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Assessment of Finfish Aquaculture Impact on the Benthic Communities in the Mediterranean Sea

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ABSTRACT

This manuscript discusses the state–of-the-art investigations related with the benthic impact of marine finfish farms in the Mediterranean. We tackle the ideas from a broad perspective, mentioning issues that are of a widespread importance in marine finfish aquaculture, before putting them into the context of the Mediterranean, with its distinctive and specific characteristics. This document comments the most evident changes related with alteration of chemical parameters and community assemblages but specially focuses on the consequences for the whole ecosystem. We look at current knowledge of the particulate deposition and dispersion rates of aquaculture wastes and the existing related models, and emphasize the previously unconsidered role of wild fish. The carrying capacity of ecosystems influenced by fish farms is discussed. The benthic impact of Atlantic bluefin tuna (*Thunnus thynnus*) fattening is summarized and compared with the impact of guilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) rearing. The suitability of using a suite of tools for assessing aquaculture impact is discussed, highlighting the importance of using toxicity tests as a new complementary technique. Ideas and suggested implementations are put into the context of the European Water Framework Directive (WFD) guidelines.

Keywords: AMBI, Bentix, fish farm, mariculture, organic enrichment, particulate waste, water framework directive
Abbreviations: AMBI, AZTI Marine Biotic Index; **AVS**, acid volatile sulphides; **EQS**, ecological quality status; **FCR**, feed conversion ratio; **ITI**, infaunal trophic index; **RPD**, redox potential discontinuity; **TAN**, total ammonia-nitrogen; **WFD**, water framework directive

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INTRODUCTION

Most fishing grounds worldwide are overexploited, and fish catches are either static or diminishing. Given the continuous increase in the demand for food, aquaculture appears to be an important option for increasing the fish supply. Consequently, during recent decades, fish farming in the open sea has undergone almost exponential growth around the world (FAO 2007), including in the Mediterranean.

The main residue derived from marine finfish farming is the organic matter released to the environment in the form of uneaten feed or fish metabolic wastes (Focardi *et al*. 2005). Such organic enrichment may have environmental drawbacks, especially if organic matter and nutrients surpass the threshold of their carrying capacity. Other substances derived from fish feed, even in much lower concentrations (metals, medicines, vitamins, hormones, etc.) or from other sources associated with fish farming activity (antifoulings, external biocides for fish, etc.), may also have an impact on the benthic system (Holmer *et al*. 2008). However, little is known about these issues.

Fish farming wastes are released from the fish farms in two forms, particulate and dissolved. Due to the strong diluting effect of the sea, concentrations of dissolved wastes are rapidly reduced close to background levels, whereas particulate wastes tend to sink to the seabed. There, they may accumulate and produce important changes in sediment chemistry and, consequently, in communities that inhabit areas close to farms (Pearson and Rosenberg 1978). Thus, the impact is usually more noticeable in the benthos that in the water column, which is why most studies related with marine finfish aquaculture impact have focused on the benthic system. Benthic impact assessment of this activity, with few exceptions (e.g. Hargrave *et al*. 2008; Kutti *et al*. 2008), has suffered from the lack of a holistic approach, and studies are usually based on measuring changes in physico-

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Invited Review

Table 1 Comparison of Atlantic salmon (*Salmo salar*) farming with the most common finfish species reared in the Mediterranean: the Atlantic bluefin tuna (*Thunnus thynnus*), guilthead sea bream* (*Sparus aurata*) and sea bass** (*Dicentrarchus labrax*). All data are an approximate mean of different fish farms. FCR stands for feed conversion ratio.

	Production cycle type	Time employed in rearing species	Dynamics of biomass production	FCR	Type of diet
Salmon	Closed	19-20 months	Variable	1-2	Formulated feed
GSB*/SB**	Closed	2-3 years	Constant	1-2	Formulated feed
Tuna	Open	6-7 months	Variable	15-25	Small fish

chemical as well as macrofaunal assemblages parameters in one or few locations without considering the results in a broader perspective, such as the consequences for ecosystem functioning or inferring thresholds for exported wastes. Local changes in physico-chemical as well as macrofaunal assemblages are well reported (Karakassis *et al*. 1999, 2000; Kalantzi and Karakassis 2006; Sanz-Lázaro and Marín 2006; Aguado-Giménez *et al*. 2007) and therefore it is not our purpose to discuss these in this manuscript.

The Mediterranean Sea is oligotrophic, with a biological productivity that is amongst the lowest in the world (Cruzado 1985). The main fish species reared in the Mediterranean are the guilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*), while Atlantic bluefin tuna (*Thunnus thynnus*) fattening is also becoming an important activity. Tuna farming has experienced a significant growth in recent years, but data on the impact of this activity is very scarce (Vita *et al*. 2004a; Borg and Schembri 2005; Vita and Marín 2007). Guilthead sea bream and sea bass have similar culture conditions, with a closed production cycle, i.e. all the stages of the production are controlled, and the biomass reared is approximately constant throughout the year. These species are fed with a feed especially created for this purpose, which results in a low feed conversion ratio (FCR; usually ranging between one and two; Lupatsch *et al*. 2003) meaning that a large part of the feed supplied is transformed into increased fish biomass. However, tuna rearing differs in many aspects from guilthead sea bream and sea bass fish farming. The production cycle is open, so wild tuna is fished and then reared, with a duration of approximately 6-7 per year. The species are mainly fed with pelagic fish of low economic value, and the FCR is very high (around 20; Aguado-Gímenez *et al*. 2006), which means that most of the feed supplied is wasted and not converted to fish biomass (e.g. to increase the weight of a reared tuna by 1 kg, it is necessary to provide 20 kg of fish feed) (**Table 1**).

The increase in aquaculture facilities is accompanied by a growing concern to make aquaculture a sustainable activity, environmentally sound and in accord with the ecosystems in which the activity takes place (Naylor *et al*. 2000). However, improved aquaculture management can only be accomplished by increasing research effort into these issues, in an attempt to improve our understanding of the effects and interactions of this activity in the environment.

The aim of this manuscript is to review the existing knowledge on the assessment of finfish aquaculture impact on benthic communities in the Mediterranean Sea. Firstly, we comment on the existing modelling of aquaculture particulate waste export. We discuss some of the parameters that may play an important role in benthic impact, which are not taken into account in current models, especially the effect of wild fish as exported wastes sink, and how to adapt models which were mainly developed for salmon farming to reared Mediterranean species. Following this, we provide an overview of different approaches to couple particulate waste export with benthic status, according to the sensitivity and conservation priority of the different benthic ecosystems. Then, we focus on tuna farming since little information is available on the impact of this activity. Afterwards the methods for assessing marine aquaculture impact are briefly discussed and a set of tools is proposed, including sea urchin toxicity tests, as a part of this approach.

THE IMPACT OF ORGANIC ENRICHMENT ON THE BENTHIC SYSTEM

The deposition of particulate matter from net-pens has been identified as the main cause of the negative environmental impact associated with aquaculture (Gowen *et al*. 1991; Read and Fernandes 2003). The principal particulate wastes are uneaten feed, fish excretion wastes and, to a lesser extent, debris from dead cultured fish and fouling communities. These wastes are the source of benthic enrichment and have potentially deleterious consequences for the seabed communities next to the fish farm facilities.

The ultimate fate of suspended particulate matter is to sink and settle on the sea floor, where it is incorporated into the sediment stratigraphy. Although the sources of the particulate matter that make up the benthic habitat vary spatially and temporally, with few exceptions the microbial and faunal communities of the benthos intimately depend on the productivity of the surface waters of the oceans (Solan and Wigham 2005).

The Mediterranean Sea is considered to be oligotrophic (Cruzado 1985; Gacia *et al*. 2002). Hence, particulate matter input from the water column to the seabed is low and benthic communities are supported by relatively minor rates of organic matter and nutrient flux. Therefore, great quantities of organic matter input derived from husbandry practices can greatly increase natural rates [perhaps more that ten-fold according to some authors (Holmer *et al*. 2007)], clearly exceeding the carrying capacity of the ecosystem and producing important changes in the sediment chemical parameters, as well as, in the benthic communities.

Organic matter overload consumes the oxygen of the surface of the seabed, producing hypoxia, and, if the carbon flux is large enough, the seafloor can reach anoxic conditions. The consequence of oxygen consumption is the appearance of bacteria with an anaerobic metabolism that uses compounds other than oxygen as electron acceptors to obtain energy. The predominant bacteria of such organically enriched sediments are *Beggiatoa*, which produce sulphides as residues of their metabolism (**Fig. 1**). There are other less abundant anaerobic bacteria which produce ammonium and methane.

All these by-products, especially sulphides, are toxic to the inhabiting fauna at high concentrations, and, along with the oxygen exhaustion of the pore water, may lead to the depletion of the more sensitive species, sometimes resulting in the total defaunation of the sediment (Brooks and Mahnken 2003b; Heilskov *et al*. 2006).

Most benthic species play a major role in the process of benthic nutrient regeneration, affecting primary production by supplying nutrients directly and enhancing rates of pelagic recycling (Grall and Chauvaud 2002). The species inhabiting the sediment, especially the macrofauna, produce bioturbation of the sediment. Basically, this process consists of moving and mixing the sediment, thereby redistributing the particles, as a result of different activities, such us motion, feeding, tube construction, etc. The results of bioturbation may vary substantially from simple to very complex structures such us galleries, hollows or tubes of many different types depending on the bioturbating traits of the species involved (Aller and Aller 1998).

Sediment bacteria play a key role in the biogeochemical cycling of nutrients. The bioturbation of macrofauna may impact the diversity, structure and function of such bacteria (Findlay *et al*. 1990) and, in turn, have a significant effect

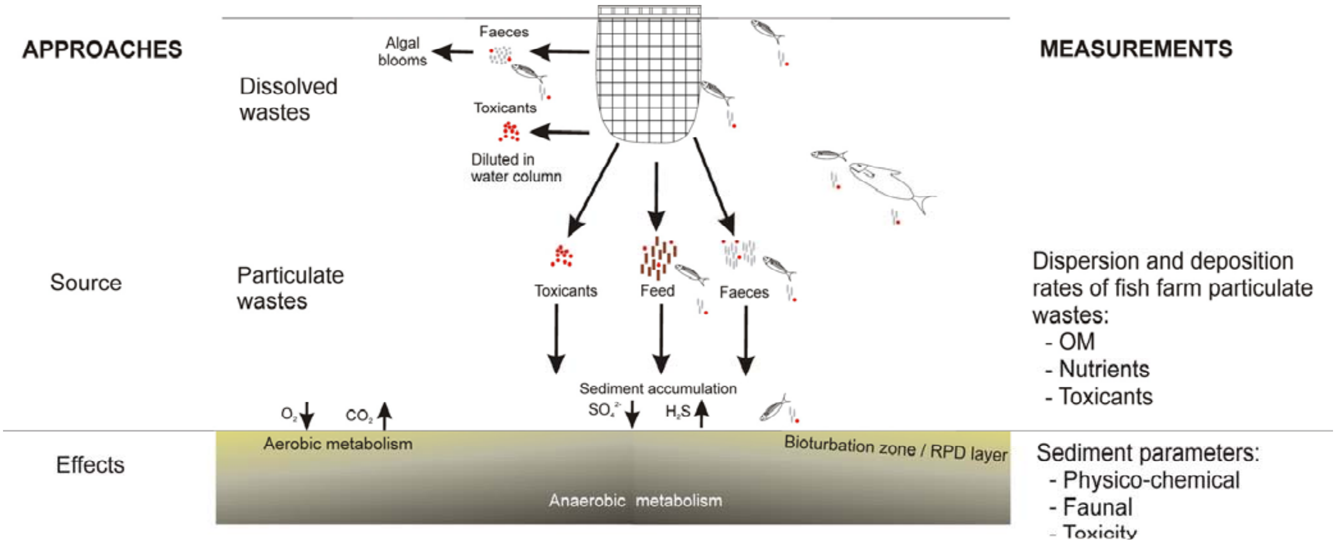


Fig. 1 Scheme of the fate of marine finfish aquaculture wastes, sediment biogeochemical alterations and the different feeding habits of wild fish. Note that for anaerobic metabolism only sulfate reduction is taken into account, since it is the most representative. The scheme also shows the approaches used to assess benthic impact and the set of parameters measured in each approach.

on many ecosystem processes (Biles *et al*. 2002). The result of this activity greatly increases the sediment area exposed to water from the surface, which is able to flush into deeper layers of the seabed, where there is a low microbial activity. This process will increase carbon oxidation of anaerobically refractory organic matter (Kristensen 2001), resulting in increased nutrient release, which will support bentho-pelagic primary production, thus enhancing benthic production (Solan and Wigham 2005). This flushing may have profound effects on the biogeochemistry of the sediment, especially in diffusion-dominated systems (fine sediments/low interstitial flow rates) (Mermillod-Blondin and Rosenberg 2006).

Since the bioturbation potential of the infauna depends on the abundance, biomass and particular bioturbating trait of each species, any reduction in the macrofauna (partial or total), may reduce the bioturbation activity and therefore fauna-mediated irrigation, subsequently reducing benthic production. Above the natural particulate organic input levels of a given community, the amount of organic enrichment load is positively correlated with the narrowing of the redox potential discontinuity (RPD) layer (the depth from the seabed surface down to where an environment with oxygen persists), and if organic input is great enough, all the sediment up to its surface can be left in anoxic conditions (Pearson and Rosenberg 1978; **Fig. 1**).

Therefore, under organic enrichment impact, important changes occur in the sediment, not only in accordance with changing chemical (oxygen lessening and stimulation of anaerobic sediment metabolism) and biological (community succession to one with more opportunistic species) parameters, but also related with main functions of the ecosystem, such as nutrient recycling and organic matter mineralization.

In marine finfish aquaculture, the quantity and quality of the feed are the most important factors determining organic matter and nutrient loss to the environment, since these factors determine both feed wastage and excretion loss (Cho and Bureau 2001). However, other substances that are released during fish farming activity may have negative effects on the functioning of the ecosystem, e.g. metals, drugs (antibiotics, pesticides, vitamins, hormones, etc), nutrients, disinfectants (formalin and iodophors), etc. If aquaculture is to be properly managed from an environmental point of view, two main points need to be known: (1) the flux of organic matter, nutrients and other potentially toxic substances (mentioned above) exiting the fish farms and reaching the seabed and (2) the carrying capacity of the different benthic ecosystems affected by these exported wastes.

MODELLING FINFISH FARMING IMPACT I: QUANTIFYING AND FORECASTING FINFISH FARMING PARTICULATE WASTES

The organic carbon, nitrogen and phosphorus loads alter the sediment characteristics beneath and close to the fish cages (Hall *et al*. 1990; Holby and Hall 1991; Hall *et al*. 1992; Brooks and Mahnken 2003b; Vita *et al*. 2004b). Particulate wastes exported from a fish farm can be calculated directly by means of sediment traps or also by using models of different complexities. However, directly measured information on the deposition and dispersion of wastes from marine finfish farms is scarce (Sutherland *et al*. 2001; Cromey *et al*. 2002; Tsutsumi *et al*. 2006), especially in the Mediterranean area (Vita *et al*. 2004a, 2004b; Holmer *et al*. 2007). Nevertheless, several aquaculture waste deposition and/or dispersion models have been developed.

Models that simulate the input and fate of waste material from marine cage finfish farms are considered cost-effective tools within management strategies to evaluate biomass limits in terms of environmental capacity, to set quality standards, aid in selecting the most suitable sites for leasing new aquaculuture facilities and for predicting environmental impact. Models have different degrees of complexity according to the number of parameters that they integrate.

Many of these models are based on the approach of Gowen *et al*. (1994), who linked dispersion distance with current speed, water depth and particle settling velocity. Since the above study, models have greatly evolved to include different sub-models, such us bathymetry variation (Hevia *et al*. 1996; Jusup *et al*. 2007), settling velocities for feed and faecal components (Cromey *et al*. 2002), the use of GIS technology (Pérez *et al*. 2002) and the effects of cage movement (Corner *et al*. 2006), among other parameters.

Despite these developments, models are still to some extent inaccurate, mainly due to the sources of uncertainty in model forecasts. According to Higgins (2003), uncertainty is a measure of the confidence of the result in a forecast. Certainly, the accuracy of a model greatly depends on initial conditions, model type and parameter estimates. Two types of uncertainty are especially linked to this sort of model. Model uncertainty*,* which is caused by uncertainty in the representation of ecological processes, and parameter uncertainty, which is uncertainty in parameter estimates derived from data and is consequently a function of sample size.

Fig. 2 Picture showing wild fish aggregation in a fish farm in the Mediterranean Sea (Spain).

Model uncertainty

All models are simplified approaches to explain reality. A useful model will be one that takes into account all the parameters that play an important role in a process within a restricted domain. Deficient models miss key processes, and may consequently yield misleading forecasts. In the context of aquaculture, sub-models used to define deposition and dispersal rates are uncertain, because of the numerous parameters involved and the difficulty of integrating all of them. Many processes that act on the waste material, and which could significantly influence benthic conditions and confound predictions are not simulated within present day models (Chamberlain and Stucchi 2007).

An example of this scenario is the wild fish effect. Coastal aquaculture net-pens have a strong aggregative effect on wild fish (Dempster *et al*. 2006; **Fig. 2**). Fish farms covering an area of just 1 to 4 ha may have up to 40 tonnes of wild fish around them (Dempster *et al*. 2004). In Australia, in shallow waters (3 to 4 m depth), wild fish consumed between 40 and 60% of the total particulate nutrient fluxes released by an experimental finfish aquaculture facility (Felsing *et al*. 2005). In the Mediterranean, wild fish consume aquaculture food pellets (Black 1998). Similarly, particulate organic wastes released by a fish farm can be reduced by up to 80% of the total (Vita *et al*. 2004b) and nutrients by almost 50% (Sanz-Lázaro *et al.* unpublished data). To the best of our knowledge, no waste dispersion model has taken this effect into account. The consumption of marine fish farm particulate wastes by wild fauna may be significant, and the magnitude of this organic matter and nutrient sink needs to be assessed and integrated into models if waste loading on the seabed is to be predicted accurately (Hevia *et al*. 1996; Dempster *et al*. 2005; Felsing *et al*. 2005; **Fig. 3B**).

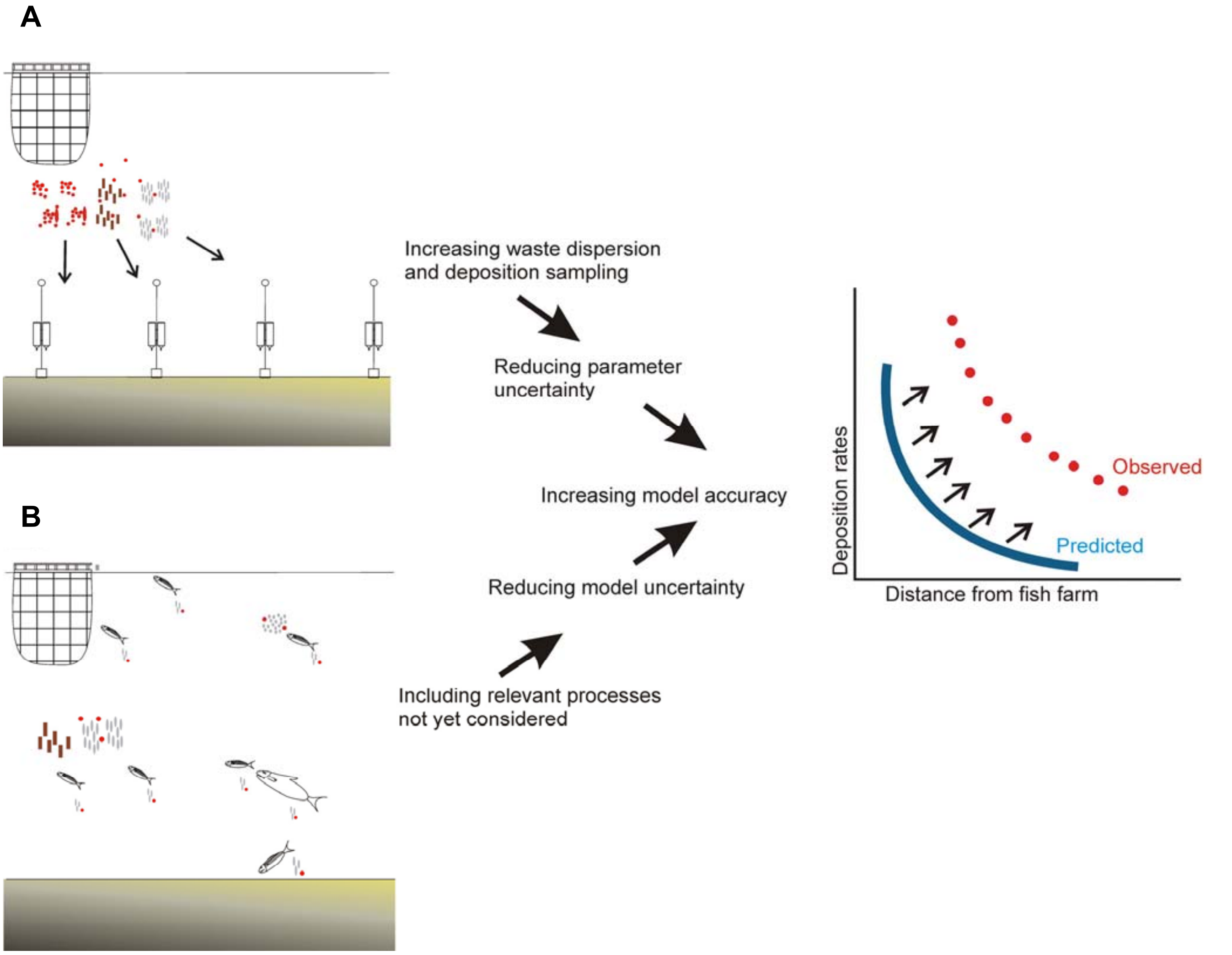
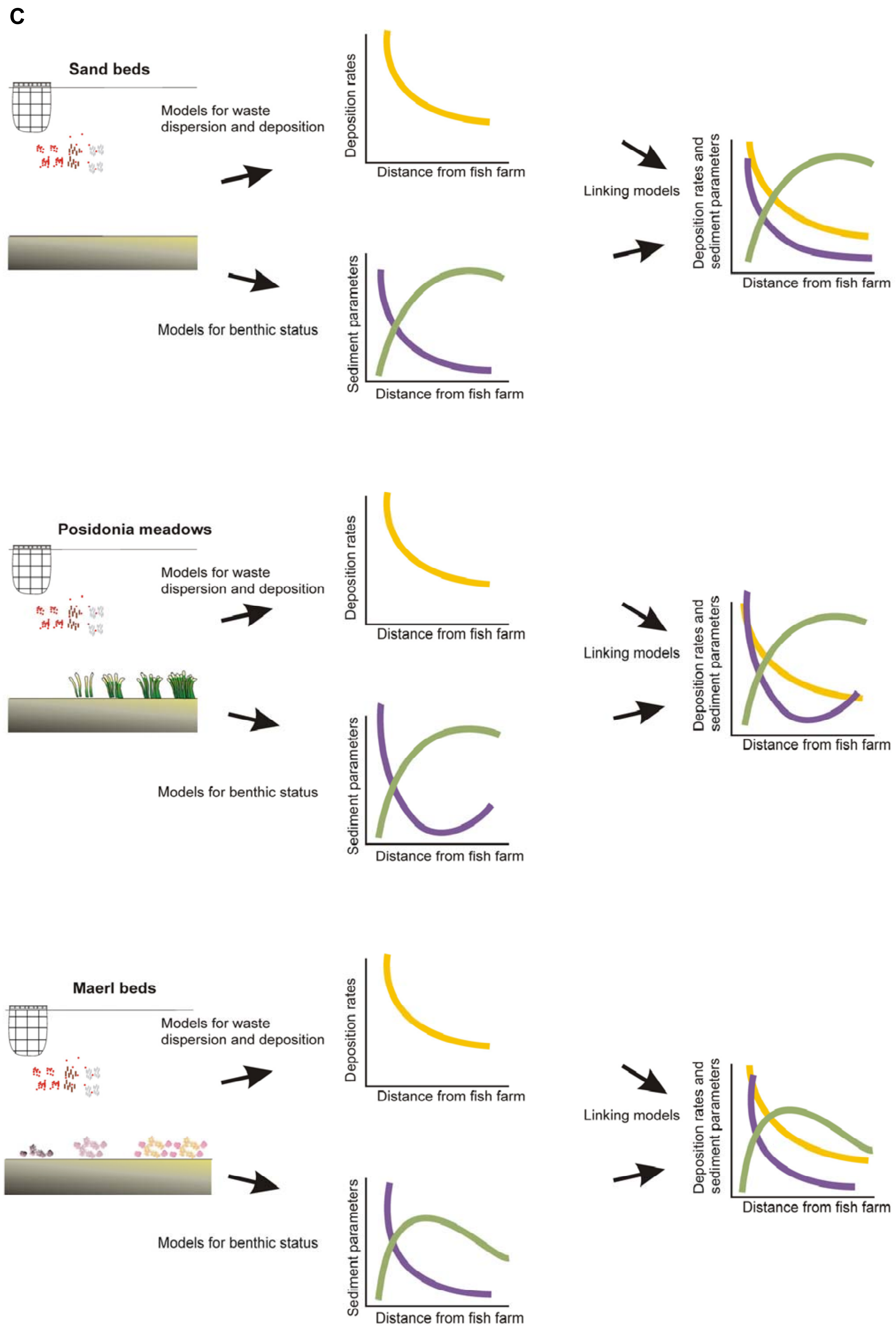


Fig. 3 Steps to improve current models related with marine finfish aquaculture: (**A**) Obtain more field measurements; (**B**) Integrate unconsidered variables in models; (**C**, next page) Link aquaculture waste deposition dynamics (sources) with benthic status (effects) and establish thresholds for each benthic ecosystem.


C
Sand beds
Models for waste dispersion and deposition
Models for benthic status
Deposition rates
Distance from fish farm
Sediment parameters
Distance from fish farm
Linking models
Deposition rates and sediment parameters
Distance from fish farm
Posidonia meadows
Models for waste dispersion and deposition
Models for benthic status
Deposition rates
Distance from fish farm
Sediment parameters
Distance from fish farm
Linking models
Deposition rates and sediment parameters
Distance from fish farm
Maerl beds
Models for waste dispersion and deposition
Models for benthic status
Deposition rates
Distance from fish farm
Sediment parameters
Distance from fish farm
Linking models
Deposition rates and sediment parameters
Distance from fish farm

Parameter uncertainty

Parameter uncertainty is a measure of how sensitive the probability of a parameter estimate is to changes in the parameter estimate. After field data collection, calibration and sensitivity analysis must be performed. This process of comparing field with predicted data may help to improve the conceptual model. It is very important to have a substantial bulk of field data for different scenarios if parameters are to be optimized, since parameter estimates are very uncertain when sampling is limited.

Moreover, parameter estimates are only as good as the statistical models used to estimate them – that is, parameter estimation cannot be divorced from model uncertainty. Statistical models require appropriate specification of the ecological processes, sources of error in these processes, and observation of the error. Provided the statistical model is adequate, parameter uncertainty can be reduced by more sampling (Higgins *et al*. 2003).

Nevertheless, robust and defensible information is not available for some of the key model parameters, such us deposition and dispersion rates of organic matter and nutrients (Islam 2005), which is where the accuracy of models shows great variations [e.g., (Cromey *et al*. 2002) ±20% and ±13%; (Corner *et al*. 2006) ±58.1%; (Chamberlain and Stucchi 2007)]. Hence, more data related with aquaculture wastes sedimentation dynamics are needed to reduce parameter uncertainty (**Fig. 3A**).

There are important parameters that are hard to measure and/or which are expected to be very site specific. For example, the FCR, which is usually taken as a fixed parameter, may vary due to other parameters, such us fish density or feeding strategy, which may not be taken into account in the models.

This fact is especially important in feed loss, which is a transient within-cage process and depends on the physical, biological and feeding characteristics at a farm site (Corner *et al*. 2006). Feeding strategy, stress level, prevailing weather conditions, current speed, water quality, water temperature variation with season, thermocline establishment and diseases, among other factors, will all influence feed loss over varying temporal scales. In addition, models consider feed loss to occur uniformly across all hydrographic measurements, but actually feed loss is limited to feeding periods only. Subsequently, there is a difficulty in assuming that the feed element of any deposition model is an accurate depiction of actual settlement (Corner *et al*. 2006).

Another example of a parameter with high uncertainty is the effect of wild fish as a sink of particulate wastes exported by fish farms. The sedimentation 'footprint' of temperate net-pen fish farms may vary depending on (1) the species and biomass of associated wild fish, and (2) where these fish are distributed in the water column (Dempster *et al*. 2005) and in the benthos (Vita *et al*. 2004b). Fish assemblages exhibit temporal and spatial variability patterns in the fish farms to which they are associated (Dempster *et al*. 2004; Valle *et al*. 2007). Wild fish abundance and biomass may be determined by a series of parameters that may be highly site specific. These parameters may be the fishing effort in the surroundings of the fish farm, fish farm size (larger fish farms may support greater wild fish biomass), the fish assemblages itself, since assemblages of different species have different preferences as regards the different depths they occupy in the water column and different feeding habits, among others. Wild fish may eat different aquaculture wastes, such as whole uneaten feed, disaggregated feed, faecal pellets, epibiotic species attached to the fish nets, sedimented organic wastes in the seabed, other wild fish, etc (**Fig. 1**). This variability suggests high uncertainty in the modelling of organic waste dispersal. Therefore, more research effort should be paid to understanding the role of different species and their distribution through the water column and benthic system in reducing fish farming impact.

Within European countries, the extent to which models are used varies greatly (Henderson *et al*. 2001). In Scotland, DEPOMOD (Cromey *et al*. 2002) is now widely used for Environmental Impact Assessment. Further testing of the model at a range of farm sites will provide the necessary data to assess the suitability for generic application of model predictions (Chamberlain 07).

Models have mainly focused on salmon aquaculture but they can be adapted to guilthead sea bream and sea bass fish farming, because of the similarities that the rearing of these species share with salmon. Key data related with the settling velocity of feed pellets and faeces of guilthead sea bream and sea bass are available (Vassallo *et al*. 2006, Magill *et al*. 2006). However, it must be taken into consideration that guilthead sea bream and sea bass production is more or less continuous with no fallow period. Also, the particular conditions of the Mediterranean Sea should be taken into account. The Mediterranean has a very low tidal range, and so parameters such as cage movements due to tides should be excluded from the models that take this into account. A parameter that is not included in salmon-based models is a summer thermocline, which normally occurs in temperate-warm regions like the Mediterranean. The seasonal fluctuation patterns of organic flux to the sea floor below the fish farm may not necessarily coincide with the amount used in the fish farm. The largest organic flux to the sea floor may occur when the vertical mixing of the water had just started, as it has been found to happen in Japan (Tsutsumi *et al*. 2006).

MODELLING FINFISH FARMING IMPACT II: LINKING AQUACULTURE WASTE DEPOSITION DYNAMICS WITH BENTHIC STATUS

The objective in the management decision framework is generally to assess the potential negative effect compared with natural conditions. So, to be of use, model outputs, in the form of a predicted waste flux, must be correlated with a measure of 'actual' or 'change to' benthic status, which itself is an important step in model development. Even so, very few models attempt an interpretation of the consequent effect on benthic condition (Chamberlain and Stucchi 2007).

A small number of models have gone one step further and have tried to correlate the effect of increased sedimentation from aquaculture activity with benthic status, including benthic oxygen demand (Findlay and Watling 1997; Tsutsumi *et al*. 2006), carrying capacity (Stigebrandt *et al*. 2004) or benthic community descriptors and indices of benthic status (Cromey *et al*. 2002). When such relationships between predicted flux and benthic status can be demonstrated to be significant, model predictions of the degree and spatial extent of the effect may be made at other locations showing similar community assemblages, as well as, biogeochemical, bathymetric and hydrographic conditions. However, generic application of these models to all locations may not be appropriate as the assumptions and limitations used are generally narrow in scope (Chamberlain and Stucchi 2007).

Of the models available, only those of Stigebrandt *et al*. (2004) and Cromey *et al*. (2002) have focused on biological status. Stigebrandt *et al*. (2004) proposed a model for estimating the holding capacity (equivalent to the carrying and assimilative capacity), in the Modelling–Ongrowing fish farm–Monitoring system. According to the authors, the holding capacity related with the benthic system is estimated with regard to three basic environmental requirements: (1) the benthic fauna at a farm site must not be allowed to disappear due to the accumulation of organic material, (2) the water quality in the net-pens must be kept high and (3) the water quality in the areas surrounding the farm must not deteriorate.

Following this approach, the holding/carrying capacity of the seabed is the maximum rate of sedimentation of organic matter that does not lead to extinction of the benthic infauna. For this reason, the above model considers that the carrying capacity of the ecosystem has not been surpassed, even though the macrofaunal community may have changed into a typically enriched assemblage where small opportunist species such as Capitellids prevail.

Such an approximation of carrying capacity does not seem to be a suitable approach since it does not meet the requirements of the Water Framework Directive (WFD; 2000/06/EC). According to Read and Fernandes (2003), the WFD takes a holistic approach, which is aimed at maintaining of the integrity of the ecosystem characteristics. The WFD requires the development of Catchment Management Plans as means of integrated management. These will contain defined environmental objectives to promote "good status" within the catchments. The WFD places emphasis on ecological status, which is defined as the "quality of the structure and functioning of aquatic ecosystems associated with surface waters". Good status is the second of five quality classes and is the minimum requirement for all waters by 2010. Such quality will be achieved through the control of water contaminants from human activities. In the context of marine aquaculture, the Dangerous Substances Directive will be integrated within the WFD and, in regulating marine aquaculture, Member States will be required to ensure compliance with these Directives in coastal and territorial waters. Compliance will be achieved through a combined approach including Emission Limit Values, Environmental Quality Standards and the application of a Best Available Technology approach. As with the Species and Habitats Directive, the WFD includes a consideration of the assimilation capacity of water bodies.

A change in benthic faunal structure next to fish farms towards less diverse communities with smaller and opportunistic species may have a strong impact on microbial communities and the biogeochemistry of the system, resulting this in slower mineralization rates and the potentially increased accumulation of organic waste products (Heilskov *et al*. 2006). Therefore, change is to be expected not only in the structure but also in the functioning of the ecosystem. Consequently, the assimilation/carrying/holding capacity should fit into the holistic approach of the WFD in order to maintain the integrity of the ecosystem characteristics. According to the WFD: "Waters showing evidence of major alterations to the values of the biological quality elements for the surface water body type and in which the relevant biological communities deviate substantially from those normally associated with the surface water body type under undisturbed conditions, shall be classified as poor". So the Stigebrandt *et al*. (2004) approach does not match the WFD since "good status" is the minimum level that all water bodies must meet.

Therefore, the carrying capacity of benthic ecosystems should be clearly defined by reference to the level of change in the structure of the community and the degree of ecosystem functionality decrease that can reasonably be allowed. Different ecosystems have different carrying capacities, but they also have different statuses from a conservational point of view, according to characteristics such as uniqueness, the biodiversity they sustain, the resilience rate (i.e. time it takes for an ecosystem to recover after suffering a perturbation) and the functions they carry out.

Examples of ecosystems highly sensitive to aquaculture impact

Seagrass meadows share the above mentioned characteristics. They are valuable ecosystems and have important functions since: (1) they are primary producers, and therefore have an important function in sequesterering CO_2, (2) they support a wide biodiversity (Díaz-Almela *et al*. 2008) and (3) they are important nursery zones for many species of fish (Bell and Pollard 1989). Another important feature of seagrass meadows it is their low resilience and, following impact from aquaculture, they may take several decades or even centuries to totally recover (Ruiz *et al*. 2001).

Another important ecosystem which has been neglected in studies is maerl beds, which are formed by the accumulation of unattached living or dead coralline red algae. These are commonly found in zones with strong currents at considerable depth within the photic zone (between 15 and 60 m depth, approximately), and are particularly abundant in the Mediterranean Sea (Foster 2001). Maerl beds share some of the values given to seagrass meadows: (1) they are also primary producers, and so they have an important function in sequestering CO_2, (2) they support an enormous benthic biodiversity, providing a variety of ecological niches for a diverse range of seaweed and invertebrate species, some of which may be confined to the maerl habitat (Hall-Spencer *et al*. 2006); (3) they also provide potential benefits for commercial fisheries as nursery zones for some species (Kamenos *et al*. 2004).

In addition, maerl beds themselves can be considered as key elements of the carbon and carbonate cycles in the shallow coastal waters where they are found (Martin *et al*. 2006). Maerl beds have a even smaller growth rate than seagrass meadows, approximately 1 mm (0.5–1.5 mm) per year (Bosence and Wilson 2003). Consequently, the resilience of this ecosystem is considered to be markedly low, and maerl beds can be very sensitive to aquaculture impact (Wilson *et al*. 2004).

Such ecosystems (as well as others with similar relevance but not mentioned in this manuscript) therefore, should be priority conservation targets and restrictive carrying capacity thresholds [at least compared to those proposed by Stigebrandt *et al*. (2004)] should be applied in order to ensure their conservation.

Similarly, the type of sediment beneath a farm is a major factor determining both the extent and the severity of aquaculture impact (Kalantzi and Karakassis 2006). Sand beds of differing grain size are expected to have naturally different RPD layer thicknesses and different community structures. Besides, the source of sediments may influence their iron content. Carbonate sediments, which predominate in the Mediterranean are characterized by a lower iron content than terrigenous sediments, which are typical of estuarine systems. Iron can reduce the toxic effects of sulfides by binding to them and reducing their bioavailability to benthic organisms (Holmer *et al*. 2005). So, aquaculture impacted sediments with a high iron content are expected to have fewer toxic effects, implying a lower impact on the benthic system.

On the other hand, sediments with a low iron content are especially prone to sulfide toxicity when enriched with organic matter from fish farms (Holmer *et al*. 2002) and a more deleterious effect on the benthos may be expected. The carrying capacity of benthic communities related to organic matter input in the Mediterranean may be less than in colder temperate regions such as fjords and lochs (Sanz-Lázaro *et al*. unpublished data). Therefore, grain size and sediment source should be taken into consideration when comparing carrying capacities of different benthic ecosystems.

Aquaculture management should bear in mind these issues, otherwise the resulting environmental impact may differ from what is expected. As an example, in Scotland, there is a trend to move fish farming operations to areas with more open conditions and strong currents, in an attempt to reduce the impact caused by aquaculture activity, since the amount of particulate wastes will be reduced below and next to the fish farms. However, this measure may be inadvisable since these new zones are constituted by maerl beds, and this community is more sensitive that other benthic ecosystems such us sand beds. Therefore the opposite effect to that expected may result, the impacted area actually expanding (Hall-Spencer *et al*. 2006).

Returning to the models merging deposition rates with benthic status, Cromey *et al*. (2002) established semi-empirical quantitative relationships between the predicted accumulation of solids and observed faunal benthic indices. This kind of approach seems to be on the right track since it tries to couple aquaculture wastes input with benthic status, comparing the effects on the benthos according to distance from the loading point (**Fig. 3 C**). However, Cromey *et al*.

(2002) found it difficult to describe relationships for the whole suite of benthic indices except for total individual abundance and for Infaunal Trophic Index (ITI), which its reliability in assessing community status is arguable (Maurer *et al*. 1999; Aguado-Giménez *et al*. 2007). Uncertainties in model parameterization further obscure potential patterns in predicted flux-benthic response coupling (Chamberlain and Stucchi 2007).

TUNA FARMING

Tuna accounts for more than 10% of the world international trade in seafood. Even though the completion of the life cycle of some tuna species has been achieved (e.g. Sawada *et al*. 2005), at present, all tuna farming is based on wild capture and fattening, mainly in Australia, Central America and the Mediterranean. The main capture-based aquaculture producers in the Mediterranean Sea are Spain, Malta and Croatia, which, together, produced more than 11,000 tonnes in 2001. The tuna farming industry is expanding rapidly not only in the Mediterranean area, but also in Australia, Mexico and Japan; the world production of farmed tuna reached 20,000 tonnes in 2001 (FAO 2004; Vita and Marín 2007 and citations there in).

During the months of May and June Atlantic bluefin tuna (*Thunnus thynnus*) enter the Mediterranean, are captured by purse-seine fleets and are then taken to the aquaculture facilities. There, tuna is usually fattened until the end of the year. During this time, the main goal is not to achieve important gains of weight in the tuna but to improve the fat content of the flesh. Tuna with a high fat content is highly valued in Japan, to which the whole production is exported. Croatia is the only Mediterranean country where small size tuna is captured and truly reared for up to 20 months (Matijevic pers. comm.).

Particulate waste output from tuna fattening is qualitatively and quantitatively different from that produced in the culture of other Mediterranean fish such as guilthead sea bream and sea bass fish (Aguado-Gímenez *et al*. 2006). While the latter are fed with formulated feeds, tuna is fed with small pelagic fish mainly pilchard, herring, bogue, sardines, mackerels, anchovies and cuttlefish. Hence, tuna farming, requires a much higher biomass of food compared with other fish rearing activities since tuna has a very high FCR, ranging from 15.3 to 24.8 (Aguado-Giménez and García-García 2005).

In the Mediterranean Sea, the capture-based aquaculture of tuna is a relatively new activity and little is known about its environmental impact on the marine ecosystem. Tuna farming has been the target of criticism from environmental and other pressure groups due to the perceived impact of the industry on the environment (FAO 2004). For adequate environmental management in tuna fattening, there is a need to fully understand the impact of this activity.

As in most cultured species, unconsumed feed and fish faeces are the main source of solid and soluble wastes and represent the major sources of pollution, although many of the data required for environmental impact assessment and the environmental monitoring of tuna ranching are simply unknown. For example, spatial and temporal scales of impact need to be established before the regulation of this new type of aquaculture can be properly implemented and for it to be considered, at least to some extent, a more environmentally sound activity.

The particulate wastes from tuna farming consist of two components that differ greatly from the wastes of other species: (1) uneaten feed (fish), which has a much higher deposition rate than formulated feed, and (2) tuna faeces, which are much more soluble and less dense than in the case of other species such as guilthead sea bream and sea bass fish (Vita *et al*. 2004a), so the settling velocity of the faeces is expected to be lower.

The only study to date (to the best of our knowledge) related with tuna farming nutrient sedimentation rates pointed to small quantities of N and P reaching the seabed beneath the fish farm facility (Vita *et al*. 2004a) compared with other reared species in the Mediterranean (Holmer *et al*. 2007). This may be due to two factors: (1) the above mentioned texture and density of tuna faeces, which are much more soluble than those of other fish species; (2) the sediment traps used to collect the organic wastes released from the fish farm are designed to measure particulate fluxes in other finfish aquaculture facilities where formulated feed is provided. These sediment traps are useful for collecting formulated feed but they were too narrow to retain the fish used for feeding because of their size (Holmer *et al*. 2008; Vita pers. obs.) .This type of trap is not able to collect all the wastes but only small amounts of particulate uneaten food (disaggregated fish) and the heaviest and more compact part of tuna faeces. Tuna fattening is characterised by a notably high FCR ratios and the use of fish as feed deposits substantial waste loads in the ecosystem (Islam 2005).

Another way of estimating nutrient output from tuna fattening installations is the nutritional approach, which only calculates the metabolic wastes (faeces and excretion) of tuna since it assumes that all feed supplied is consumed by the tuna. The quantity of N and P collected below the fish cages by means of the sediment traps by Vita *et al*. (2004a) was only 11.7 and 13.0% of the total amount of N and P released by the reared tuna, according to Aguado-Gimenez *et al*. (2006). This low percentage of faeces below the fish cage of tuna farming facilities may be due to the higher solubility of tuna faeces compared with salmon, guilthead sea bream and sea bass. Since the dispersion dynamics of the particulate wastes are very different from other cultured fish, the extent of the impact may also be different.

The impact of tuna farming has been little studied (Vita *et al*. 2004a; Borg and Schembri 2005; Fernandes *et al*. 2007; Vita and Marín 2007; Holmer *et al*. 2008; Matijevic *et al*. 2008). The assumption that the nutrient flux measured is underestimated (Vita *et al*. 2004a) is lent weight by comparing the impact that this type of fish farming has on the seabed. The only benthic impact assessment using both chemical and biotic parameters (to our knowledge) found the same impact characteristics as produced by salmon, guilthead sea bream and sea bass fish culture but with a different reach (Vita and Marín 2007). Macrofaunal community indices, as well as some chemical parameters such us acid volatile sulphides (AVS) and total ammonia-nitrogen (TAN), pointed to a clear impact in the form of organic enrichment. However, the values of these parameters rapidly fell with distance and soon reflected the absence of any impact. The same behaviour was observed in the multivariate analysis of the benthic assemblages. These results match those of an impact assessment only using macrofaunal community parameters, which also found that the impact of tuna farming was limited to the area below the fish cages (Borg and Schembri 2005). This, to some extent, differs from the impact resulting from the rearing of salmon and guilthead sea bream and sea bass fish, where the effect of organic enrichment is usually still noticeable at least around in a radius between 50 to 100 m from the fish farm.

The impact of tuna farming on benthic communities seems to be more spatially restricted that the impact of other finfish species such us guilthead sea bream and sea bass (Aguado-Giménez *et al*. 2007, Marín *et al*. 2007). The impact observed in the seabed during the production period in a tuna farm is quite pronounced very close to the fish farm. Even if the impact of tuna aquaculture on the benthic system seems to have a more reduced reach than is the case for guilthead sea bream and sea bass fish, organic enrichment of the water column will be much more intense since the amount of dissolved nutrients released by tuna is much greater than that generated by guilthead sea bream and sea bass (Aguado-Gímenez *et al*. 2006). This nutrient excess in the water column should be studied when quantifying the magnitude of the possible impact, and management should aim to minimize algal blooms and other negative impacts derived from water column eutrophication.

It should also be borne in mind that the production dynamics of tuna fattening is also different from guilthead sea bream and sea bass fish rearing, since tuna aquaculture involves a fallow period that may last 6 to 7 months of the year, during which the benthic conditions influenced by organic enrichment from the fish farming should improve. Even so, this time span does not seem to be sufficient for the seabed to completely recover (Vita and Marín 2007). Data point to complete or extensive recovery in low impacted areas (next to the fish cage) during the fallow period, but the organic enrichment footprint was still noticeable directly below the fish cage, due to the above mentioned nature of the wastes. These results are in accordance with recovery studies of benthic non-vegetated systems after guilthead sea bream and sea bass fish aquaculture abatement, where recovery takes more than a year to be complete (Karakassis *et al*. 1999; Sanz-Lázaro and Marín 2006).

Modelling tuna farming waste deposition dynamics by deriving models from salmon rearing, does not seem to be as easy as for guilthead sea bream and sea bass, since there is no data available comparing the very different settling dynamics of feed pellets and faeces. These are the main parameters that need to be studied, along with other particular characteristics of this type of aquaculture: for example, the fallowing period (different from salmon) and some environmental characteristics specific to the Mediterranean Sea, mentioned above.

To conclude this section, specific sediment traps should be designed with a wider diameter container for storing the collected sedimented material, since only in this way will all the particulate waste material released by tuna farms be collected. In this way, organic waste sedimentation rates and dispersion could be quantified more accurately. In addition, more estimates of waste dispersal and benthic impact are needed if we are to have a more realistic picture of the impact of this activity on the seabed. These studies would help to improve the management of this activity and so minimize the deleterious environmental effects that this activity may produce

In order to accurately model waste dynamics of tuna farming, great research effort is needed to quantify organic waste sedimentation rates and dispersion. For example, specific sediment traps should be designed that are capable of collecting all the particulate waste material released by tuna farms.

METHODS FOR ASSESSING FINFISH FARMING IN ACCORDANCE WITH WFD IMPLEMENTATION

The benthic impact due to marine fish farming has classically been assessed by sampling the seabed communities and their physico-chemical parameters. Although meiofaunal and bacterial assemblages (Mirto *et al*. 2002; Vezzulli *et al*. 2002) have also been used in marine fish farm environmental impact assessment, the use of macrofaunal assemblages has become the benchmark for this purpose.

Some physico-chemical analyses have demonstrated their usefulness for assessing benthic impact (e.g. AVS, redox potential, TAN, total organic C and N, total P, C:N atomic ratio and C and N isotopes), the most appropiate being those related with the consequences of organic enrichment. Both, AVS and redox potential, along with macrofaunal diversity, are among the most suitable parameters for assessing benthic fish farm impact (Giles 2008).

AVS is a measure of the principal by-product of the anaerobic bacterium *Beggiatoa*, which ubiquitously appears when oxygen depletion occurs, and has shown itself to be one of the most sensitive parameters in aquaculture benthic impact assessment (Sanz-Lázaro and Marín 2006; Aguado-Giménez *et al*. 2007). Redox potential is also a measure that correlates well with changes in macrofaunal assemblages (Kalantzi and Karakassis 2006) due to fish farm impact, providing a direct measure of the depth/thickness of the RPD layer, which, it must be borne in mind, may vary naturally according to grain size in unaltered zones. As mentioned above, grain size will influence the thickness of the RDP layer, since a coarser grain will result in a greater number of cavities between the grain particles, and so greater communication between pore water and oxygenated water from the pelagic system will occur, thus increasing the RPD layer. Likewise, a grain size analysis should be performed with redox measurements to prevent the assessment from being skewed by the natural physical characteristics of the sediment.

Implementation of the WFD has produced, on the one hand, a series of common concepts, terminologies and tools, and on the other, what can only be described as a race to develop new indices (Dauvin 2007). Indeed, a whole new suite of indices has appeared and, together with previous ones, they have been tested for suitability within the scope of the WFD. A complete review of the main indices proposed was published by (Pinto *et al*. 2008). Of all these indices, the AZTI Marine Biotic Index (AMBI) (Borja *et al*. 2000) and the biotic index Bentix (Simboura and Zenetos 2002) are probably the most widely used. Both indices are straightforward to apply. They gather species into ecological groups according to life-history traits related to the degree of sensitivity to pollution and, by means of an algorithm, provide a classification of the ecological quality status (EQS) of the ecosystem in accordance with the terminology defined in the WFD. AMBI and Bentix have been tested in different ecosystems and impacted scenarios and seem to be useful [e.g. (Pranovi *et al*. 2007; Marchini *et al*. 2008; Simboura and Reizopoulou 2008)].With regard to finfish farming impact, very few studies have been performed in the Mediterranean using indices related with WFD implementation (Muxika *et al*. 2005; Carvalho *et al*. 2006; Sanz-Lázaro and Marín 2006; Aguado-Giménez *et al*. 2007). These studies have shown the suitability of these indices, even though, sometimes not being sufficiently accurate, giving for a location different EQS depending on the index.

As with the physico-chemical tools, many of these indices are still dependent on the Pearson–Rosenberg model (1978) for organic enrichment and must be tested for other stressors, such as chemical pollution (Quintino *et al*. 2006).

Following the recommendations of the WFD, the assessment of pollution must follow an integral approach and take into account all contaminants: "In identifying priority hazardous substances, account should be taken of the precautionary principle, relying in particular on the determination of any potentially adverse effects of the product and on a scientific assessment of the risk". Marine finfish farming wastes, as mentioned above, comprise mainly organic matter and nutrients, but also metals, vitamins, medicines, hormones, etc. Therefore, any environmental assessment should take into account all these contaminants to provide a thorough and accurate environmental assessment. WFD guidelines recommend the use of bioassays with algae, daphnia, fish and other representative organisms of the ecosystem to follow environmental quality standards and protect the aquatic biota from pollutants listed in the Annex VIII (which includes metals and biocides). Sulphides are the main toxic source in the benthic system following organic enrichment. The accumulation of other contaminants, such as disinfectants (formalin and iodophors) and some metals (Cu, Zn and Cd) derived from food and antifoulants has been described in the sediments influenced by fish farms (Henderson and Davies 2000; Brooks and Mahnken 2003a; Dean *et al*. 2007). Accumulation of these, may have toxic effects on benthic organisms (Morrisey *et al*. 2000), but the Bentix and AMBI may not be suitable for assessing such contamination (Marín-Guirao *et al*. 2005).

Two or more contaminants together may have complex toxic interactions. Sulphides influence sediment toxicity in their own right: by reducing metal toxicity, by forming metal sulphide solids and/or complexes and by affecting animal behaviour (Wang and Chapman 1999). Therefore, the presence of sulphides together with metals that may produce pollution (e.g. Cu, Zn, Cd, Pb, etc.) may result in an antagonistic contamination effect, thus reducing the bioavailability of both kinds of contaminants. The same reactions may occur between sulphides and metals that are not toxicants, such as iron, which might result in the reduction of sulphide contamination (Holmer *et al*. 2005). When sulphides, iron and other metals that are contaminants occur together, predicting pollution becomes a more difficult task. The affinity of sulphides to bind iron or other metals, the concentrations of each compound and environmental parameters are some of the factors that may strongly influence the final amount of bioavailable toxicants.

Given this complicated scenario of possible interactions, measuring the concentration of each contaminant in the sediment will probably provide little information as to the possible biological effects of the contaminants (Chapman *et al*. 1998). Toxicity bioassays have been applied worldwide to assess and monitor sediment quality, since only the responses of living systems are able to integrate the complex effects of contaminants. The internationally recognised role of toxicity bioassays is related with their ability to provide quantifiable information about the potential for biological damage (toxic hazard) caused by bioavailable multi-factorial contamination. Toxicity bioassays also offer a spatial–temporal resolution that is usually considered better than that provided by other bioassessment tools (Wells *et al*. 1989).

Experiments involving sea urchin eggs and embryos are straightforward, rapid and extremely sensitive, providing results of great uniformity and accuracy. The embryonic and larval development of sea urchins is regularly used in toxicity assays for monitoring and assessing risks (Warnau *et al*. 1996). For example, the toxicity of sediment-associated contaminants has been assessed using embryos and larvae of the sea urchin *Paracentrotus lividus*, and it is known that sea urchin embryonic stages (from fertilization to gastrula) are particularly sensitive to the presence of metals, ammonium and sulphides (Cesar *et al*. 2004).

Toxicity tests using sea urchin embryos have been performed in the Mediterranean Sea related with guilthead sea bream, sea bass and tuna farming. Sea urchin larval toxicity has been shown to significantly correlate with fish farm pollution, confirming that embryo–larval bioassays using the sea urchin *P. lividus* represent a sensitive tool for describing the environmental impact of fish farming (Marín *et al*. 2007). Taking into account the advantages of toxicity tests and their recommendation by the WFD, embryo–larval bioassays with the sea urchin *P. lividus* could be used as a supplementary tool to assess the ecological quality status of benthic ecosystems influenced by aquaculture.

However, it needs to be considered that sea urchin gametes are not available in wild populations year round. Tank-based or photoperiod-manipulated urchins could be used to provide a continuous supply of gametes, but controls would be required to ensure egg quality.

The complementary use of different indices or methods based on different ecological principles is highly recommended for determining the environmental quality of a system (Dauer *et al*. 1993; Salas *et al*. 2004). This option seems preferable when assessing the EQS of an area in order to take the complexity of the ecosystem into consideration and to minimize errors (Alden *et al*. 2002; Dauvin 2007).

In order to determine EQS according to WFD guidelines, an integrative set of indicators should be used to assess the biological quality elements as well as the physico-chemical parameters, taking into account the diversity and functioning of the ecosystems and the adverse effects of all the contaminants present in them. In addition, reference values should be clearly defined for each different ecosystem. To achieve a holistic picture of the ecological status of the ecosystems affected by aquaculture, final assessment of the EQS according to WDF guidelines needs to be based on a set of tools. These could include both kinds of indices, based on species composition (e.g. Shannon-Wiener) and on life-history traits (e.g. AMBI and/or Bentix), physico-chemical parameters (e.g. AVS, redox potential and grain size analysis) and sea urchin embryo toxicity tests.

CONCLUSIONS AND RECOMMENDATIONS

Aquaculture management should work towards setting a general limit value to the amount of wastes (organic matter, nutrients, metals, drugs, etc) that a fish farm may produce, not only focusing on the biomass reared but on the physical, chemical and biological characteristics of the site and on the benthic communities that may be influenced. For each specific location, thresholds should be calculated based on the above points, perhaps following Cromey *et al*. (2002) approach. However, modeling in this area still needs improvement.

Models related with the deposition and dispersion of aquaculture particulate wastes and benthic impact could be improved in several ways (**Fig. 3**): (1) by integrating parameters that may play important roles in aquaculture waste input into the benthic system, such as the wild fish effect, (2) by obtaining more real data of deposition and dispersion dynamics for aquaculture wastes in order to reduce parameter uncertainty, (3) by tailoring a definition of the carrying/holding capacity of the different ecosystems that fits the holistic approach of the WFD in order to maintain the integrity of ecosystem's characteristics and (4) by linking the increases in aquaculture organic fluxes with the responses of the benthic communities in different affected ecosystems.

Models have mainly focused on salmon aquaculture but they can be adapted to guilthead sea bream and sea bass fish farming, because of the similarities that the rearing of these species share with salmon. Key data related with the settling velocity of feed pellets and faeces of guilthead sea bream and sea bass are available (Magill *et al*. 2006; Vassallo *et al*. 2006). However, it must be taken into consideration that guilthead sea bream and sea bass production is more or less continuous with no fallow period. Also, the particular conditions of the Mediterranean Sea should be taken into account, such us its low tidal range and the occurrence of the summer thermocline.

In order to achieve improvements in models of deposition and dispersion dynamics, deposition and dispersion rates, along with the benthic status in different ecosystems at different sites should be measured. In this way, more accurate models merging deposition dynamics with benthic status might be achieved, allowing specific thresholds to be forecast according to the physical, chemical, biological and ecosystemical characteristics of each particular site. In the mean time, models need to be used with caution, since uncertainties are always present.

In the case of tuna farming, specific sediment traps should be designed with a wider diameter container for storing the collected sedimented material, since only in this way can all the particulate waste material released by tuna farms be collected. In this way, organic waste sedimentation rates and dispersion can be quantified more accurately. In addition, more estimates of waste dispersal and benthic impact are needed if we are to have a more realistic picture of the impact of this activity on the seabed. These studies would help to improve the management of this activity and so minimize the deleterious environmental effects that this activity may produce.

In order to meet the WFD requirements, the adverse effects of all contaminants derived from aquaculture should be tested. Toxicity tests have emerged as an integrative tool for measuring the toxicity of the residues generated by marine aquaculture. In our opinion, to achieve a complete picture of the EQS of the benthic ecosystems affected by aquaculture, a suite of different tools should be employed, that should include: indices based on macrofaunal assemblages (Shannon-Wiener and AMBI or Bentix), physico-chemical parameters (AVS and redox potential, including a grain size analysis) and sea urchin embryo toxicity tests.

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