from the Pacific region (89 % of the total), having been introduced either directly with Japanese oyster imports or by shellfish transfers (oysters, mussels and clams) from other aquaculture areas (attached to shells or on the packing materials). An introduction or co-introduction by shipping, *via* the harbour of Sète, is considered possible for only few species. Although no extensive study was carried on the fauna, several exotic invertebrates have also been identified in the Thau Lagoon (Zibrowius, 1991, 1994, and pers. comm.).

Approximately thirty years after the accidental introduction of a first contingent of Pacific macrophytes along with massive importations of *Crassostrea gigas* from Japan, new Asiatic species are still discovered. This provides evidence that importations of oysters (spat or adults) from the NW Pacific have occurred in Europe after 1977. In 1994, illicit imports of Korean oysters have effectively spread in Europe (Verlaque, 1996). The high number of oyster farms and the difficulty in controlling the origin of the oysters did probably increase the risk of this type of importation.

The Thau Lagoon is one of the world's hot spots of marine macrophyte introduction, as it comes far before other major introduction sites, such as New Zealand (21 introduced marine macrophytes), Australia (20) and San Francisco Bay (6) (Ribera and Boudouresque, 1995; Carlton, 1996). This result is a cause for worry as the Thau Lagoon is also an important exportation site of living bivalve molluscs (*C. gigas, Ostrea edulis, Mytilus galloprovincialis, Tapes* spp.) towards other French regions and abroad and, in light of the legislation currently in force, the introduced algae present in the Thau Lagoon have a high-probability of being spread throughout Europe and other Mediterranean countries.

## The Arcachon Basin (N.E. Atlantic)

The Arcachon Basin is another important oyster-farming area of France. Since the massive *C. gigas* imports from Japan and British Columbia in 1970s, oyster transfers with the other European and extra-European shellfish basins regularly occur (Auby, 1993), for example:

Arcachon (spat) → Thau (or) Ireland (or) Brittany (ou) Normandie → Arcachon.

Arcachon (spat) → The Ebro Delta (Mediterranean, Spain) → Thau.

During the PNEC Program, 21 exotic macrophytes have been identified (Verlaque *et al*., 2006, amended). The main possible vector of introduction and the main donor region are the shellfish transfers (oysters, mussels and clams) and the Pacific, respectively (Table 3). Among the 16 taxa that also occur in the Thau Lagoon, two Rhodophyta, *Pterosiphonia tanakae* and *Herposiphonia parca* are known only from these two localities in Europe, and three other species, *Dasya sessilis, Heterosiphonia japonica* Yendo and *Ulva pertusa,* have been identified close to other European oyster-farming areas in Brittany and in Holland (Stegenga, 1997; Maggs and Stegenga, 1999; Stegenga and Mol, 2002; Pe_a and Bárbara, 2006; Christine Maggs and Frédéric Mineur, unpublished data).

As for the Thau Lagoon, excepting the oldest introductions for which shipping cannot be excluded, the shellfish transfer (oysters, mussels, clams and the packing materials) and the Pacific appear as the most probable vector and origin of introduction respectively (Table 3).

## The Mediterranean coastal lagoons

Stressed environments are easily colonized by alien species. Coastal lagoons exhibit at once natural stress (variable salinity), lower diversity, abrupt changes in dominant species and high human-induced disturbances through organic enrichment, pollution, physical habitat alterations, ship traffic and extensive aquaculture (Occhipinti-Ambrogi and Savini, 2003). Consequently, a bibliographical analysis of the flora of 34 Mediterranean coastal lagoons has been carried out to inventory the exotic species. For each lagoon, we considered both the presence of shellfish farming and the number of introduced species.

Table 3. Exotic taxa of the Arcachon Basin. All the suspected introductions have been considered. For each taxon, the class, possible origin and vector of primary introduction and secondary dispersal are mentioned. Phyla: **R** = Rhodophyta; **O** = Ochrophyta; **C** = Chlorophyta. Origin: **A** = Atlantic; **I** = Indian Ocean; **M** = Mediterranean; **P** = Pacific. Vectors: **Shell** = shellfish transfers; **Ship** = shipping (hull fouling, ballast waters). An exotic macrophyte can have several possible origins and vectors (from Verlaque *et al.,* 2006, amended).

| Species | Phyla | Origin | Vector |
|---|---|---|---|
| *Anotrichium furcellatum* (J. Agardh) Baldock | R | P | Ship - Shell |
| *Antithamnionella spirographidis* (Schiffner) E.M. Wollaston | R | P | Ship - Shell |
| *Antithamnionella ternifolia* (J.D. Hooker & Harvey) Lyle | R | P | Ship - Shell |
| *Caulacanthus okamurae* Yamada | R | P | Ship - Shell |
| *Centroceras clavulatum* (C. Agardh) | R | A - M - IP | Ship - Shell |
| *Dasya sessilis* Yamada | R | P | Shell |
| *Gracilaria vermiculophylla* (Ohmi) Papenfuss | R | P | Shell |
| *Grateloupia subpectinata* Holmes | R | P | Shell |
| *Herposiphonia parca* Setchell | R | P | Shell |
| *Heterosiphonia japonica* Yendo | R | P | Shell |
| *Hypnea valentiae* (Turner) Montagne | R | P | Shell |
| *Lomentaria hakodatensis* | R | P | Shell |
| *Neosiphonia harveyi* (Bailey) M.-S. Kim, H.-G. Choi *et al.* | R | P | Ship - Shell |
| *Pterosiphonia tanakae* Uwai *et* Masuda | R | P | Shell |
| *Rhodothamniella codicola* (Børgesen) Bidoux & F. Magne | R | P | Ship - Shell |
| *Colpomenia peregrina* (Sauvageau) Hamel | O | P | Ship - Shell |
| *Sargassum muticum* (Yendo) Fensholt | O | P | Shell |
| *Codium fragile* (Suringar) Hariot | C | P | Ship - Shell |
| *Kornmannia leptoderma* (Kjellman) Bliding | C | P | Shell |
| *Monostroma obscurum* (Kützing) J. Agardh | C | P | Ship - Shell |
| *Ulva pertusa* Kjellman | C | P | Shell |

Table 4. Shellfish farming activities (Yes/No) and number of exotic macrophytes in 34 Mediterranean coastal lagoons. In bold: lagoons with introduced macrophytes (from Verlaque, 2005, amended).

| COUNTRY | COASTAL LAGOON | SHELLFISH FARMING | NUMBER OF EXOTIC MACROPHYTES |
|---|---|---|---|
| **CROATIA** | Veliko and Malo Jezero | N | 0 |
| **FRANCE** | Arnel | N | 0 |
| | **Bages Sigean** | **N** | **1** |
| | **Berre** | **N** | **2** |
| | Biguglia | N | 0 |
| | **Diane** | **Y** | **2** |
| | Ingril | N | 0 |
| | Mauguio | N | 0 |
| | Palo | N | 0 |
| | Pérols | N | 0 |
| | **Prévost** | **Y** | **1** |
| | **Salses-Leucate** | **Y** | **11** |
| | **Thau** | **Y** | **58** |
| | Urbino | Y | 0 |
| | Vic | N | 0 |
| **GREECE** | Agiasma, Eratino, Fanari, Keramoti and Vassova | N | 0 |
| **ITALY** | Lesina | N | 0 |
| | **Mar Piccolo** | **Y** | **10** |
| | Orbetello | Y | 0 |
| | **Stagnone di Marsala** | **N** | **1** |
| | Vendicari | N | 0 |
| | **Venice** | **Y** | **25** |
| **MOROCCO** | **Mar Chica** | **Y** | **1** |
| **SPAIN** | Addaia Bay | N | 0 |
| | Buda (Ebro delta) | Y | 0 |
| | **Mar Menor** | **N** | **1** |
| **TUNISIA** | **Bizerte** | **Y** | **3** |
| | Ghar El Melh | N | 0 |
| | Lac of Tunis | N | 0 |

Table 5. Vectors of introduction and donor regions of the exotic macrophytes reported in Mediterranean coastal lagoons. An exotic species can have several possible vectors and donor regions, which explains a sum of percentages > 100 % for each category (from Verlaque, 2005; amended).

| | | N | % |
|---|---|---|---|
| Vectors of primary introduction and secondary dispersal | Canals | 2 | 3.0 |
| | Fishing baits | 2 | 3.0 |
| | **Shellfish transfers** | **64** | **94.0** |
| | Shipping | 21 | 31.0 |
| Donor regions | Atlantic | 19 | 28.0 |
| | Cosmopolite | 1 | 1.5 |
| | Indian Ocean | 3 | 4.4 |
| | **Pacific** | **53** | **78.0** |
| | Tropical seas | 1 | 1.5 |

Using present-day taxonomy, the exotic flora reported in these lagoons reaches a total of 68 taxa (42 Rhodophyta, 19 Ochrophyta and 7 Chlorophyta) (Table 2). Exotic taxa have been reported from twelve Mediterranean coastal lagoons (Table 4). The exotic flora is the lowest (one or two taxa) in the lagoons without aquaculture activities, whereas its richness is maximum in the leading Mediterranean shellfish-farming areas as the Thau Lagoon and the Lagoon of Venice, with 58 and 25 exotic taxa, respectively.

After a primary introduction in Europe, the Asiatic taxa were probably secondarily dispersed with the frequent shellfish transfers between the Atlantic and the Mediterranean Sea and between the different Mediterranean shellfish-farming areas like Thau and Venice (Occhipinti Ambrogi, 2000).

An additional mode of transportation is with the direct importation of marketable products from a source country to a host country where the shellfish is sold in local markets (Blake, 1999; Carlton, 2001; Weigle *et al*., 2005). For example, France regularly imports large livestock of adult mussels for the seafood trade from Spain, Italy and other Mediterranean regions. Although the re-immersion ("*retrempage*") in coastal waters of such livestock is strictly prohibited, this practice is frequent. Moreover, when quarantine tanks exist, the effluent seawater discharged is rarely sterilized.

## THE EXPERIMENTAL EVIDENCE

Despite the presumed importance of oyster transfer in species introductions, only a few studies were devoted to the epibionta of shells (Schodduyn, 1931; Korringa, 1951; Gruet *et al*., 1976; Haydar and Wolff, 2004). As part of the ALIENS Program, the risk of transferring native and non-indigenous macrophytes in association with oysters, from one farming site to another, was assessed (Mineur *et al*., 2007a). Several transfers of oysters were simulated. The experimental donor area was the Thau Lagoon. The simulation involved conditions likely to be experienced during surface transport (by road) to most other European oyster farming sites. Several durations of emersion of the shells were tested. We also tested two realistic methods (i.e. immersion in hot seawater and immersion in brine) to reduce potential risks of macrophyte transfers. Immersion in freshwater was not tested because it is inefficient (Gruet *et al*., 1976). After a simulated transfer, the oyster shells were maintained in culture tanks until the epiflora reached a suitable size for identification.

The simulation showed that oysters visually cleaned of epibionts can still bear a high diversity of viable macrophyte propagules. A total of 57 taxa belonging to 17 orders were recorded across all treatments and experiments, including 16 exotic species. By comparison, only seven macrophyte orders were found during a survey in the same area (harbour of Sète) from the hull fouling of 23 large standard commercial ships coming from all over the world (Mineur *et al*., 2007b). The period of aerial emersion did not reduce the number of taxa nor the total 'propagule pressure' measured as the cumulative number of shells fouled by each taxon. The abundance of macrophyte propagules on the shells may be due to the fact that after cleaning, the oysters are re-immersed for two weeks in plastic net bags in order to decrease stress and to allow removal of moribund individuals prior to transport.

Immersion for short periods (3 seconds) at high temperatures (80 to 85 °C) had a lethal effect on nearly all macrophyte propagules, except for tubular *Ulva* spp. Under brine treatment, the reduction of macrophyte propagules was significantly less and some resistant Chlorophyta (*Cladophora* spp. and tubular *Ulva* spp.), Ochrophyta (ectocarpalean species and *Scytosiphon lomentaria*) and Rhodophyta (*Porphyra* sp. and *Stylonema alsidii)* were able to survive.

## Discussion and conclusion

In 1994, a bibliographic review identified the Suez Canal as the major vector of introduction of macrophytes into the Mediterranean Sea (Verlaque, 1994). The ALIEN and PNEC Programs showed that the oyster transfer is a more efficient vector. This is especially true when one considers that the list of introduced macrophytes recognized likely represents the "tip of the iceberg". Indeed, the number of introduced species is probably underestimated since one introduction can remain undetected when it concerns a cryptic species that is similar to a native one (Carlton, 2001). Likewise, when native species are present in a large but fragmented area (e.g. the Atlantic and the Mediterranean), gene introductions from remote populations must occur. Such types of introduction, which are very difficult to detect, constitute an important biological pollution to be considered. Thus, when compared to the Mediterranean lagoons without aquaculture activities, the diversity of the Thau Lagoon flora is abnormally high; this situation might reflect undetected introductions from the Atlantic.

Consequently, the remark by Elton (1958) "The greatest agency of all that spreads marine animals to new quarters of the world is the business of oyster culture" also holds true for marine macrophytes.

The ALIENS and PNEC Programs demonstrated the high efficiency of oyster transfers as vector of macrophyte introduction. When compared to the constraints imposed by other major vectors like hull fouling and ballast waters (e.g. long travel, changes in latitude, darkness, anti-fouling paintings, pollutants, etc.), the conditions of marine livestock transfers appear very soft, non selective and favorable to the survival of many organisms (Weigle *et al*., 2005). Aquaculture acts as a "low-cost" vector for the hitchhiker species, particularly for the macrophytes.

The ALIEN experiment involved four simulated transfers of 320 oyster valves each (i.e. only 160 oysters, more or less equivalent to 15 kg), a very small quantity compared to those transferred every year by European oyster farmers (e.g. 205 million of juvenile *C. gigas* at Thau in 2001; Girard *et al*., 2005). Likewise, in France the oysters frequently change rearing basins before their marketing. In 2001, these transfers represented several tens of thousand metric tons of young and adult *C. gigas* and 2,000 metric tons of *Ostrea edulis* (Girard *et al*., 2005).

Before the 1960s, the ecological consequences of the large-scale, deliberate introduction of exotic shellfish species were in general disregarded. But the growing awareness that shellfish imports could be accompanied by the import of pests, parasites and devastating diseases as well as the observed effects on native communities, led to a number of measures since. Codes of conduct were introduced in several countries (see Utting and Spencer, 1992, for the United Kingdom). Quarantine measures have been introduced as well. In addition, hatchery production of marine bivalves became technically and economically feasible, thus diminishing the necessity to import seed shellfish from the wild and often from other parts of the world. However, large quantities of shellfish are still being transported from one culture area to another within Europe. The European Common Market even encourages this practice (Wolff and Reise, 2002). The inadequacy of current legislation is such that these transfers occur with accidental primary introduction and secondary dispersal of marine species (Martel *et al*., 2004; Verlaque *et al*., 2005, this study).

Relatively simple changes to the shellfish transfer practice can reduce the risk of species introductions. Heat treatment is an efficient way to kill macrophyte propagules (Mountfort *et al*., 1999; Mineur *et al*., 2007a). Certain French oyster farmers already commonly use such a treatment to remove small oyster spat and other fouling organisms from medium-sized oysters. Immersion in saturated brine for a short period is another effective method of control of various invasive organisms such as *Crepidula fornicata* (Linnaeus, 1758) and *Sargassum muticum* (Hancock, 1969; Franklin, 1974; Lewey, 1976; Ruellet, 2004; Mineur *et al*., 2007a). Other preventative methods

involve toxic chemicals (MacKenzie and Shearer, 1959; Barber, 1997; McEnnulty *et al*., 2001; Ruellet, 2004). However, the use of toxic substances is not suitable for shellfish production aimed at human consumption. Hitchhiking species, pests, parasites and diseases are not confined to the shell exterior alone but also occur within the shell, the mantle cavity and tissues as well as within the vacant spaces of dead oysters.

Table 6. Guidelines to reduce the unintentional introductions by aquaculture.

- Awareness of farmers concerning the risks associated with uncontrolled importation has to be increased.
- Aquaculture should be based on native, local stock whenever possible. Imports and transfers of stock should be minimized, thoroughly inspected, and quarantined for an appropriate observation period.
- Special attention would have to be paid during aquaculture trials with new exotic species (even with livestock from hatcheries).
- Non-native livestock for introduction has to be produced in hatcheries.
- Live products destined for consumption, processing, and aquarium or display should not be placed into the natural environment.
- In the case of livestock transfers (including interregional ones), decontamination processes and/or quarantine as proposed by the ICES (2005) have to be followed.
- Efficient treatment (e.g. hot-seawater for oysters) to avoid introduction or secondary dispersal of exotic or native species would have to be carried out prior to each transfer that is to say after the period of re-immersion preceding the transfer and would have been repeated on arrival.

Comprehensive guidelines for preventing introductions of exotic species are available through IUCN (Shine *et al*., 2000) and ICES (2005) (Table 6). Widespread adoption of these policies is urgently needed to stem the rising tide of aquatic invasions (Naylor *et al*., 2001; Occhipinti Ambrogi, 2001; Cohen, 2005; Weigle *et al*., 2005).

**Acknowledgements**

This study was supported by grants from the Programme National sur l'Environnement Côtier (PNEC) "Lagunes Méditerranéennes" and a Fifth Framework Program of the European Community (ALIENS: 'Algal Introductions to European Shores'). We thank Isabelle Auby, Martin Plus and the IFREMER Station of Arcachon for their collaboration during the study at Arcachon, and Michèle Boudouresque for bibliographical assistance.

# Parasite and disease transfer between cultured and wild coastal marine fish

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## Abstract

Mariculture cage structures characteristically attract surrounding wild fish, modifying community structure, fish behavior and feeding habits. The intermingling of cultured and wild fish also provides opportunities for pathogen exchange. However, the routes of disease transfer between cultured stocks and wild fish populations have so far not been amply studied and are still poorly understood. In recent years, infections and diseases of cultured and wild fish in the Gulf of Eilat (Israel, Red Sea) were investigated, to determine if and to what degree such disease exchanges actually took place. Particular attention was devoted to the infection dynamics of lethal bacterial pathogens typical of farmed fish, of the myxosporeans *Enteromyxum leei* and *Kudoa iwatai*, and of VER (Viral Encephalopathy and Retinopathy). Significant bacterial infections by *Mycobacterium marinum* and *Streptococus iniae*, typical of cultured stocks, were found to cause morbidity and mortality also in wild fish. While determining the initial source was not unequivocally possible, intensive sea cage farming acted as pathogen "amplifiers", with diseases transmitted in both directions between feral and cultured fish populations, and specificity - or lack of it - of each pathogen for a host playing an essential role.

In present day land-based and sea-cage mariculture, cost-effectiveness is high on the priority list and therefore fish are often reared at the highest possible densities, producing unnatural and highly stressful conditions in the confined spaces. Such conditions also promote pathogen transmission, amplification and spread of diseases to the surrounding environment. Frequently, disease occurrences in these mariculture systems break out quite abruptly and unexpectedly. Sea caged cultured stocks may also display receptiveness to pathogens originating in the surrounding environment. Indeed, disease interactions between cultured and wild fish have been shown repeatedly to occur in a variety of geographical regions (Diamant *et al*., 2000; Kent, 2000; McVicar, 1997; Nowak *et al*., 2004; Paperna, 1998; Sepulveda *et al*., 2004). However, the effect of such interactions is poorly understood and there are conflicting views regarding their actual impact on the environment (Brackett, 1991; McVicar, 1997). Cage structures characteristically attract wild fish from the surrounding area (Sánchez Jerez *et al*., this volume), hereby modifying the local community structure of the natural fauna, altering fish behavior, feeding habits and, not less importantly, influence composition of the associated parasite assemblages. The forces that drive disease interactions between cultured and wild fish, and the impact which this phenomenon is likely to have on both sides need to be much better understood than they have been to date.

In the Gulf of Eilat, on the northern Red Sea coast of Israel, a relatively small mariculture industry has developed, currently producing approximately 2,000 tons/year. The main cultured species are the gilthead sea bream *Sparus aurata* and, to a lesser extent, the European sea bass *Dicentrarchus labrax*, both of which were introduced in the early 1970s from the Mediterranean Sea. Sea bass lower production is mainly due the extreme susceptibility that this species has shown to *Mycobacterium marinum* (Colorni, 1992). In 1999 a widely publicized debate on the allegedly adverse impact of mariculture cage farm activity on Eilat's coral reef ecosystem broke out in Israel. Consequently, the Israeli government appointed an International Expert Team (IET) to evaluate the current ecological condition of the Gulf and recommend steps to ameliorate the situation for better conservation of the native coral reefs (Atkinson *et al*., 2001). One of many concerns that were raised dealt with the ostensible increase in the incidence of infections and diseases in local wild fish populations, often associated with mortalities. Claims were made that the fish farms had transformed into dangerous hubs of infection, and that increased incidence of diseases in the wild fish was linked with the mariculture activity, where most of the regional fish disease research has been carried out (Figure 1). In 2002-2003, a survey commissioned by the IET was carried out to critically evaluate the available information on fish diseases in the region. In addition, supplementary hard data were needed and collected from various sites along the Israeli Red Sea coast (Figure 2) aiming at determining whether or not the cage farms were a responsible factor for spreading diseases to the surrounding wild fish fauna (Diamant *et al*., 2004). The results of the survey indicated that indeed, some pathogens were shared by both cultured and wild fish (Diamant *et al*., 2004).

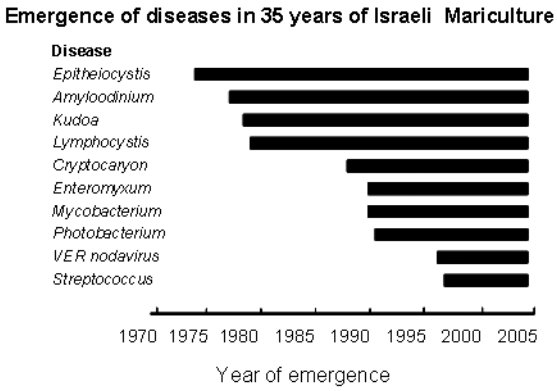


Fig. 1. Emergence with time of diseases in 35 years of Israeli mariculture.

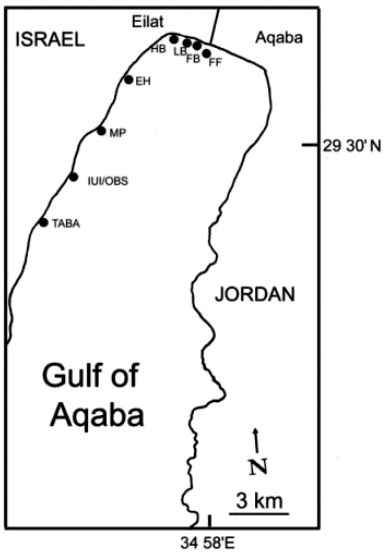


Fig. 2. Sampling sites in study area. FF – Mariculture cage farms; LB – Lagoon Beach; HB – Hotel Beach; EH – Eilat Harbor; MP – Marine Pollution Station; OBS – IUI - Underwater Observatory/Interuniversity Institute; Taba – Taba frontier station.

In the IET study, several significant pathogens were found in the feral fish (Figure 3): *Mycobacterium marinum*, *Streptococcus iniae, Lactococcus garvieae* and *Enteromyxum leei* (Colorni *et al*., 2003; Diamant *et al*., 2004). All four are recognized pathogens of farmed fish in Israel (Colorni, 1992; Colorni *et al*., 2002; Colorni *et al*., 2003; Diamant *et al*., 1994), but only *M. marinum* was present at a significant level.

*M. marinum* is considered one of the most serious pathogens of cultured fish. The molecular attributes of the local strains in Israel have been thoroughly studied (Knibb *et al*., 1993; Ucko *et al*., 2002). Whether the bacterium may have been unintentially introduced into the Red Sea with farm stocks or, conversely, is an endemic Red Sea strain and particularly sensitive exotic fish were exposed to it, could not be verified. Two localities were identified in previous studies as *M. marinum* "hot spots" in wild rabbitfish populations in the Eilat area (Diamant *et al*., 2000; Diamant, 2001). The most recent data show that many additional wild Red Sea fish species are infected with this pathogen and that the disease is widely distributed along the Israeli Red Sea coast (Figures 4, 5) (Diamant *et al*., 2004). *M. marinum* in the present survey was established by the presence of granulomatous lesions containing acid-fast rods (Ziehl-Neelsen stain) in histological sections of fish spleen, liver and/or kidney.

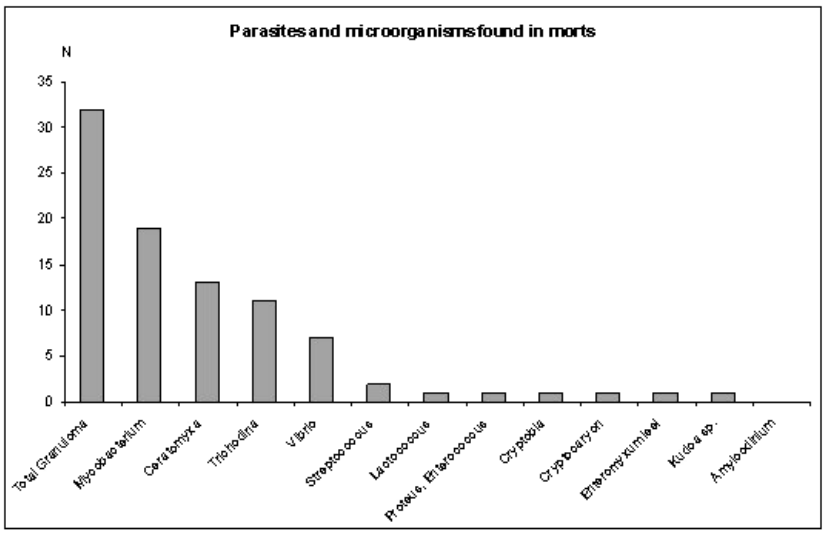


Fig. 3. Granulomas, parasites and microorganisms diagnosed in dead/dying wild fish ("morts") recovered from the surveyed coastal sites on the Red Sea coast of Israel (Eilat).

A second bacterial pathogen, the gram-positive *Streptococcus iniae*, was repeatedly isolated from feral fish found dying in the wild (Colorni *et al*., 2002). As was also detected in Mediterranean Sea cage farms by Zlotkin *et al*. (1998), who observed transmission of *S. iniae* between wild and cage-cultured fish, and due to the frequent transfer of fingerlings from hatcheries based on the Mediterranean coast of Israel to the Red Sea farms, it is hypothesized that its appearance in the Red Sea was most likely due to an accidental introduction. Mortalities due to this pathogen have been reported in wild fish elsewhere in the world (Yuasa *et al*., 1999; Ferguson *et al*., 2000).

*Lactococcus garvieae* has so far been found only once in the Red Sea area, in a wild, moribund Red Sea fish (Colorni *et al*., 2003).

*Enteromyxum* is a myxosporean genus that has the unusual capacity (unique among the Myxosporea) to transmit directly between fish, a route which contradicts the usual heteroxenous life cycle of the group that utilizes annelid worms as alternate hosts (Diamant, 1997). *Enteromyxum leei* initially emerged in the Mediterranean in the late 1980s and it is believed to be an unintentional introduction into the Red Sea (Diamant, 1992, 1997). Infections with this myxosporean were found in sea-caged sea bream as well as in 10 different wild fish species living in cage farm vicinity (EU project Myxfishcontrol, unpublished data). While the phenomenon of escapees from sea cages may have genetic implications on wild populations (Garcia-Vazquez *et al*., this volume;

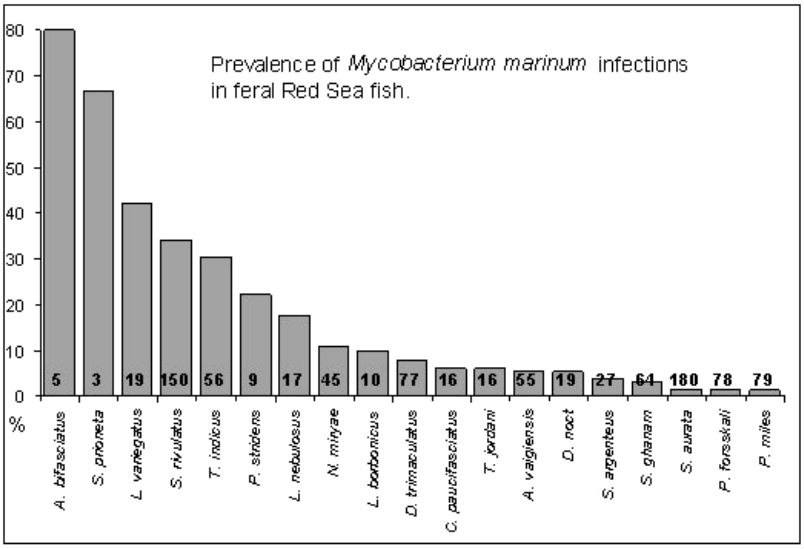


Fig. 4. Prevalence of *M. marinum* infection (based on granulomas containing acid-fast rods) in sampled wild Red Sea fish species (numbers are fish sample sizes).

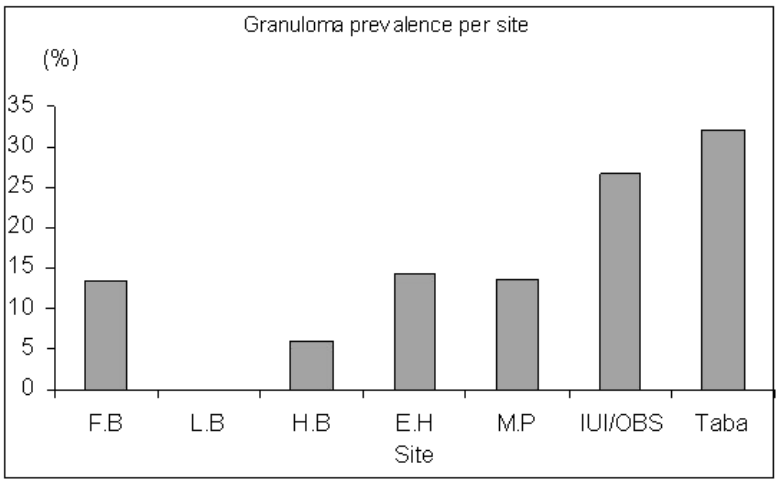


Fig. 5. Granuloma prevalence – in most cases lesions were associated with acid-fast rods *(Mycobacterium marinum)* in wild Red Sea fish at the different sampling sites.

Triantafyllidis, this volume), they may also be assumed to carry infections they acquired at the culture systems. We collected sea bream escapees infected with *E. leei* as far as 5 km away from the sea cages (EU project Myxfishcontrol, unpublished data). However, we found no evidence that these escapees have transferred the infection to wild fish at the site. In addition, the prevalence of this parasite in wild populations near the cage farms themselves was low, with infections being limited to fish living inside or in the immediate surroundings of sea-cage confines. It is interesting to note that the dinoflagellate *Amyloodinium ocellatum* and the ciliate *Cryptocaryon irritans*, which often cause serious losses in land-based sea bream and sea bass cultures in Eilat, have very seldom been found to infect cage-cultured stocks, and the results of our recent studies confirm that both cage stocks and associated wild fish have a very low prevalence of *C. irritans* and virtually no *A. ocellatum* infections (Figure 3; Diamant *et al*., 2004).

Two additional fish pathogens were given particular attention. One is a multivalvulid myxosporean, *Kudoa iwatai*, and the other, a neuropathic betanodavirus (known as either VNN, Viral Nervous Necrosis or VER, Viral Encephalopathy and Retinopathy).

*Kudoa* sp. is known from Red Sea cultured sea bream since the early 1980s (Paperna, 1982). It was recently identified as *Kudoa iwatai*, a species previously known only from Japan. Our molecular analyses suggested that in all probability this is a widely distributed Indo-Pacific species that it is probably native also to the Red Sea, where it has been isolated from several species of reef fish, and was likely transmitted from feral fish to cage farm stocks (Diamant *et al*., 2005). This assumption is supported by the absence of any reports to date of this parasite infecting gilt-head sea bream, wild or cultured, in the Mediterranean.

The appearance of new fish pathogens in the Gulf of Eilat in recent years is consistent with an emerging global pattern of disease affecting marine organisms appearing in areas subject to intense anthropogenic impact (Harvell *et al*., 1999; Ward and Lafferty, 2004). At the same time, the distribution of marine microorganisms is changing worldwide as a result of various causes, including global movement of ballast water by ships (Ruiz *et al*., 2000; Wonham *et al*., 2001). All in all, the emergence of *M. marinum*, *S. iniae, L. garvieae* and *E. leei* over a relatively short time span in wild fish populations in the northern Red Sea is worrisome. Since ichthyopathological studies have been conducted in wild and cultured fish in the Eilat area since the early 1970s, and these failed to detect any of the 4 disease agents until recent years, it follows that a) either these agents were very rare and we are currently witnessing a surge in their virulence, or b) their appearance is the result of anthropogenic (mariculture) activity.

In the last decade, great efforts have been invested by our Institute in the domestication of the white grouper, *Epinephelus aeneus*. This Mediterranean species has considerable market value and a high commercial potential. Efforts to culture *E. aeneus* were continuously frustrated by the appearance in both broodstock and offspring of a severe viral infection. The agent is a betanodavirus that affects the brain, retina and other nervous tissue of the fish, producing neuromotory imbalance, blindness, etc. A phylogenetic comparison of the sequences of the coat protein gene of VNN/VER isolates from five maricultured species from both Red Sea and Mediterranean coasts of Israel during 1998-2002 showed that Red Sea sequences were significantly different from the Mediterranean. While all Israeli isolates belonged to the RGNNV type, which infects a variety of warm-water marine fish species**,** our results showed that VER infections of Israeli maricultured fish in recent years are caused by a number of viral strains, and no host-specificity was observed (Ucko *et al*., 2004). The possibility that the VNN/VER sequences from the Gulf of Eilat belong to a strain endemic to the Red Sea region cannot be ruled out. VNN/VER has been detected in the past in cage-cultured sea bass in the Mediterranean and transmission to wild fish in the farm vicinity was suspected (Le Breton *et al*., 1997). Thus, the appearance of the virus could be the consequence of an introduction. In the case of the white grouper, *E. aeneus,* isolated sequences, indicate that the broodstock had been reared from a group of wild individuals caught on the Mediterranean offshore waters of Israel. It was therefore assumed that some individuals were carriers of the disease and that the Mediterranean isolates were introduced into the Red Sea with these stocks.

During the winter of 2006/2007, dozens of freshly caught adult fish were caught in the Mediterranean coast of Israel and brought to NCM. Some of the fish were PCR tested soon after their arrival and produced positive results. These results confirmed that the virus is widespread in white grouper populations in the Mediterranean Sea, supporting the results of a survey recently carried out in Sicilian waters by Ciulli *et al*. (2006). These authors detected VNN/VER infections in at least five species of wild fish. Carrying our routine monitoring program in coastal marine ecosystems wherever farmed stocks in cage farms are present is essential for maintaining an up-to-date knowledge of the local wild fish health situation. Although the impact of fish farming may not be as far-reaching as previously believed (Diamant *et al*., 2000, 2004; Diamant, 2001) and determining the initial source of each pathogen was not always possible, intensive sea cage farming acted as pathogen “amplifiers”.

# Escapes of marine fish from sea-cage aquaculture in the Mediterranean Sea: status and prevention

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## Abstract

Escapes are widely regarded as a key environmental problem in the culture of fish in the marine environment. At present, standing stocks of both cultured sea bream and sea bass number close to 500 million fish in the Mediterranean Sea. Escapes of these two species occur when farms break-down during storms, through holes in the netting created by biting by predators or possibly by the cultured fish themselves, through holes created during farming operations, or as a result of spills during handling and harvesting of fish. While escape events have been reported in the popular press, no official statistics exist to assess their frequency or size. This critical lack of knowledge makes it difficult to gauge the possible effects of escapees on wild fish populations through genetic and ecological interactions. Here, we present the status of escapees in the Mediterranean Sea and discuss techniques that may be successful in reducing escapes and mitigating possible negative consequences. Measures to prevent escapes include regulatory tools such as the implementation of reporting requirements and independent certification of fish farms to design and dimensioning standards. The use of more robust net-cage constructions through strengthening mooring and floater technologies or strengthening nets may reduce escapes through farm break-down during storms or escapes through holes in the netting. Measures to mitigate the impacts of escapees include the possible use of sterile fish, siting farms away from areas of particularly high importance to wild fish stocks, such as known points of natural aggregation for feeding, spawning areas or migratory pathways, and designing specific technologies for effective recapture of escapees.

## Status of Mediterranean sea-cage fish farming and escapes

The sea-cage aquaculture industry is expanding throughout the Mediterranean, with production now 160 000 t/yr in hundreds of sea-cage farms (FAO, 2005). Greece, Turkey and Spain are the leading producers, with farms present in numerous countries in the Mediterranean. Approximately 500 million sea bass and 450 million sea bream are believed to be held in sea cages at any time (ICES WGEIM report, 2006a, b). Wild sea bream and sea bass populations are not fully known; however wild fish numbers are believed to be considerably lower than the standing stock in sea-cages (e.g. 210 million wild sea bass; ICES WGEIM report (2006a)). Overfishing of these stocks has been reported from some areas of the Mediterranean (Sanchez-Lamadrid, 2002). As mariculture grows, the risk posed to wild stocks by escapees in terms of genetic mixing or

ecological interactions increases as more farms means more escapees. Moreover, a great variety of marine fish species (>20) are under trial or are beginning to be cultured in sea-cages in the Mediterranean as the industry attempts to diversify into new species.

Escapes of sea bream and sea bass from sea cages in the Mediterranean have been sporadically recorded (e.g. Dempster *et al*., 2002; Figure 1), although no requirements exist for farmers to report escape events. Storms have caused damage to farms in Spain and Greece resulting in mass escapes. Sea bream are thought to nibble on net cages and create holes, as described for the Atlantic cod (Moe *et al*., 2007), so it is likely that escapes through holes may be similarly important. In addition, manual harvesting techniques in sea bream and bass farming in the Mediterranean are a further source of escapes through losses and spills.

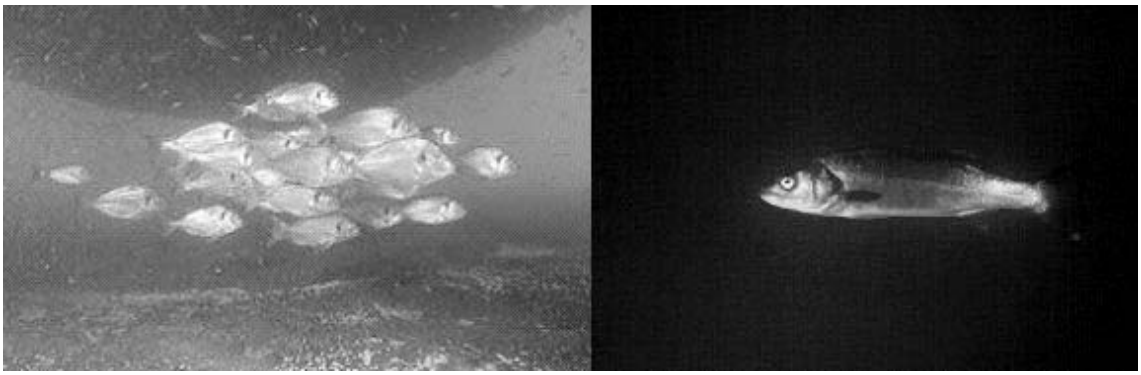

Fig. 1. Escaped sea bream beneath a sea cage (left) and an escaped sea bass (right).

The proportion of escapes that are due to storms, holes in the netting, or spills through handling of fish is difficult to determine. Extensive documentation of salmon escapees exists in some countries due to mandatory reporting requirements (e.g. Norway, Canada). Roughly 0.2-0.3% of salmons held in cages are reported to escape each year (Norwegian Fisheries Directorate 2006), although the real number that escape is thought to be much higher (Torrisen, 2006). Storms and fish farm breakdown now account for over 80% of salmon escapes by number. However, for new marine species, there is gathering evidence that the reasons for escape are markedly different from salmon due to differences in the way that these species interact with cages. For example, Moe *et al*. (2007) estimated that a minimum of 0-6% of cultured Atlantic cod were reported to have escaped in Norway in any particular year from 2000 to 2005. Approximately half the number of reported cod escapees were due to storms with escapes through holes in the net also important (Figure 2).

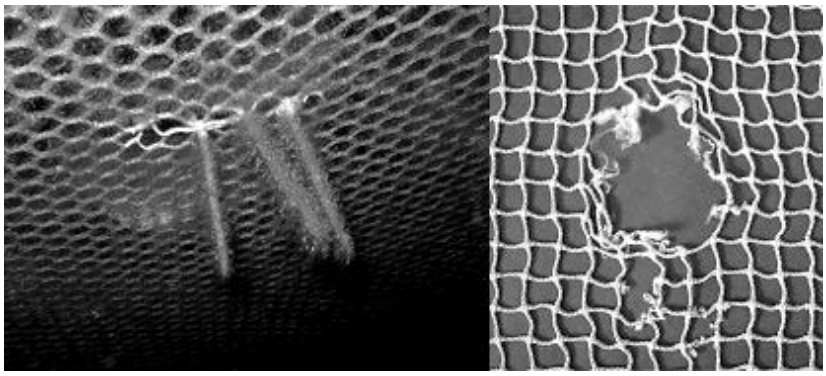

Fig. 2. Hole in a sea bream net cage in the Mediterranean Sea, repaired by divers with a series of plastic cable ties (left), and a net cage hole through which several thousand Atlantic cod *(Gadus morhua)* are known to have escaped through at a farm in Norway (right).

In general, escapes from storms will be more likely to be reported, as damage is noticeable and escapes from storms are typically large. However, escapes through holes in the net are less likely to be noticed and farmers may have little idea how many fish have escaped if a hole in the netting is discovered. Combined, this may lead to substantial under-reporting of numbers escapes through holes in particular. Certain behaviours will heighten the likelihood of escape through holes, including biting of the net which creates holes and continuous close interaction with the net wall, as for Atlantic cod (Ås, 2005; Moe *et al*., 2007). Sea bream also exhibit such behaviours (ICES WGEIM report, 2006b), which increase the risk of escape through holes.

Holes may also be created by predators, such as the bluefish *Pomatomus saltatrix*, which bites its way into sea-cages (Spain) to attack sea bream and sea bass (Figure 3). During predation episodes, escapes may occur through the holes made by these predators as the cultured fish avoid predation (Sanchez-Jerez *et al*., this volume).

Fig. 3. *Pomatomus saltatrix* predates upon sea bream and sea bass within sea-cages (left) in the Mediterranean Sea by forcing its way through holes in the cage netting (mid) that may have been created through biting with its sharp teeth (right). Holes created in the net cage by this predator may contribute to escapes.

A range of data sources from studies focused on investigating aggregations of wild fish around sea cage farms provides a tentative picture of escapes of sea bream and sea bass in the Mediterannean Sea and at the Canary Islands. Since 2001 eight studies have investigated the abundance and species composition of wild fish assemblages around sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus auratus*) fish farms (Table 1).

Table 1. Data sources for escaped sea bream and sea bass from sea-cage farms.

| Authors | Location | No. of Farms | Study duration | Sampling days | Sampling method | Escaped sea bream | Escaped sea bass |
|---|---|---|---|---|---|---|---|
| Dempster *et al*. 2002 | MS | 9 | 2 mo | 27 | Pelagic DVC | Y | Y |
| Vita *et al*. 2004b | MS | 1 | 3 mo | 9 | Pelagic + benthic DVC | N | Y |
| Valle *et al*. 2007 | MS | 1 | 1 yr | 12 | Pelagic DVC | Y | Y |
| Fernandez-Jover pers. comm. | MS | 3 | 2 yr | 72 | Pelagic DVC | Y | Y |
| Dempster *et al*. 2006 | MS, CI | 5 | 2 mo | 15 | Pelagic + benthic DVC | Y | N |
| Boyra *et al*. 2004a | CI | 2 | 1 yr | 24 | Benthic DVC | Y | Y |
| Tuya *et al*. 2005 | CI | 3 | 1 mo | 3 | Benthic DVC | Y | N |
| Tuya *et al*. 2006 | CI | 1 | 2 mo in 2 yr | 16 | Benthic DVC | Y | N |

MS = Mediterranean Spain, CI = Canary Islands. DVC = Diver visual count.

Sea bream and/or sea bass were observed directly beneath 10 separate farms out of a total of 15 farms censused by this group of studies. Small groups <50 fish were most frequently observed (e.g. Figure 1), with schools of thousands of individuals observed on three separate occasions. Further, we have directly observed small escapes of fish during harvesting procedures (tens to hundreds of individuals). These results suggest that losses of sea bass and sea bream periodically occur at many farms. From our visual observations (Dempster *et al*., 2002, 2006; Tuya *et al*., 2005, 2006; Fernandez-Jover, pers. comm.), fish were within the same size range and they exhibited similar morphological characteristics as the caged fish (reduced caudal fin size and general stockiness of the body indicative of aquacultured fish which have a high weight-to-length ratio).These fish therefore were likely to have been escapees. Furthermore, wild sea bream and sea

bass are not known to aggregate around natural floating structures (Castro *et al*., 2002). Wild sea bream prefer rocky reefs and sea grass habitats which differ from the sandy or muddy sea floors which lie beneath fish farms (Tuya *et al*., 2005). Detailed comparisons of bony-structure chemical compositions (e.g. otolith microchemistry; Gillanders and Joyce, 2005), growth rates, muscle-lipid biomarkers (e.g. Fernandez-Jover *et al*., 2007a) or genetic composition (Triantafyllidis, this volume) between farmed fish, fish beneath farms and wild populations distant from farms, or a combination of techniques, are required to test this assumption rigorously. At the Canary Islands, all sea bream and sea bass observed may be considered to be escapees, or their offspring, as wild stocks do not naturally occur there in significant numbers.

## Possible environmental impacts of escapes of marine species

As limited information on the extent of escapes in the Mediterranean exists, assessment of the potential genetic and ecological risks to the population is difficult (see Bonhomme, this volume; Triantafyllidis, this volume; Garcia-Vazquez and Moran, this volume). Contention exists as to whether marine fish species, which generally have large reproductive capacities and populations that mix over large distances, can be expected to be affected similarly by escapees as anadromous species that have comparatively small breeding populations and limited mixing (see Bonhomme, this volume). In reviewing the literature on the genetic effect of salmon escapees, Weir and Grant (2005) concluded that while little data existed to test the direct effects of escapees on the demographics of wild fish populations, several studies provide evidence that escaped salmon have lower fitness than their wild counterparts, as measured by survival and reproductive success. Further, numerous studies provide strong evidence of genetic and phenotypic differences between farmed and wild salmon, presumably because of selection for high growth and survival in the artificial sea-cage environment.

Two recent risk analyses have been produced by ICES on the potential for interbreeding between wild and escaped sea bass and sea bream (ICES WGEIM report, 2006a,b). The potential for genetic mixing exists in the Mediterranean for both species. Moreover, large-scale escapes of thousands of individuals in restricted coastal areas may lead to ecological effects such as competition for food with wild stock or the spread of pathogens (Diamant, this volume). Genetic differences exist between wild and cultured stocks (Allegrucci *et al*., 1997; Lemaire *et al*., 2000). As breeding programs continue (e.g. sea bream: Gorshkov *et al*., 2002), differences will become more pronounced.

An emerging issue regarding escapes is that certain fish species are being raised to sizes within sea-cages at which, if they become sexually mature, they are capable of spawning. This requires the concept of "escape from mariculture" to be redefined to include the escape of reproductive gametes into the environment. Spawning of cultured cod in sea-cages has recently been demonstrated in Norway. Jørstad and van der Meeren (2006) allowed 1000 gene tagged cod to spawn within a small fjord system in Norway. Upon sampling larvae in the waters surrounding the farm two weeks later, 25% were traced back to caged parents. This indicates that if spawning occurs within commercial cod farms where the numbers of animals are far greater, the contribution of 'escaped' larvae to cod recruitment within fjords may be substantial. Spawning of sea bream within sea-cages has also been observed in Greece (Somarakis *et al*., pers. comm.) and sea bass are also known to spawn in sea cages (ICES WGEIM report, 2006b). If breeding programs shift the genetic diversity of aquacultured fish away from wild stocks, the extent of spawning within sea cages and whether larvae subsequently survive and recruit into natural populations in significant numbers will likely greatly affect the siting of farms.

## Preventing escapes through regulation and enhanced technology

Given the expansion of the sea bream and sea bass industries and the possibility of negative genetic and ecological interactions, more effective strategies to prevent escapes are required.

### *– Regulatory instruments*

In the Mediterranean, statutory reporting requirements of the number of individuals that escape and the suspected cause of escape is necessary before the likely impacts of escapes can be fully gauged. In Norway and Canada, reporting systems have been operating for over a decade and much

knowledge from their operation could be readily transferred. A standard for sea-cage fish farm certification is in operation in Norway (NS 9415: 'Marine fish farms - Requirements for design, dimensioning, production, installation and operation'), which is principally aimed at reducing escapes from storm damage through correct engineering of fish farm technology. As technology currently used in the Mediterranean for sea bream and sea bass culture is similar to salmon farming, implementation of this standard (after modification to Mediterranean conditions) may significantly reduce escapes due to storm events. The NS9415 standard is likely to become an international standard (ISO) in the future. In addition, in late 2006, Norway initiated the first permanent 'Escapes Commission', which is mandated to determining the causes of escape events, so changes to technology and operations can be made accordingly. All of these instruments may have a place in the Mediterranean context.

**– *Submerged cages*** avoid the strong physical forcing at the ocean surface caused by storms as most surface wave energy (95%) dissipates within the first 10 m in the open sea. Thus, they may allow use of a range of offshore sites distant from the coast and could also reduce the number of escapes of cultured fish, which are principally due to storm damage. At present, surface cage technologies are cheap and dominate the marketplace. Submersible or semi-submersible cages are currently used for the culture of sea bream (*Sparus auratus*) in Italy (Refa-med leg tension cages, Sea Station cages: Mirto, pers. comm.), Pacific threadfin (*Polydactylus sexfilis*) in Hawaii (SeaSpar cages) and cod (*Gadus morhua*) off New Hampshire (in Sea Station cages) (Ryan, 2004). Widespread adoption of submerged cage technology by industry, however, will require solutions to several technological and operational obstacles (Chambers and Howell, 2006). Further, it must be rigorously demonstrated to industry through research that their use does not have negative economic consequences through diminished growth rates, poorer food conversion ratios or reduced welfare of the cultured fish in comparison to standard surface systems. Finally, the fact that holes in the nets of submerged cages will be more difficult to monitor through diving must be countered through the development of a new system to monitor net integrity.

For marine species that interact closely with the net-cage, Moe *et al*. (2007) suggested that the solution to developing sea-cages with lower incidences of escape lay within the following three areas or their combination: 1) using stronger net cage constructions; 2) developing an uninteresting or 'repulsive' net cage wall; and 3) providing a more stimulating cage environment.

**– *Stronger net cage construction*** is a broad concept that may be achieved through strengthening mooring and floater technologies or strengthening nets. Technological solutions that account for all component elements of sea-cage farms are required, so that solving a problem achieved through modifying one element does not create a new problem in a separate element. For example, the use of new 'bite-resistant' netting materials to reduce hole formation must take into account whether such materials change the response of a farm to waves and current, hence necessitating a modification to the mooring components or a modification to the handling techniques used for netting at farms. In addition, whether changes to the netting also induce other undesirable effects, such as increased biofouling leading to reduced water flow through the net, must also be tested.

**– *Developing an uninteresting or 'repulsive' net cage wall*** may be achieved by providing a stiff or taut net cage with a smooth surface. Fish have strong senses, and it may be possible to use taste, smell, sound, colour and shape to prevent biting at the net cage or discourage behaviour near cage walls that leads to the discovery of holes.

**– *Providing a more stimulating cage environment*** may encourage the natural behaviour of fish and distract fish from biting at the netting and thereby reduce escape. Present day sea cages are stimulant-poor; small changes may therefore significantly improve the culture environment for fish. For many other species of cultured animals, creating a stimulating environment has involved satisfying the need for challenges and adventure and preventing boredom (Young 2003).

Considerable applied research effort is required within each of these three areas to determine solutions that are economically viable for the industry.

## Reducing the environmental impacts of escapees

### – *Sterile fish*

The use of sterile fish is a possible solution to reduce the potential for genetic mixing of escapees with wild stock. Sea bass triploids have been experimental in France and sea bream triploids have been produced by some fish farms (ICES WGEIM report, 2006a,b). Breeding was successfully inhibited in 98% of sea bass. However, the industry has not adopted the use of triploid fish as they were economically unviable due to poorer performance in the grow-out environment.

### – *Siting of fish farms*

Assessment of the risk that escapees and other effects pose to wild populations when placing farms has been suggested (Naylor *et al*., 2005; WWF, 2005). During farm placement, consideration must be given to the proximity of the site to areas that may be of particularly high importance to wild fish stocks, such as known points of natural aggregation for feeding, spawning areas or migratory pathways. Declaration of the 'national salmon fjords' throughout Norway in 2003 and the consequent restriction on placing new fish farms in these areas is an example of considering important wild fish stocks when locating farms (Sivertsen, 2006). Particular areas of coastline may be considered of such high ecological importance to wild fish populations (e.g. known spawning areas, key feeding habitat) that sea-cage aquaculture may be excluded.

### – *Recapture*

At present, specifically designed technologies to recapture escapees do not exist. Nets that can be rapidly deployed or mobile feeding stations to attract fish before using nets to recapture them have both been proposed. Key to successful recapture is an understanding of the movements of fish immediately after they escape and their period of residence in the vicinity of farms. Sea bream and sea bass escapees appear to remain in the vicinity of farms for short periods only (Dempster *et al*., 2002), so attempts to recapture them may require rapid deployment of recapture equipment within days of an escape event. However, any recapture technology must take into account the considerable by-catch of other wild fish that may occur as Mediterranean farms are hotspots of wild fish aggregation (Dempster *et al*., 2002, 2006; Sanchez-Jerez *et al*., this volume).

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# Under which condition should we be afraid of the genetic consequences of escapees in the marine world ?

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Aquaculture escapees raise several concerns for local populations of the same species :

- dissemination of pathogens harmful for the residents;
- ecological competition for resource;
- eventual genetic swamping lowering the average fitness of the wild residents through cross-breeding;

to which a fourth concern is added by some:
- denaturation of the "aboriginal" state of the resident population by incorporation of new ecophenotypic characteristics.

These concerns stem mainly from the experience gained from continental species such as game species (partridges, hares, etc.) or fish (trouts, salmons, etc.) where some of these adverse effects have often been advocated and sometimes been demonstrated.

I will not discuss the first point, that needs undoubtedly to be considered. The second point is an issue only if the escapees are released in number of the same order of magnitude as the resident population and if their survival and space occupancy resemble that of the residents. The third point will depend, as a supplementary condition, on their ability to mate and on the fitness of their offspring. The fourth point is not devoid of ideological background (what exactly means "aboriginal"?) and can be criticized in the light of what we know about evolutionary fluctuations (no ecosystem has ever been stable, especially throughout the Quaternary fluctuations) and long range gene flow that percolates through populations (populations continuously borrow from each other genetic novelties through gene migration).

As a first step, I will make a distinction between species with high fecundity, large population sizes and high dispersal capabilities through larval stage, typical of most marine species, and others, like salmons for instance, which have very small local breeding sizes, have no larval dispersal and a strong adult philopatry. The latter behave much as continental species, and are thus sensitive to some extent to the above-mentioned risks. This has been much documented and debated already (see for instance papers by McGinnity *et al*., 2003; Verspoor *et al*., 2006; Einum and Fleming, 1997) and will not be considered further. We note however that most if not all the literature concerning the potential adverse effects of aquacultured animals on wild stocks concern salmonids, and therefore the emerging picture may be flawed and not applicable to typical marine species.

As for these fully marine species (i.e. sea bream, sea bass, oysters, mussels, etc.) I will argue that the genetical risks are very low, because of Fisher's fundamental theorem of natural selection, which explains that the average fitness of a population cannot do anything but increase with time because selection cannot favour detrimental genes, unless there are strong epistatic interaction between genes. The conditions under which the native gene pool may be altered to the point that the average fitness or characteristics are modified in anything but a transient fashion are probably rarely met in the sea (like having literally a genetic swamping with an extremely strong and dissymmetrical gene flow from the cultivated side relatively to the wild one, which is quite improbable unless very special conditions).

This may happen eventually for oysters in some very peculiar places where the biomass of cultivated animals may be extremely large, but even in this case, the resilience will be extremely rapid if the gene flow ceases. In other cases, the detrimental domesticated genes have hardly a chance to establish locally except in a transient fashion as every model of underdominance and negative epistasis indicates. Highly fecund marine species may expose ten of thousands of zygotes per capita to natural selection at each generation, which translates in a tremendous potential to sieve the wheat from the chaff. Indeed, domesticated genes effects are almost invariably inferior in nature to their wild counterparts for intrinsic reasons linked to the domestication process itself. Domestication consists in a selection of tame behaviours and a derivation of energy metabolism toward growth rather than other fitness components. Like with a two-bladed sword, for the very same reason that people fear the impact of inferior domesticated x wild F1s, their genes will not last very long in nature unless genetic swamping is massive and occurs repeatedly.

A good indirect example of this may be found in the natural situations where two different genetic entities interact and produce underfit F1s and back-crosses as is the case in the blue mussels hybrid zone (Bierne *et al*., 2006): despite a large potential for hybridisation throughout their range, these species still maintain themselves as two recognisable entities which do not seem to be threatened by each other.

As to the last point, I will argue that in the very improbable event where a domesticated gene proves to be slightly better than a wild one, a condition required for this gene to establish locally, then there is no ground on which to fight against it: good and bad genes are constantly been brought about by long range gene flow, especially in those marine species under consideration, and they are constantly added or removed locally by a very strong selection potential. Hence, the concept of "locally adapted populations" that should be conserved as pristine jewels has not much to do with the reality of what those populations are, unless one understands "locally adapted" in a very dynamic way.

# Aquaculture escapes: potential risks for gene pool integrity of native species

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## Abstract

In this article we analyse the potential risk of increasing interspecific hybridization due to aquaculture escapes. Three cases of species inhabiting different ecosystems are considered: carps (freshwater), Salmonids (diadromous) and mussels (marine). We conclude that evaluation of potential for interspecific hybridization should be considered in all impact assessments of aquaculture.

## Introduction

One of the consequences of widespread aquaculture throughout the world is the dissemination of exotic species in wild ecosystems (Bartley and Subasinghe, 1996; Cambray, 2003), due to escapes or even deliberate releases. There are many examples of invasions originated from introductions of exotic species, in both freshwater (for example Salmonids in New Zealand and South America; Townsend, 2003; Baigun and Ferriz, 2003) and marine environments (Semmens *et al*., 2004). Many interactions between domestic exotics and wild native species are due to competition for resources, and may lead to extinction or endangering of the natives (Bryan *et al*., 2005).

Hybridization between animal species is relatively frequent in the wild (Mallet, 2005). Although hybrids usually exhibit low fitness due to sterility or reduced fertility (Garcia-Vazquez *et al*., 2004), many factors have been identified for maintaining relatively high hybridization rates in natural populations, from alternative mating strategies (Garcia-Vazquez *et al*., 2001; Redenbach and Taylor, 2003) to secondary contact between formerly isolated species that may contribute to break reproductive barriers and lead to introgressive hybridization (Verspoor and Hammar, 1991; Kinziger and Raesly, 2001). In aquaculture stocks, deliberate interspecific hybridization is carried out for many fish and shellfish (Hulata, 2001). Inter-specific hybrid fishes have been produced for aquaculture and stocking programmes (Bartley *et al*., 2000). Some of their many applications are to increase growth rate, transfer desirable traits between species, combine desirable traits of two species into a single group of fishes, reduce unwanted reproduction through production of sterile fish or mono-sex offspring, take advantage of sexual dimorphism, increase harvestability, increase environmental tolerances, and increase overall hardiness in culture conditions. Experimentation with new hybrid fishes is ongoing, especially in marine culture systems where sterile fish may be preferred because of the concern that fish may escape into the marine and coastal environment.

Despite widespread use of interspecific hybridization in aquaculture, its impact on wild populations has rarely been addressed. However, domestic releases may promote (or increase) interspecific hybridization between native and foreign stocks. As hybrids are fertile in many species (Argue and Dunham, 1999), introgression may occur as a further consequence of interaction between domestic stocks and native populations. Introgressive hybridization can lead to species extinction (Rhymer and Simberloff, 1996; Epifanio and Nielsen, 2000). Thus if hybridization increases as a consequence of aquaculture escapes, it can be a major threat for native populations. In this paper we review some relevant cases where interspecific hybridization has been reported as a consequence of interaction between domestic stocks and native populations, and explore the need of considering this aspect when evaluating aquaculture impacts.

## Two fish examples: carps and Salmonids

In fish, hybridization and introgression rates may increase as a consequence of deliberate or accidental (escapes) releases of domestic individuals in the wild, thus intermingling stocks that were previously isolated and subject to different selective pressures (Berrebi *et al*., 2000; Allendorf *et al*., 2001; Docker *et al*., 2003).

In carps, a typical freshwater genus, introgressive hybridization between native crucian carp (*Carassius carassius*) and introduced goldfish (*C. auratus*) and common carp in the U.K. has been demonstrated employing microsatellite DNA markers (Hänfling *et al*., 2005). These authors suggest that release or escape of closely related exotic cyprinids poses a problem to the genetic integrity and associated local adaptation of native species, and may also contribute to shifts in community structure through competitive interactions.

Interspecific hybridization derived from escapes or aquaculture releases has been known for long in species, like Salmonids, inhabiting both freshwater and marine habitats. Increased rate of hybridization between Atlantic salmon and brown trout in different northern countries (Scotland, Norway, Ireland) showed associations with the presence of escaped farm salmon (Youngson *et al*., 1993; Hindar and Balstad, 1994). In fact interspecific hybridization is one of the risks attributed to escaped salmon from aquaculture (Naylor *et al*., 2005).

Domestic individuals of both sexes can be involved in heterospecific crosses. Domestic salmon females tend to mate with wild brown trout males, increasing first-generation hybridization rates (Matthews *et al*., 2000). Recent findings in Spain (Figure 1) demonstrate that not only hybridization but also interspecific introgression occurred with the introduction of domestic genomes into wild populations of sympatric Atlantic salmon *Salmo salar* and brown trout *Salmo trutta*. In this case, hybridization involving principally brown trout of domestic origin was deduced from the genetic composition of pure species, first-generation and second-generation hybrid individuals (Table 1).

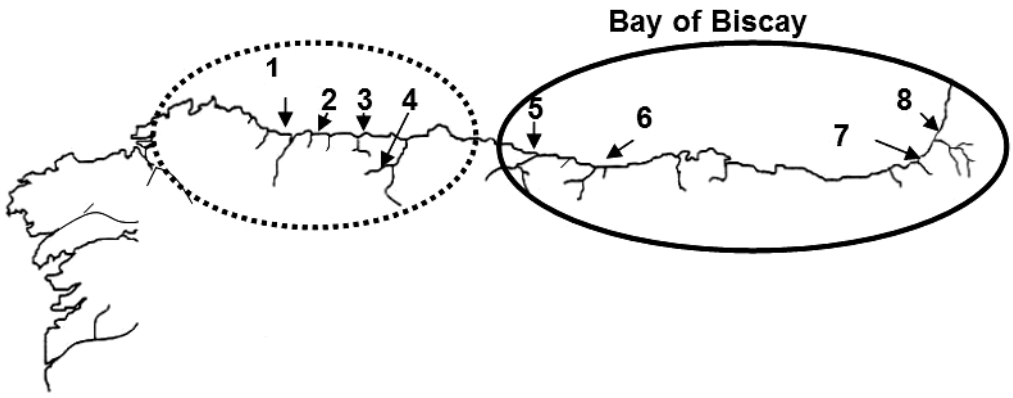


Fig. 1. Map showing populations of Salmonids genetically analyzed in Spain (north Atlantic coast). Dotted circle, 0% interspecific introgression (populations exhibiting 0.5% and 1.7% domestic genomes of brown trout and Atlantic salmon). Solid circle, 2.45% interspecific introgression (populations exhibiting 6.8 % and 29.5% domestic genomes of brown trout and Atlantic salmon respectively).

Table 1. Percent of individuals of domestic and wild origin in pure species individuals and $F_1$ and post-$F_1$ hybrids.

| Species | Domestic brown trout | Domestic Atlantic salmon | Wild stocks |
|---|---|---|---|
| Pure species | 1% | 17.1% | 81.9% |
| F1 hybrids | 11.1% | 0 | 88.9% |
| Post-F1 hybrids | 22.2% | 25% | 52.8% |

## In marine shellfish: widespread hybridization in mussels

Mussels are a heterogeneous group of many genera within the family *Mytilidae*. Some species inhabit freshwater ecosystems; others inhabit marine environments. Many of them are farmed, for example *Aulacomya ater* (ribbed mussel), *Choromytilus chorus* (giant mussel), *Mytilus edulis* (Blue mussel), *Mytilus galloprovincialis* (Mediterranean mussel), *Mytilus trossulus* (foolish mussel), *Mytilus chilensis* (Chilean mussel), *Mytilus californianus* (Californian mussels), *Perna perna* (Brown mussel) and others. Many species have been introduced out of their natural distribution range due to market demands.

All *Mytilus* species exhibit a natural marked ability to generate hybrid individuals when two species are in contact. This has been extensively demonstrated for different species and in different regions (i.e. Daguin *et al*., 2001; O'Mullan *et al*., 2002). Interspecific introgression has also been demonstrated (Bierne *et al*., 2003). Mussels are generally cultivated in open facilities, without barriers stopping the movement of individuals or gamete movement to wild environments. Thus transferring of these species across the world for purposes of mariculture poses a clear threat to the genetic integrity of the native species. Identification of interspecific hybrids around aquaculture sites should be considered a priority. However, the use of genetic tools for species identification is limited to some genera (Inoue *et al*., 1995; Ohresser *et al*., 1997; Toro, 1998; Rego *et al*., 2002; Santaclara *et al*., 2006). Further efforts should be developed to identify individuals and populations in order to monitor introgression of genes from cultivated mussels into natural populations.

## Discussion - Learning from birds

Although there are not many examples of interspecific introgression derived from domestic escapes in mariculture, it is always possible to learn from other taxa. For example, from birds. Native to North America, ruddy ducks *Oxyura jamaicensis* now occur in 21 countries in the western Palaearctic (including Iceland) and their expanding population threatens the native white-headed duck, *Oxyura leucocephala*, through hybridization and possibly competition for food and nest sites (Muñoz-Fuentes *et al*., 2006). Limited genetic diversity of the European ruddy duck population is consistent with a founder population as small as seven birds released from a captive population in the UK, which traces to seven individuals imported from the USA in 1948. Hybrids between the two species are fertile and produce viable offspring in backcrosses with both parental species. Unless effective control of ruddy ducks is continued, genetic introgression will compromise the unique behavioural and ecological adaptations of Spanish white-headed ducks and consequently their survival as a genetically and evolutionary distinct species (Muñoz-Fuentes *et al*., 2007).

## Conclusions

In both fish and shellfish hybridization and introgressive hybridization can be considered as factors of potential impact derived from aquaculture escapes. Although information about actual effects of mariculture on alien domestic gene introgression into wild native populations is scarce, there is a need to include hybridization studies when assessing aquaculture impact.

# Aquaculture escapes: new DNA based monitoring analyses and application on sea bass and sea bream

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## Abstract

The rapid development of marine cage culture has raised concerns about the impact of escaped and/or stocked fish on wild populations. Much of the research has involved salmonid fish, while little is known on the interactions between cultured and wild individuals of sea bass and sea bream, two economically important fish within the Mediterranean. This paper looks into i) opportunities that arise for the monitoring of individuals with new DNA based analyses (mostly focusing on microsatellites), ii) what is currently known on the genetic structure of wild and hatchery populations of the two species, iii) whether if the new statistical approaches can help in the identification of escapees, and iv) future research priorities for successful future monitoring of the two species.

## Introduction

Global decline of wild fin-fish and shell-fish stocks is well documented as well as the parallel increase in aquaculture production, which has more than doubled over the past 15 years. Aquaculture activities now produce more than a quarter of all fish/shellfish directly consumed by humans. However, the effects of farming practices on aquatic resources have been very controversial and the subject of intense debate. As stated in the Code of Conduct for Responsible Fisheries (FAO, 1995) "… efforts should be undertaken to minimize the harmful effects of introducing non-native stocks used for aquaculture, especially where there is a significant potential for the spread of such non-native stocks into waters; and whenever possible minimize effects of escaped farmed fish on wild stocks". Most arguments deal with the potential negative genetic impacts of escaped farm-fish/shellfish and/or deliberate introductions of farmed and non-native organisms into wild stocks (Bonhomme, this volume). Caged fish can escape and therefore interbreed with wild conspecifics in the natural environment (Sanchez Jerez *et al*., this volume). In addition to unintentional escapes, deliberate restocking of farm-reared or non-native individuals takes place in an attempt to supplement recruitment in wild populations and increase subsequent harvests (e.g. in the case of gilthead sea bream, Dimitriou, 2000).

There is therefore growing concern that aquaculture may pose a threat to biodiversity due to detrimental impacts on wild populations and on the ecosystem through ecological interactions and interbreeding (Garcia-Vazquez and Moran, this volume; Karakassis, this volume). Escapees and restocked individuals are genetically divergent from wild individuals for a number of reasons. First, they are usually not of local origin, bearing alleles "adapted" to different geographical

conditions. Additionally, the domestication process leads to changes in allele frequencies as a response to selection to the specific culture conditions, and due to stochastic genetic changes (genetic drift). As a consequence, cultured organisms often display poor survival in the wild. Yet, some individuals do survive and interbreed with wild conspecifics. The resulting hybrids may also display reduced survival and reproduction success, thereby lowering the overall fitness of the wild population (McGinnity *et al.,* 2003). With continued releases, weakened populations may enter an extinction vortex. Other factors that should be taken into consideration are disease interactions between cultured and wild fish (Diamant, this volume) as well as the EC regulation 104/2000 which requires traceability and geographical labelling of fish products. Therefore the right of the consumers to know the origin of the fish they eat both at species and geographical level is now required.

## DNA-BASED DETECTION ANALYSES OF ESCAPEES

Molecular markers are increasingly employed to monitor genetic variation in domestic stocks and for identifying domestic individuals in the wild (Liu and Cordes, 2004). Molecular markers are permanent markers and since most of them are nowadays DNA-Polymerase Chain Reaction (PCR) based, tissue sampling is non-invasive, not requiring animal sacrifice. Additionally, their main advantage as opposed to morphological markers is that they are inheritable, enabling even the identification of the offspring of aquaculture individuals interbreeding with wild populations and therefore are traceable into further generations. The main genetic markers that have been used are allozymes, mitochondrial DNA and microsatellite DNA. Future markers include coding DNA variation based on single nucleotide polymorphisms (SNPs) and / or using DNA microarrays (Schlötterer, 2004).

Of the above markers, microsatellites are undoubtedly the most popular genetic markers nowadays (Schlötterer, 2004). Microsatellites or simple sequence repeats (SSRs) are tandemly repeated motifs of 1-6 bases, abundantly distributed within genomes. They are characterized by a high degree of length polymorphism, and thanks to the advent of polymerase chain reaction (PCR) technology, products of different length can be easily amplified with primers flanking the variable microsatellite region. Recently, due to the availability of high-throughput capillary sequencers or mass spectrometry the sizing of alleles has also become a very fast process. Using a panel of several microsatellite loci, a unique combined SSR genotype profile can be produced for each individual tested. The genotype profile is highly discriminating, which suggests that a random individual would have a low probability of matching a given genotype. In the field of fisheries and aquaculture, microsatellites are useful for the characterization of genetic stocks, for paternity and relatedness analysis of wild populations, hatchery broodstock selection, constructing dense linkage maps, and mapping economically important quantitative traits (Liu and Cordes, 2004; Chistiakov *et al.,* 2006).

The most important advance in recent years in the statistical analyses of molecular markers in general, but with most applications so far in microsatellites has been the development of powerful analytical methods/statistical programmes, which has enabled analyses to shift from populations to individuals. It is now possible to assign or exclude individuals originating from a claimed population, with applications in the identification of the genetic origin of specific individuals (Primmer *et al.,* 2000), the identification of immigrants (Paetkau *et al.,* 2004), the occurrence of hybridization or admixture (Choisy *et al.,* 2004), the success of stock enhancement programmes (Bravington and Ward, 2004) and, most importantly, the assessment of introgression of hatchery individuals into wild populations (Hauser *et al.,* 2006; Renshaw *et al.,* 2006).

These new individual-centred programs aim to detect the "strange" individuals, using the fact that these individuals will present different multilocus genotypes than expected for native individuals (Excoffier and Heckel, 2006). Some of them attempt to allocate individuals to predefined populations (classification problems, e.g. GeneClass2, Piry *et al.,* 2004), others to 'virtual' populations, for which allele frequencies are also iteratively estimated (clustering problems, e.g. STRUCTURE, Pritchard *et al.,* 2000). Assignment of the individuals to predefined samples is usually done with three methods: using a Bayesian-based approach, based on reference population allele frequencies and on genetic distance (see Manel *et al.,* 2005, for more information). However,

current programs have been developed under a restrictive set of assumptions concerning mutation and demographic models. Approximate Bayesian methods, that have been recently developed, deal with more complex models. More work is still needed to assess the statistical properties of these models (Excoffier and Heckel, 2006).

## THE CASE OF SEA BASS AND SEA BREAM

While much of the research on the genetic impacts of farm escapes and restocking has involved salmonid fish, especially Atlantic salmon and brown trout, there is also a need to assess genetic impact on wild fish populations within the Mediterranean Sea (Youngson *et al.,* 2001). The European sea bass (*Dicentrarchus labrax*) and the gilthead sea bream (*Sparus aurata*) are two of (if not) the most important species in Mediterranean fish culture, with a yearly production of more than 100,000 metric tonnes per species, the biggest percentage of which are cultured in floating sea cages, in nearshore locations. The rapid development of marine cage culture has raised concerns in the Mediterranean about the impact of escaped fish on wild populations.

### Sea bass

The European (or common) sea bass, *Dicentrarchus labrax* L. (Moronidae, Perciformes) is found in coastal waters of the eastern North Atlantic Ocean from southern Norway to Morocco and throughout the Mediterranean Sea and the Black Sea. It is a fish with high commercial value both from capture from wild stocks, and in the last 25 years from aquaculture production (Haffray *et al.,* 2006).

The genetic structure of European sea bass populations has been extensively studied using numerous molecular markers including allozymes (Allegrucci *et al.,* 1997; Sola *et al.,* 1998; Lemaire *et al.,* 2000), mitochondrial DNA (Allegrucci *et al.,* 1999; Lemaire *et al.,* 2005) and microsatellites (Garcia de Leon *et al.,* 1997; Naciri *et al.,* 1999; Bahri-Sfar *et al.,* 2000, 2005; Lemaire *et al.,* 2000; Castillo and Ciftci, 2005; Katsares *et al.,* 2005). It is obvious from these genetic studies that the European sea bass consists of well-defined stocks throughout its distribution range. Sea bass populations group into three genetically distinct areas: the north-eastern Atlantic Ocean including the Sea of Alboran, the western Mediterranean and the eastern Mediterranean Seas. Subtle genetic structure has been only found within the eastern Mediterranean stock, which is consistent with the subdivision of the region into several basins, e.g. the Adriatic, Ionian and Aegean Seas, the Libyco-Tunisian Gulf and the Levantine basin (Bahri-Sfar *et al.,* 2000; Castilho and Ciftci, 2005).

Microsatellite markers have been able to detect cases where (mostly Eastern Mediterranean) population samples did not cluster according to their geographic origin, but with western Mediterranean samples. This is not surprising, since many hatcheries around the Mediterranean, used broodstock, eggs or fingerlings originating from the western basin, when sea bass aquaculture began (Haffray *et al.,* 2006). Therefore, it is evident that some wild stocks may have already been affected by escapees (Bahri-Sfar *et al.,* 2005).

The use of the new individual based analyses should therefore succeed in genetically identifying immigrants and/or escapees in cases where hatchery broodstock was transferred between the three main geographic areas, as evident from the works of Bahri-Sfar *et al.* (2000, 2005). Some success is also probable for populations/individuals within the Eastern Mediterranean area (Castilho and Ciftci, 2005). However, there is still a lot of information missing on the genetic structure of sea bass populations. Future work should include the genetic analysis of additional wild populations, mainly from the eastern Mediterranean and the limit of the Atlantic distribution of the species (Morocco to Norway). Little information also exists on the origin of hatchery broodstock. Nowadays, many hatcheries use local individuals for broodstock, though in the past it has been reported that Western Mediterranean breeders were used throughout sea bass hatcheries. Since the application of selective breeding in captive broodstocks is recent, it can be assumed that the genetic differentiation between wild and farmed populations will be limited, which would hinder identification of escapees with new DNA based analyses. However, recent results (Triantafyllidis *et al.,* unpublished) show that at least in some Greek areas, wild populations are well differentiated from local hatchery populations.

It should be noted that mitochondrial and allozyme loci have also proven to be very informative as regards monitoring of *D. labrax* stocks and can help in the identification of hatchery escapees in some cases. Mitochodrial analyses clearly differentiate Atlantic from Mediterranean stocks (Lemaire *et al.,* 2005). A survey of Greek *D. labrax* individuals (Triantafyllidis *et al.,* unpublished) has shown no evidence of escapees or of progeny of Atlantic origin breeders within Greek waters. Additionally, some allozyme loci in *D. labrax* seem to be under some sort of selection shaped by adaptation in different environments (Lemaire *et al.,* 2000). When data from microsatellite and allozyme markers in Mediterranean lagoon and marine populations were compared, there was evidence that some allozyme loci seemed to be implicated in the differentiation between marine and lagoon samples.

**Sea bream**

The gilthead sea bream *Sparus aurata*, is found in the Mediterranean and the Black Sea (rare), and in the Eastern Atlantic, from the British Isles, Strait of Gibraltar to Cape Verde and around the Canary Islands. This is also a fish with high commercial value (Sola *et al.,* 2006).

Molecular markers that have been used to study gilthead sea bream include allozymes (Alarcon *et al.,* 2004; Ben Slimen *et al.,* 2004; Rossi *et al.,* 2006), AFLP (Miggiano *et al.,* 2005), mitochondrial DNA (Alarcon *et al.,* 2004; Funkenstein *et al.,* 1990; Magoulas *et al.,* 1995) and microsatellite loci (Alarcon *et al.,* 2004; De Innocentiis *et al.,* 2004, 2005; Miggiano *et al.,* 2005; Karaiskou *et al.,* 2005; Triantafyllidis *et al.,* 2006). Nevertheless, the picture on the genetic structure of the species is still not clear. In the most widespread study of sea bream populations to date, Alarcon *et al.* (2004) discovered a slight degree of differentiation (mostly with microsatellites) but this was not associated with geographic or oceanographic factors. Additional studies (De Innocentiis *et al.,* 2004; Rossi *et al.,* 2006) have also revealed some genetic differentiation among populations, though this again does not seem to follow some geographic scheme. It seems that, though some main assemblages can be identified, the pattern of population subdivision is not correlated to an isolation-by-distance model.

In this species considerable work has been done on the genetic differentiation of wild from hatchery populations. Hatchery populations seem to be differentiated from local populations (Alarcon *et al.,* 2004; Karaiskou *et al.,* 2005; Triantafyllidis *et al.,* 2006) indicating low genetic flow between them. Additionally, geographic assignment of breeders has revealed a mixed and highly heterogeneous origin of broodstocks, with a high percentage of Atlantic individuals among breeders (De Innocentiis *et al.,* 2005). More importantly a simulation study (Miggiano *et al.,* 2005) demonstrated that molecular tags (AFLP, microsatellites) allow the identification of hatchery escapees with the prerequisite that sampling of hatchery broodstocks and the basal wild populations is available.

The above results seem a little contradictory, since despite absence of clear genetic structuring of wild *S. aurata* populations, DNA-based genetic assignment of individuals has been quite successful. However, a lot of information is lacking on the genetic structure of sea bream populations. Future work is urgent to cover the whole geographical range of the species and to fill the sampling gaps. At the same time it is important to create a database of the genetic variability of hatchery broodstocks in main hatcheries. At least for sea bream it seems that broodstock of Atlantic origin are highly appreciated (De Innocentiis *et al.,* 2004), however their genetic identification is not as straightforward (like in sea bass) since there does not seem to be high genetic differentiation at mitochondrial DNA level (Alarcon *et al.,* 2004; Triantafyllidis *et al.,* unpublished). Genetic tagging of sea bream broodstocks might be a suitable tool to monitor the genetic impact of fish farm escapes/releases. In this way it would also be possible to trace, record and quantify ”gene flow” in the industry through the exchange of fry and broodstock among producing countries.

## CONCLUSIONS

Without a doubt microsatellites are the most promising markers for detecting escapes of farmed sea bass and sea bream individuals in the wild or for monitoring stocking success. The success of these efforts depends on the existence of baseline populations to which the different individuals

assayed will be assigned, especially in the case of sea bream which presents low genetic differentiation among populations; a lot of this information is still missing on the genetic makeup of wild and hatchery populations of both species. It should be kept in mind nevertheless that even the use of numerous, highly polymorphic loci cannot guarantee success. The big genomic programmes in progress for both species (AQUAFIRST, BASSMAP, BRIDGEMAP, MARINE GENOMICS EUROPE, WEALTH) should identify the genes of domestication, i.e. the changes in the genetic architecture of wild populations when brought under farming practices. This should allow in the future the identification of the true functional differences of wild to farmed individuals and should therefore facilitate the identification of farmed escapees based on the genes that matter with microarray technology (Roberge *et al*., 2006) and not only with supposedly neutral markers.

**Acknowledgements**

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# Implications of infauna harvesting at inter-tidal flats on nutrient cycling, Ria Formosa - Portugal

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## Abstract

Clam harvesting causes physical disturbance of sediments, modifying sediment-water exchanges of nutrients. To assess this alteration overlying water was collected above the inter-tidal sediments during the first 60 min of tidal inundation. Ammonium and phosphate in water overlying undisturbed sand and mud flats showed concentration peaks with the inundation, being more accentuated in re-worked sediments. Otherwise, a clear retention of phosphorous was observed in re-working mud. The estimation of advective fluxes points to additional transport of ammonium and phosphate to the water column when permeable sediments are re-worked for clam harvesting. In less permeable substrates opposite signals were found, since ammonium is intensely exported and phosphorus highly retained in sediments. The fluxes with opposite directions were examined as N/P ratio, showing low values (<3) except in re-worked mud (N/P=450). This means an excess of nitrogen transported to the water column eventually modifying the ecosystem ecology.

## Introduction

Harvesting of marine invertebrates from inter-tidal areas is widespread as commercial and subsistence activities (Kaiser and De Groot, 2000). Bivalve molluscs are harvested mostly for human consumption (Ferns, *et al*., 2000; Lenihan and Micheli, 2000), while other benthic organisms, such as polychaetes and sipunculids, are collected as bait for fishing (Beukema, 1995; Kaiser, *et al*., 1996). The physical disturbance of sediment enhances by harvesting causes the mixing of freshly deposited organic matter and reduced compounds (Aller and Yingst, 1985; Boudreau, 1984; Hall and Harding, 1997), induces the oxygenation in the re-worked layers, modifies the microphytobenthos distribution (De Jonge and Van der Bergs, 1987), and causes the destruction of *Zostera noltii* colonies and of macro/meiofauna burrows (Aller, 1982). Harvesting occurs mainly in shallow tidal-driven ecosystems with extensive areas of inter-tidal sediments. The excursion of tidal water over inter-tidal sediment induces ammonium export to the water column (Falcão and Vale, 1995; Rocha, 1997) and supplies oxygen to deeper layers (Huettel, *et al*., 1998; Kerner and Wallmann, 1992), which oxidises pore water Fe(II) and Mn(II) in a minute time scale (Caetano, *et al*., 1997) and retains phosphate (Slomp *et al*., 1998).

Ria Formosa is a shallow meso-tidal coastal lagoon located in the south of Portugal, with a wet area of 10,500 ha including several channels and an extensive inter-tidal area constituted by salt marshes (3,500 ha), muddy flats colonized by *Z. noltii* (2,500 ha), and 1,000 ha of sandy sediments. It is estimated that sediments supply most of the daily N and P requirements of phytoplankton (Falcão and Vale, 1998), driven by the molecular diffusion and advection created by the daily flooding

water over extensive inter-tidal sediments (Caetano *et al*., 1997; Falcão *et al*., 2005). Approximately 800 ha of the inter-tidal area are used for farming the clam *Ruditapes decussatus*. At the present time, the biomass of cultivated clams varies from 1 to 2 Kg $m^{-2}$ and the annual production reaches 5,000 ton per year. The sediments are re-worked by fishermen to collect clams on a daily to weekly basis (Figure 1), creating the opportunity to examine the effect of sediment re-working on the nutrient cycling. This paper presents field data evidencing the changes of advective fluxes of nutrients associated with the infauna harvesting, and examines the effects in terms of sustainable management of the ecosystem.

Fig. 1. A view of clam harvesting on the inter-tidal flats of Ria Formosa, Portugal.

## Material and methods

Two inter-tidal zones were selected in Ria Formosa: a natural muddy zone covered by *Z. noltii* and a sandy clam-rearing zone. Within each selected zone two areas of 20 $m^2$ were delimited, one was completely re-worked using an artisanal fishing gear (harvesting knife), which penetrates 10 cm into the sediment, while the other was left undisturbed. Overlying water was collected 2 cm above the sediment surface directly with pre-cleaned syringes at both sites in February/March 2001 over a short time interval during flooding: at 2, 5, 10, 15, 20, 30 and 60 min. The water was filtered through 0.45 µm Millipore filters and stored at 4 ºC until analysis. Ammonium ($NH_4^+$) and phosphate ($HPO_4^{2-}$) were determined using a "SKALAR" autoanalyser (Grasshoff, 1983). The detection limits were 0.2 µM for ammonium and 0.05 µM for phosphate.

The advective flux (F) of phosphate and ammonium from sediment to overlying water was calculated by the expression $F = \Sigma(|C_{t+1} - C_t|)(h_{t+1} - h_t)/2$, where $C_{t+1}$ and $C_t$ are the concentration of $HPO_4^{2-}$ or $NH_4^+$ in the flooding water at times t+1 and t and $h_{t+1}$ and $h_t$ are the water depth at the same times (Caetano *et al*., 1997). It was observed that during field measurements, water depth in the inter-tidal areas increased in average 1 cm per minute of inundation. The transport of $HPO_4^{2-}$ and $NH_4^+$ was calculated for the first 60 min of inundation. Since inter-tidal sediments are inundated twice a day, the daily advective flux was obtained multiplying F by a factor of 2.

## Results and discussion

### Nutrient concentrations

Figure 2 shows the ammonium and phosphate concentrations in overlying water during the first 60 min of tidal inundation. Undisturbed and re-worked sandy and muddy inter-tidal areas were considered in this study. In the first two minutes of inundation, levels of ammonium in water overlying sandy and mud flats were elevated (1.5±0.5 µM and 0.9±0.1 µM, respectively) decreasing to almost constant concentrations after 60 minutes (0.4 µM). However, the concentrations in re-worked sediments exceeded the values registered in the undisturbed sediments (maximum of 2.6±0.5 µM in sand and 8.2±1.2 µM in mud). The increases indicate an additional export of ammonium from re-worked sediment pore waters to the water column. These results

follow previous works that showed an export of ammonium from undisturbed inter-tidal sediments (Falcão and Vale, 1995; Caetano *et al*., 1997). A different variation was found for phosphate: water overlying sandy sediments showed similar levels at undisturbed (0.65-0.31 µM) and re-worked (0.64-0.24 µM) conditions, while phosphate concentrations over undisturbed mud flats (0.59-0.07 µM) exceeded the values at re-worked mud (0.05-0.07 µM).

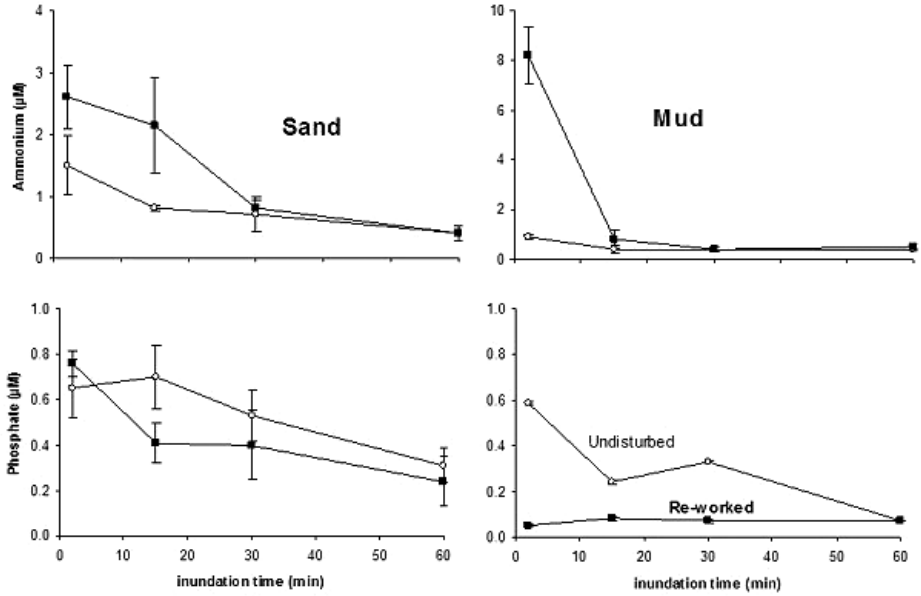


Fig. 2. Time-course variation of ammonium and phosphate concentrations (µM) in water overlying undisturbed and re-worked sands and muddy sediments at Ria Formosa, during the first 60 minutes of tidal inundation.

## Advective fluxes

On the basis of the measured concentrations of ammonium and phosphate in overlying water, the advective fluxes associated with the tidal inundation over undisturbed and re-worked sediments were calculated (Figure 3). The obtained fluxes show that re-working more permeable sediments implies an additional transport of ammonium and phosphate to the water column. In less permeable substrates opposite signals were found, since ammonium is intensely exported and phosphorus

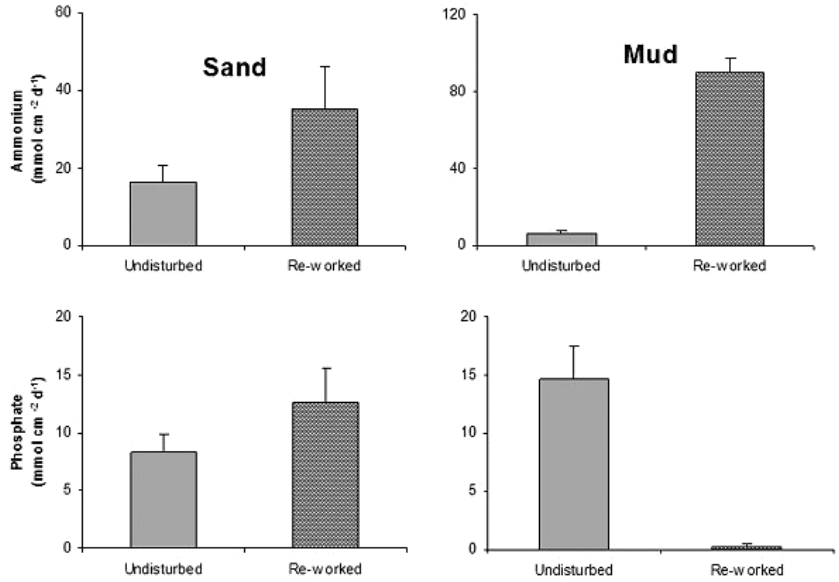


Fig. 3. Calculated advective fluxes of ammonium and phosphate from undisturbed and re-worked sands and muddy sediments at Ria Formosa to the water column, during the first 60 minutes of tidal inundation.

highly retained in sediments (almost null flux). Therefore, re-working muddy areas increases the sediment P-buffering capacity due to adsorption onto iron oxides (Krom and Berner, 1980; Sundby *et al*., 1992; Slomp *et al*., 1998). Similar findings were observed when the seabed was disturbed by mechanical clam dredging (Falcão and Vale, 2003).

### N/P ratio

The contrasting advective fluxes may be examined in terms of N/P molar ratio. This ratio was calculated dividing the amounts of ammonium and phosphate transported to the water column. In undisturbed and re-worked sands the N/P ratio was lower than 3, clearly below the Redfield ratio (N/P=16), which indicates a deficit of nitrogen. A similar picture was observed for undisturbed mud (N/P=0.4), but in re-worked mud, the ratio reached as much as 450, meaning an excess of nitrogen transported to the water by advective fluxes.

## Concluding remarks

These results illustrate the effect of harvesting clams produced at inter-tidal areas on the nutrient cycling, and therefore on the ecosystem ecology. The magnitude of this effect is greatly dependent on the extent of this type of aquaculture and the ecological status of the water body.

# Ecological relationship between wild fish populations and Mediterranean aquaculture in floating fish cages

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## Abstract

Floating fish farms are associated, with important aggregations of wild fish around them. Several studies have shown that there is an important aggregation around all of the Mediterranean farms throughout. The most abundant families are clupeids, sparids, carangids, mugillids and pomatomids, although the dominant species varied markedly among farms and seasons. This important aggregation affects the behaviour and physiology of the local ichthyofauna, and may have important consequences for wild fish populations and local fisheries on a regional scale. Aggregated fish change diet, using aquafeed as main resource, which increases the biological condition but also affects the fatty acid composition, increasing vegetal fatty acids. Floating cages also aggregate post-larvae and juveniles of several pelagic and benthic species which find protection around the floating structures. Wild fish reduce environmental impact on the benthic communities by feeding on the lost food pellets. In addition, protection of this aggregation around Mediterranean fish farms can promote biomass exportation and enhance local fisheries. Therefore, the aggregation of wild fish around fish farms is an important aspect to take into account for coastal managers.

## Introduction: fish cages work as 'mega' FADs

Fish are attracted towards a high variety of natural and artificial objects which stimulate the formation of aggregations (Dempster and Taquet, 2004). These objects are defined generally as Fish Aggregation Devices (FADs). This behaviour occurs throughout the different developmental stages of fish, from larvae to adults. One of the most important artificial structures in Mediterranean pelagic systems are floating fish farms, which attract great numbers of wild fish. The phenomenon is widespread and large aggregations around fish farms have been described across the Mediterranean Sea (Dempster *et al*., 2002; Thetmeyer *et al*., 2003; Machias *et al*., 2004).

The effect of attraction seems to be higher around farms than around traditional FADs due to the availability of food, with up to 2,800 times more wild fish in their immediate vicinity than in areas without farms (Dempster *et al*., 2002). A single sea-cage farm covering a sea surface area of just one hectare may have up to 40 tons of wild fish beneath it. Comparison of fish assemblages before and after fish farms deployment in Greece showed that the overall abundance of the fish assemblage increased by a factor of four and the average trophic level of the fish community increased from 3.59 to 3.79 (Machias *et al*., 2004).

In the Mediterranean, the production of the two main species farmed reaches over 140,000 t $yr^{-1}$ and due to the increasing demand of cultured species, production will continue to increase significantly in the future. Therefore, ecological effects of mariculture on wild fish which aggregate around fish farms in coastal areas may become a global concern.

## Fish assemblages associated with Mediterranean fish farms

In a study carried out around nine locations in autumn 2001 in SE Spain (Dempster *et al*., 2002), fish farms had greater abundance (52 to 2,837×), biomass (2.8 to 1,126×) and number of fish species (1.6 to 14×) than control counts at all locations. During the study, 28 species belonging to 14 families were recorded. Two families, Carangidae (four species) and Sparidae (12 species), were the most represented. Only fourteen species occurred at fish farms, 13 species were seen in both farm and control counts, and 1 species (*Mola mola*, one individual) was seen at one control location only. *Sardinella aurita* was the most common species observed in the control counts, although the number per count (20 to 200 individuals) was far less than at fish farm locations. Furthermore, the fish assemblage has a marked seasonal variability (Figure 1), mainly dominated by migrations of some species such as *Boops boops*, *Sardinella aurita* and *Trachurus mediterraneus* (Valle *et al*., 2007).

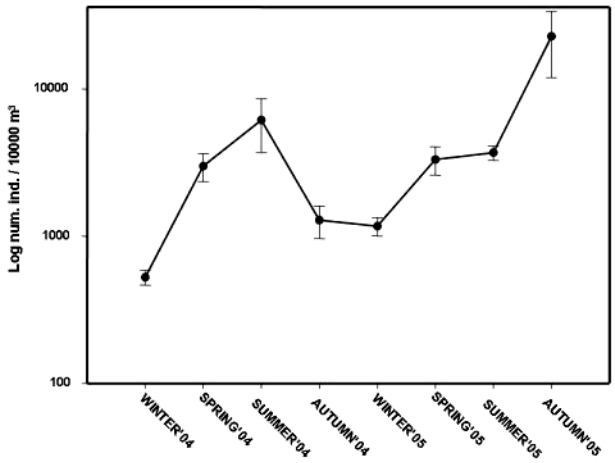


Fig. 1. Seasonal pattern of fish abundance around three fish farm on SE Spain. Each value corresponds with the mean of six visual counts at three fish farm, carried out two random times per season.

## Fish larvae attraction to fish farms

The vast majority of demersal teleost fishes have a pelagic larval stage which has major implications for the dynamics of fish populations and fisheries management. Fish farms also aggregate fish larvae around their floating structures, mainly in the upper 2 m of the water column. A survey carried out during 2006 (Fernandez-Jover *et al*., unpublished data) showed that there is a permanent recruitment of post-larvae (individual smaller than 1 cm), mainly belonging to the Sparidae, Mullidae and Atherinidae families. Around a single cage of 12 m diameter *Boops boops* larvae could number several hundreds and up to 1,800 in December 2006 (Figure 2).

The influence of fish cages on the pelagic larval stage could affect the connectivity between larval and adult populations, through a spatial modification of the habitat and altered mortality, due to high aggregations of predatory adult fishes during this pelagic stage.

## Changes in diet and physiological effects

Many fish species which aggregate around coastal sea-cage fish farms use food pellets as food, which alter the natural diet. Changes in diet can in turn affect the condition index. Fernandez-Jover *et al*. (2007a) demonstrated that Mediterranean horse mackerel (*Trachurus mediterraneus*) captured at fish farms had a significantly higher condition index than its non-associated counterparts due to a diet based on the lost food pellets. Wild fish that fed around the cages had a

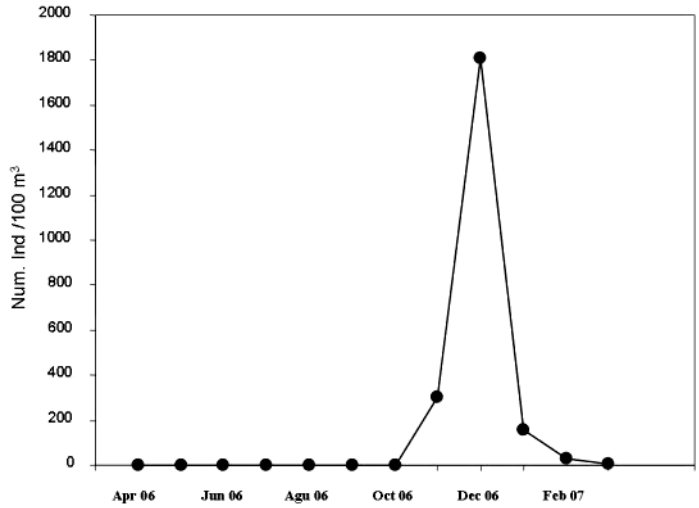


Fig. 2. Temporal trends of *Boops boops larvae* abundance (< 3 cm) around a single farm at SE Spain (El Campello, Alicante).

significantly higher body fat content than the control fish (7.30±1.8% and 2.36±0.7% respectively). This change in body condition due to the extra feeding may lead to changes in their reproductive capacity.

The composition of the food pellets affects the fatty composition of wild fish. Food is composed of fish protein, but also of vegetable-derived proteins and fats. The fatty acid composition differed between farm-associated and control fish, principally because of the significantly higher levels of linoleic (C18:2ω6) and oleic (C18:1ω9) acids (vegetable-derived fatty acids) and lower levels of docosahexaenoic acid (C22:6ω3) in farm-associated fish. Because of the changes in fatty acids, the concentration of ω3 is cut down when wild fish feed on food pellets thus affecting to the taste for human consumers (Skog *et al*., 2003). Furthermore the fatty acids compositions could also serve as biomarkers to infer the influence of a fish farm on the local fish community.

## Reduction of benthic impact by reduction of lost food

Some studies have detected the effect of aggregated wild fish in reducing the impact on the benthos. Aggregated wild fish reduced the sedimentation of the total organic wastes at one Mediterranean farm by up to 80% (Vita *et al*., 2004b). There are models that include wild fish as removers of feed wastes (<http://www.meramed.com>) but they are only considered as a sink of organic matter. Wild fish that aggregate around fish farms and feed on the lost food pellets influence the environmental impact by excreting nitrogen and carbon to the water column, thereby reducing the input to the benthos. Experimentally, it has been demonstrated that there is an important input of $NH4^+$ and DOC from the faeces of wild fish to the pelagic system, which occurs very quickly in the first minutes (Fernandez-Jover *et al*., 2007b). This reduces the quantity of organic matter that reaches the seafloor because uneaten food pellets start leaching nutrients as soon as they contact water; however, they are rapidly eaten by wild fish in a high proportion.

Based on the conceptual model for nutrient mass budget of Islam (2005), we can estimate the influence of wild fish on the total amount of nitrogen lost to the environment. Using a Food Conversion Ratio of 1.79 for sea bream (Lupatsch and Kissil, 1998), without the influence of wild fish, 22.6 kg of nitrogen would sediment (at 25 °C) for one ton of sea bream production, including both feed and faeces nitrogen. Therefore, there is a severe impact on the surrounding benthic communities. However, if we consider a scenario where wild fish consumed 80% of lost food pellets (Vita *et al*., 2004b) and apply the results on faeces leaching rates (Fernandez-Jover *et al*., 2007b; assuming that all the aggregation is composed of *T. mediterraneus*, only 0.28 kg of nitrogen will reach the sediment. This load of nutrients could be dispersed or assimilated by the pelagic communities and therefore reduce the impact on the benthic environment around fish farms.

### NEGATIVE INTERACTION: PREDATION OF *POMATOMUS SALTATRIX* ON CULTURED FISH

The bluefish, *Pomatomus saltatrix*, is an abundant marine piscivorous fish that occurs in all oceans except the eastern Pacific, from oceanic to coastal environments. Bluefish have been shown to be important piscivores, as their diet consists of a variety of fish species of commercial and recreational importance (Buckel *et al*., 1999). Adults are in loose groups, often attacking shoals of mullets or other fishes and destroying numbers apparently far in excess of feeding requirements. Permanent aggregations of bluefish occur around several fish farms in SE Spain, with abundances of 1,000s of individuals (Fernandez-Jover *et al*., unpublished data). Gut contents analysis showed a predominance of *Sardinella aurita*, which are very abundant around fish farms in the diet of the bluefish which also feeds on benthic species such as *Serranus cabrilla* and *Mullus surmuletus*. One of the fish farms studied suffered entries of bluefish from several individuals up to 400 tons. Entry into sea-cages very negatively affects culture conditions because of the aggressive behaviour of *P. saltatrix*. Seabream stop eating and mortality increases dramatically due to predation. Further, it has been detected that escapes increase during such episodes, as the sea-bream more actively seek to escape to avoid predation and do so through the entry holes made by the bluefish.

### BENEFIT OF LOCAL FISHERIES BY PROTECTION OF AGGREGATED POPULATIONS

Wild fish that gather at farms tend to be large adults (Dempster *et al*., 2002). This is important as the 'big ones' do most of the spawning and produce the next generation. The constant supply of high protein food when feed is lost through the cages also means that these big fish are in better body condition than their wild counterparts elsewhere in the sea. Better conditions increase the spawning success of fish. Higher-order predators, such as large pelagic fish, rays and dolphins, are also present at farms to feed on the aggregated wild fish (Dempster *et al*., 2002, 2006; Boyra *et al*., 2004a).

Many of the fish species that occur at farms in high numbers are commercially important to coastal fisheries and are already subject to heavy fishing pressure. Fish farms produce an apparently positive effect for local fisheries (Machias *et al*., 2006). If restrictions on fishing are applied within farm leasehold areas, it has been suggested that coastal sea-cage fish farms may act as small (up to 160,000 $m^2$) pelagic no-take areas (Dempster *et al*., 2002). Groups of fish were not seen more than 50 m away from cages at any farm. This result is analogous to the association of reef fishes with artificial reefs, where a steep decline in abundance is typically observed at distances of just a few meters from the artificial structure. Therefore, wild fish aggregation around fish farms could have positive effects for local fisheries, mitigating partly the negative impact of this activity on coastal ecosystems (Dempster *et al*., 2006).

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# Sustainable aquaculture in the Mediterranean Sea: are we moving in the right direction?

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## Abstract

In 2005, FAO reported that over 1 billion people are dependent upon fish as a source of quality protein in their diet, particularly in developing countries where food supplies are inadequate. Since capture fisheries are reaching their maximum biological limits with over 80% of the world fisheries being overfished (FAO, 2004a), the world looks at aquaculture as a way to secure fish protein supply and relieve pressure on wild catch fisheries. However, intensification of aquaculture practices has revealed a spectrum of environmental problems in nearby natural ecosystems. Nowadays aquaculture is considered as a potential aquatic environment polluter and in some cases is described as an unsustainable practice (i.e., tuna fattening). Therefore, aquaculture has attracted attention of diverse societal groups, governmental authorities and non-governmental sectors in order to re-address the sector production towards a more responsible and sustainable process.

In the Mediterranean basin there is a long tradition of aquaculture, mainly fish and molluscs, based in coastal areas. Currently a large number of species are cultured, either in land-based or sea cages systems, such as seabass (*Dicentrarchus labrax*), seabream (*Sparus aurata*), sharpsnout seabream (*Puntazzo puntazzo*), turbot (*Psetta maxima*), european eel (*Anguilla anguilla*), blackspot seabream (*Pagellus bogaraveo*), red snapper (*Pagellus erythrinus*), pollack (*Pollachius pollachius*), sole (*Solea senegalensis*), meagre (*Argyrosomus regius*), tuna (*Thunnus thynnus*), tilapia (*Oreochromis* sp.), mussels (*Mytilus* sp.), oysters (*Ostrea edulis, Cassostrea* sp.), scallops (*Patinopecten yessoensis*), octopus (*Octopus vulgaris*) and mullets (*Mugil* sp.). Most of the environmental impacts (predation, waste effluents, seagrass degradation, etc.) are related to the location of the farm, but others are related to daily escapes or to aquafeed sources (trash fish, fish meal/oils). Many of these impacts could be avoided with the development of good practices to support responsible and sustainable products from aquaculture, such as those developed in the IUCN Guidelines for Sustainable Mediterranean Aquaculture.

## I- Introduction

Aquaculture currently faces a major challenge: how to alleviate the pressure that fishing fleets exercise on fish populations and the growing demand of sea products in local and international markets without generating environmental problems. Aquaculture is expected to develop widely

in the near future, in the Mediterranean, Southern and Eastern European countries. To avoid potential environmental disruption issues, it is important that the aquaculture sector disposes of clear, user friendly, scientific-based guidelines to ensure its sustainable development.

In the Mediterranean region, aquaculture has rapidly expanded over the last two decades, with an annual growth rate rising from 4% in 1980 to 13% in 2000, and with a trend towards the diversification of cultured species. Although Mediterranean aquaculture production was focused mainly on mollusc farming during the mid 1990s, the share of finfish culture continues to increase, reaching levels three times higher in 1994 than in 2003.

Table 1. Aquaculture in the Mediterranean. Production by species (FAO, 2006a).

| | |
|---|---|
| Mediterranean mussel *(Mytilus galloprovincialis)* | 147,920 MT |
| Gilthead seabream *(Sparus aurata)* | 74,078 MT |
| European seabass *(Dicentrarchus labrax)* | 43,804 MT |
| Flathead grey mullet *(Mugil cephalus)* | 42,546 MT |
| Japanese carpet shell *(Ruditapes philippinarum)* | 25,000 MT |
| Other seabass | 20,982 MT |
| Pacific cupped oyster *(Crassostrea gigas)* | 8,608 MT |
| Other marine fish | 4,894 MT |
| Trout *(Salmonids)* | 1,194 MT |
| Red drum *(Sciaenops ocellatus)* | 438 MT |

The Marine Programme of the World Conservation Union (IUCN) has been promoting best practices in the aquaculture sector, developing "Guidelines for Sustainable Development of Mediterranean Aquaculture".

These will propose recommendations for responsible and sustainable aquaculture in the Mediterranean countries, taking in consideration its specific biologic, oceanographic and social conditions.

The guidelines will be made up of a number of individual guides, each of them addressing the following issues, amongst others: The Interaction between Aquaculture and Environment; Site Selection; Species and Products Diversification; Animal Welfare and Sanitary-Ethic Aspects; Social Aspects; Food Origin and Quality; Market Aspects; Aquaculture Management. These guidelines will give support to decision makers, aquaculture producers and stakeholders in diverse Mediterranean coastal areas. The recommendations included in each of the Guides will allow producers/authorities to grow aquatic organisms in a commercial scale in such a way that will respect as much as possible the natural marine environment.

In this contribution, we shall explain the process and different stages of progress of the IUCN Guidelines, taking as an example the Guide on Interactions between Aquaculture and Environment. This document addresses finfish and shellfish culture, but mainly focuses on finfish aquaculture, and specifically cage culture which is perceived as the most polluting activity. It does not address the interaction with other human activities taking place in the same environment. Neither does it cover fresh water aquaculture, although some examples are taken from this activity. The document is the result of 6 months scientific consultation process as well as three-day workshop held in Las Palmas de Gran Canaria (26-28 October 2006), organized by the University of Las Palmas de Gran Canaria, which gathered scientists, aquaculture producers, and representatives of governmental and environmental organisations.

## II- Environmental interactions

The intensification of aquaculture, mainly finfish cages farming, along Mediterranean coasts generates in various environmental impacts, among them:

**a) Domestication**

Research is carried out to obtain species which are completely acclimatised to captivity, with faster growth rates, and resistance to stress conditions and diseases. Therefore, the process of domestication in the Mediterranean region is at present focused on large numbers of species so as to diversify aquaculture products, and to improve husbandry of current cultured species (Mylonas *et al*., 2004; Agulleiro *et al*., 2006). Part of the research efforts are centered on methods and techniques to produce non-viable varieties of species, in order to make them sterile, unable to survive in wild conditions, and incapable of reproduction and cross-breeding with wild stocks (Brake *et al*., 2004; Omoto *et al*., 2005; Cal *et al*., 2006; Gagnaire *et al*., 2006). Modern genomic technologies can help traditional selective breeding techniques by accelerating the procedures (Howard *et al*., 2004).

**b) Introduced marine species**

According to the CIESM Atlas of Exotic Species in the Mediterranean vol. 1 Fishes and vol. 2 Crustaceans (Galil *et al*., 2002; Golani *et al*., 2002), one fish species (amongst a total of 90 species of introduced fish species) was introduced for aquaculture purposes, the mullet *Mugil soiuy*. This species was introduced primarily from the western Pacific in the Sea of Azov and in the Black Sea, but is still very rare in the Aegean Sea. Among crustaceans, one species of shrimp, *Marsupenaeus japonicus*, escaped from aquaculture facilities in the Western Mediterranean, but is also rare. The same species has been introduced as well via the Suez Canal and is now very abundant, and commercially important for fisheries, in the Levant and southern Turkey. There are also two species of crabs, *Dyspanopeus sayi* and *Rhithropanopeus harrisii* which have been introduced with clam seed and are now common in the brackish waters of the Adriatic Sea where they are abundant and outnumber the autochthonous crabs.

For fish species, aquaculture can be a vector of introduction outside their natural range through escapes (ICES, 2004; Hewitt *et al*., 2006). In this sense, escapes of cultured organisms from aquaculture facilities may interact and harm local wild stocks. Some escapes may occur through normal operational "leakage" where only a few organisms are lost; large-scale escapes can occur following damage to cages caused by storms, vandalism, marine mammals or human error (McGinnity and Ferguson, 2003). When cultured organisms escape or are restocked they may interbreed with wild populations and change their genetic make-up, sometimes decreasing the fitness of wild populations (Hindar, 2001; Youngson *et al*., 2001; McGinnity and Ferguson, 2003). When the number of escapes is higher than that of wild stocks, the native genetic make-up of wild stock can change, altering local populations.

**c) Capture of wild stocks for aquaculture needs**

The wild collection practice of fingerlings is mainly made for species whose wild stock is high enough to cover the required demand without affecting the natural populations, such as the wild spat collection of some molluscs (mussels, oysters, scallops) (Davenport *et al*., 2003). It is also carried out for those species whose life cycles are not yet complete, with no way of accurately reproducing them in captivity. Examples include eels (*Anguilla* spp.), tuna (*Thunnus* spp.), yellowtails (*Seriola* spp.), groupers (*Epinephelus* spp.), octopus (*Octopus* spp.), rabbit fish (*Siganus rivulatus*), species of mullet (Hair *et al*., 2002; Ottolenghi *et al*., 2004).

The dependence on wild populations (larvae, juveniles or adults) as biological material for subsequent on-growing to marketable size, or fattening using captive rearing techniques, is known as capture-based aquaculture. This accounts for about 20% of the total quantity of food fish production through aquaculture – mainly molluscs, though carnivorous finfish are becoming more evident (FAO, 2004b). Nowadays, hatcheries in most countries are capable of producing good quality seeds of marine and freshwater species, gradually diminishing the dependence on wild-caught seed, limited to mature fish for breeding programmes to improve the quality of broodstock (FAO, 2006b).

Research is focused on breeding technologies to close the life cycles of these groups to avoid the dependence of their culture on wild stock populations. Many of such technologies have been achieved in experimental conditions, but have not yet been obtained in commercial conditions –

such breeding technologies are not yet considered effective for mass production, and are not yet cost efficient on a large-scale (Marino *et al*., 2003; Iglesias *et al*., 2004; Mylonas *et al*., 2004; García-Gomez *et al*., 2005; Van Ginneken and Maes, 2005; Jerez *et al*., 2006). In these cases, aquaculture still relies on the capture of wild juvenile stocks to cover the market demand.

**d) Organic matter in the effluents**

One of the difficulties in studying the impacts of N and P discharges from aquaculture farms on the receiving waters is that nutrient discharges can also come from other sources (river run-offs, sewages). In nutrient-limited waters, modest additions of nutrients may increase the productivity and biodiversity in an area, and could lead to eutrophication if flushing (nutrient dispersal) rates are not high.

Several studies and large scale projects (MEDVEG, MERAMED, etc.) have indicated that direct benthic effects from aquaculture are limited to within a short distance of the cages, normally not exceeding 30-50 m from the fish farms (see for example Karakassis, this volume). There are signs that pelagic fish, invertebrate and seagrass communities may be affected to a large distance (Dimech *et al*., 2000; Pergent-Martini *et al*., 2006). It is well known that fish farming releases a substantial amount of nutrients into the marine environment and therefore it would be reasonable to expect effects at larger spatial scales, particularly when a group of farms is established in a coastal bay. Data arising from large scale projects (including MARAQUA, BIOFAQs, AQUCESS, ECASA) indicate that such changes may also affect benthic and fish communities in the vicinity of aquaculture development zones and particularly in oligotrophic environments, as the Mediterranean Sea, where nutrient scarcity limits productivity.

The estimated time for the benthos to recover its species abundance, richness and biomass after the closure of fish farming has been reported from a few months to five years, depending on the scale and duration of the fish farming activity and the geography of the area (Burd, 1997; Angel *et al*., 1998; Mazzola *et al*., 2000; McGhie *et al*., 2000; Pohle *et al*., 2001; Pergent-Martini *et al*., 2006). The high organic matter supply under and close to fish cages resulted in a slight decrease of benthic meiofauna biomass and the impoverishment of species diversity. The abundance of the main meiofaunal groups (Nematoda, Harpacticoidea, Polychaeta, Turbellaria, Bivalvia) gradually increased from the fish farm to a higher level at 200 m from the cages.

In addition to solid wastes discharges, the benthic efflux of dissolved inorganic nutrients to the overlying water, following organic matter decomposition, is an important source of N and P to the surrounding waters. Excess nitrogen and phosphorus can lead to eutrophication, which is expressed as an increase in primary production, changes in algal composition, algal blooms (that could be toxic) that may lead to hypoxia and anoxia (Gismervik *et al*., 1997; McClelland and Valiela, 1998; Worm *et al*., 1999; GESAMP, 1990; Worm and Lotze, 2000; Worm *et al*., 2000). Studies carried out on shellfish farming indicate that the extent of effect of nutrients (decomposition of biodeposits) is related to oceanographic and biological parameters of the area. Those studies showed different effects in the benthic environment, ranging from no appreciable effect (Hostin, 2003), small (Buschmann *et al*., 1996; Crawford *et al*., 2003; Miron *et al*., 2005; Da Costa and Nalesso, 2006) and important (Mirto *et al*., 2000; Chamberlain *et al*., 2001; Christensen *et al*., 2003; Smith and Shackley, 2004). The study of Kovac *et al*. (2004) in the Bay of Piran (Northern Adriatic Sea, Slovenia), demonstrated long-term impacts of fish farms on meiofauna communities.

**e) Pathogen transfer**

Recently it has been emphasised that the possible introduction into the ecosystem of pathogens could be associated with the unintentional release of infected farmed organisms (native or exotic). There are still few scientific data to support or demonstrate pathogen transfer between cultured and wild stocks (De Silva *et al*., 2006). This causality is difficult to identify or correlate, because it might be associated with other factors. However, Diamant (in this volume) demonstrated the transfer of pathogens in both directions with cage culture systems in the Gulf of Eilat, with data from three different marine pathogens:

– *Mycobacterium marinum,* was first found in 1990 in cultured fish and since 1995 at high prevalence in wild fish populations, particularly rabbit fish *(Siganus* spp.);

– *Enteromyxum leei* was introduced into the Red Sea with mariculture stocks; the parasite has spread to wild native species;

– in the other direction the cultured seabream fish were infected by the local pathogen *Kudoa iwatai* from wild fish populations.

**f) Vaccines and other products**

Veterinarian research in aquaculture is focused on the production of vaccines for every known disease, and for the use of biosafe chemicals. The development of probiotics and immunostimulants agents is one of the latest research area offering perspectives to enhance the immune status of the cultured organisms (Dugenci, 2003; Rodríguez *et al*., 2003; Torrecillas *et al*., in press). At the same time, the use of new anaesthetics is also being investigated, to reduce the detrimental effects on the cultured organisms. Current complicated licensing procedures and the small size of the aquaculture industry have discouraged pharmaceutical companies to invest in the sector for licensing new products.

**g) Antifouling**

Today, copper is the main ingredient in antifouling paints in which it is used as cuprous oxide ($Cu_2O$). The slow dissolution of the oxide in water favours the gradual dispersion of the copper, thereby enhancing the antifouling effect.

Research focuses on natural repellents or on the use of biological substances that prevent the settlement of fouling organisms through the better understanding of settlement mechanisms. Investigation is also carried out on new coatings, such as silicon based fouling-releasing coatings (Baum *et al*., 2002), on spraying with antifouling solutions (acetic acid) (Carver *et al*., 2003), or nanotechnology applied to new materials. An entire European project has been dedicated to biofouling and its solutions, called Collective Research on Aquaculture Biofouling (CRAB, <www.crabproject.com>).

At present, the aquaculture sector is searching for alternatives to present coating products such as copper, and moving towards more environmentally friendly procedures. These include research on biological control using grazers, such as gastropod snails, sea urchins, or even fish, that feed on the fouling organisms (Lodeiros and García, 2004).

**h) Impact on seagrass meadows**

Many studies have indicated that visible effects from aquaculture on the benthic environment are found within a short distance, normally not exceeding 50m from the fish farms, while the biological communities of the water column may be affected at a greater distance (Grant *et al.,* 1995; MEDVEG, MERAMED, Uriarte and Basurco, 2001; Machias *et al*., 2005). Seagrass meadows are essential benthic habitats, playing a major ecological role in the Mediterranean coastal zone preventing coastal erosion, supporting biodiversity and water transparency, and oxygenating water and sediments (Hemminga and Duarte, 2000). *Posidonia oceanica* meadows are considered determining elements for the biological quality of Mediterranean coastal zones, but they are highly vulnerable to human activity, such as marine aquaculture (Delgado *et al*., 1997; Ruiz *et al*., 2001; Pergent-Martini *et al*., 2006). They suffer large-scale losses in response to nutrient enrichment (Ruiz *et al.,* 2001; Cancemi *et al*., 2003) and this may continue for several years even after the cessation of activities (Delgado *et al*., 1999). Under or near sea cages, the meadows of *Posidonia oceanica* die and the effects are not reversible, at least on a human timescale (Holmer *et al*., 2003; Pergent-Martini *et al*., 2006). Due to the sensitivity of seagrass meadows to aquaculture activity, vertical rhizome growth can be used as an early indicator of fish farm impacts on *P. oceanica* meadows (Marbà *et al*., 2006).

**i) Fauna attracted to aquaculture installations**

Aquaculture facilities attract wildlife that benefit from easily available food or shelter. It cause problems in fish farms due to predation, stress on animals, disease transfer, etc. Among the predators and scavengers attracted by the aquaculture structures, fishes are the most prevalent, but birds, marine mammals, sharks and turtles also visit aquaculture facilities. They are searching for food, which can be both the cultured organisms or organisms colonising on and around the structures (Nash *et al*., 2005). The greatest risk to any animal near the aquaculture facility is rubbish

from the site, such as plastics, feed bags or ropes, which can prove to be fatal when ingested accidentally. However, the aquaculture structure itself (e.g., ropes, lights, acoustics, buoys, nets) only poses a minimal threat to wild species thanks to the improvements that have been made in recent years (Nash *et al*., 2005).

It is well known that fish farming releases a substantial amount of nutrients into the marine environment and therefore it would be reasonable to expect effects within a larger radius of the site, particularly when a group of farms is established in a coastal bay. New studies are starting to show that such changes also affect fish communities in the vicinity of aquaculture development zones, particularly in oligotrophic environments such as the Mediterranean Sea where nutrient scarcity limits productivity and fisheries production. In this sense, the release of nutrients from fish farming in nutrient-poor systems can have a positive effect on local fisheries with no visible negative change in species composition or biodiversity (Machias *et al.,* 2005, 2006).

The effects of cages and other aquaculture structures are very different and change with time. In general, the situation can be summarised as follows:
- very strong interaction exists between aquaculture structures and local flora and fauna;
- part of the local fauna benefits from excess food accumulated below the cages;
- species richness is affected, increasing away from the cages;
- wild fish catches and landings increase near the cages; and
- interaction is mostly reversible, though not in the case of some very sensitive species such as *Posidonia,* or specific ecosystems.

In the different chapters of the IUCN Guide on Interactions of Aquaculture and Environment, there are several recommendations to reduce the negative impacts of sea cages or land-based aquaculture, with the aim to promote sustainable use of natural resources and responsible aquaculture production along Mediterranean coasts. The specific recommendations of each chapter have been defined, either from the biological or the management point of view, with the help of the Aquaculture Expert panel as well as by consultation to other experts, trying to adapt to local geographic and social characteristics.

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