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Can macrobenthic communities be used in the assessment of environmental quality of fish earthen ponds?

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The present study was undertaken in order to analyse whether macrobenthic communities can or not be used in monitoring programmes of the environmental quality of fish ponds. Functional community analysis and biotic metrics were analysed aiming at the assessment of their effectiveness in discriminating potential impacts of fish production in these systems. The macrofaunal patterns in earthen fish ponds of the Ria Formosa lagoon showed to be influenced by the input of fish food during the production cycle and by changes in abiotic parameters caused by seasonality and fish production. Polychaetes were generally dominant considering the number of taxa and abundance. The trophic functional analysis of the benthic communities showed that the deposit-feeding functional group dominated in both areas of the ponds but within water entrance areas there was an increase of suspension-feeding, carnivory and herbivory feeding modes. The presence of less trophically mixed communities within feeding areas may be related to the relative high disturbance levels of these areas. From the biotic indicators that show a differential response to organic input in fish earthen ponds, the abundance of Capitella *spp. as well as the diversity (Shannon–Wiener and Margalef species richness), evenness (Pielou) and AMBI indices seem to be the best indicators to be used in monitoring studies in similar systems. Managers should pay particular attention when* Capitella *spp. taxa are observed within the feeding areas. Nevertheless, manipulative experiments are needed in order to test the dominance levels of* Capitella *spp. and the values of those indices that are of concern.*

Keywords: macrobenthic communities, assessment, environmental quality, *Capitella* spp., fish earthen ponds

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INTRODUCTION

Semi-intensive production earthen ponds may be compared to low water-renewal areas of lagoon systems that are submitted to continuous organic inputs (Barnes, 1980; Kelly & Naguib, 1984). If moderate organic inputs are present, it is generally reflected in an increase of growth rates of phytoplankton and macroalgae and, afterwards, of zoobenthos (Gray, 1992; Bachelet *et al.*, 2000). However, when the amounts of nutrients in a system exceed those consumed by the organisms, negative effects on benthic fauna and flora may be observed, such as changes in species composition (Pearson & Rosenberg, 1978; Beukema, 1991; Perus & Bonsdorff, 2004) and mass growth of macroalgae (Raffaelli, 1999; Sfriso *et al.*, 2001). In extreme conditions, dissolved oxygen depletion and the production of toxic products, such as H_2S (Holmer & Kristensen, 1992; Cancela da Fonseca *et al.*, 2001a) may lead to the disappearance of benthic fauna, creating an azoic zone (Heilskov & Holmer, 2001).

Usually, the quality of fish production areas is monitored in the water column using physico-chemical metrics (e.g. dissolved oxygen and nutrients). However, biological metrics show a faster and more sensitive response to changes in the quality of benthic environment and ultimately of the water column (Edgar *et al.*, 2005). Moreover, most of the times, abiotic metrics have little value unless they are related to organisms. For example, a reduction in redox potential from 100 to 0 has little significance to managers unless other information is available on how such a change affects the plants and animals in the area (Edgar *et al.*, 2005). The maintenance of macrobenthic communities within earthen ponds is very important as it is widely recognized that benthic fauna plays an important role in supplying and mineralizing organic matter (Heilskov & Holmer, 2001) but may be also a food resource for the cultivated fish (Gamito, 1997). The ability of marine benthic animals to establish and maintain themselves under certain environmental conditions is mainly determined by physiological requests, such as food intake (Boström & Mattila, 1999; Cardoso *et al.*, 2007). This is in turn influenced by the feeding pattern and the ability to make use of the food potentially available in the area (Gaston, 1987). Therefore, different feeding strategies are influenced by environmental factors (e.g. stability, particle size, water, organic content and oxygen content in the sediment) and, consequently, the distribution of trophic groups among macrobenthic communities (Snelgrove & Butman,

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1994; Sfriso *et al.*, 2001; Cardoso *et al.*, 2004; Perus & Bonsdorff, 2004). Some authors have already tested the use of macrobenthos feeding guilds' distribution to assess impacts (Cardoso *et al.*, 2004) and examined the relationships between feeding guilds and environmental variables (Arvanitidis *et al.*, 1999; Bonsdorff & Pearson, 1999; Bazaïri *et al.*, 2003; Cardoso *et al.*, 2004).

Macrobenthic communities are worldwide used as bioindicators (e.g. Diaz & Rosenberg, 1995; Belan, 2003; Cardoso *et al.*, 2004, 2007; Carvalho *et al.*, 2006b, c) and were already tested regarding effects of salmon aquaculture in the environment (Haya *et al.*, 2001; Pohle *et al.*, 2001). Nevertheless, to our knowledge no information is available concerning macrobenthic seasonal patterns in semi-intensive earthen ponds, where the organisms are under a constant nutrient input resulting from the food added during the production cycle. A better knowledge of the macrobenthic dynamics may be a valuable tool for a more proper management of these production systems. In this context, a study was undertaken during a 13-month period in order to analyse the influence of sediment organic enrichment on the temporal patterns and functional properties of macrobenthic communities in fish ponds from the Ria Formosa lagoon. Macrobenthic community structure, trophic functional analysis and several biotic metrics were tested in order to assess their effectiveness in discriminating potential impacts of fish production in earthen ponds.

MATERIALS AND METHODS

The present study was undertaken in the Olhão Fish Culture Experimental Station of the INRB, IP/IPIMAR, located in the Ria Formosa lagoon (southern Portugal; Figure 1). Sampling was carried out in two *Diplodus sargus* (L.) (white sea bream) production ponds with an area of approximately 400 m^2 and a water depth of 1.5 m. In each pond the water entrance is positioned at one edge and the water exit and the automatic feeder are positioned at the opposite edge. The ponds are filled up with seawater pumped from the water reservoir, a main tank that receives water directly from the lagoon during spring tides. The daily water turnover rate varied between 20% and 40%, depending on water temperature and fish biomass. Fish from the production ponds were fed with dry pellets for 15 minutes every hour, between sunrise and sunset, using an automatic feeder.

Macrobenthic sampling was carried out monthly during a 13-month period, beginning in September 2004 in two ponds (A and B). Two sampling campaigns were undertaken before the beginning of a *Diplodus sargus* production cycle (September and October 2004). Within each pond, two areas were established, the water entrance area and the feeding area and 9 replicate corers (9 cm internal diameter) were taken within each area. Samples were washed through a 0.5 mm square mesh sieve, and the retained material was preserved in 4% buffered formalin stained with rose Bengal, in order to distinguish the organisms. Biomass, expressed as ash-free dry weight (AFDW; ±0.0001 g) was determined per area, pond and month.

For the study of the environmental parameters, 4 corers (5 cm i.d.) were taken per area, pond and sampling period. Samples were immediately frozen until subsequent analysis. The determination of chlorophyll-*a* (chl-*a*), phaeopigments (phaeo) and organic matter (LOI) were undertaken in subsamples of the top 1 cm layer. Part of the sample was dried to 80°C until constant weight was obtained and then grounded to a fine powder. The LOI content was determined by 'loss on ignition' of dried sediment, at 450°C for 2 hours. Chl-*a* and phaeo were extracted for 24 hours with 90% acetone from the upper wet sediment layer and determined by spectrophotometry according to the equations of Lorenzen (1967) adapted by Plante-Cuny (1974).

Data analysis

Macrobenthic community structure was analysed regarding abundance (N), number of taxa (S), biomass, Margalef species richness (*d*), Shannon–Wiener diversity (*H*′) and Pielou's evenness (*J*′) indices. All variables and indices were calculated for each sampling period, pond and area. Multivariate analyses were performed using the PRIMER v5.0 software package. For the trophic group analysis, the assignment of a taxon to a group was performed by first

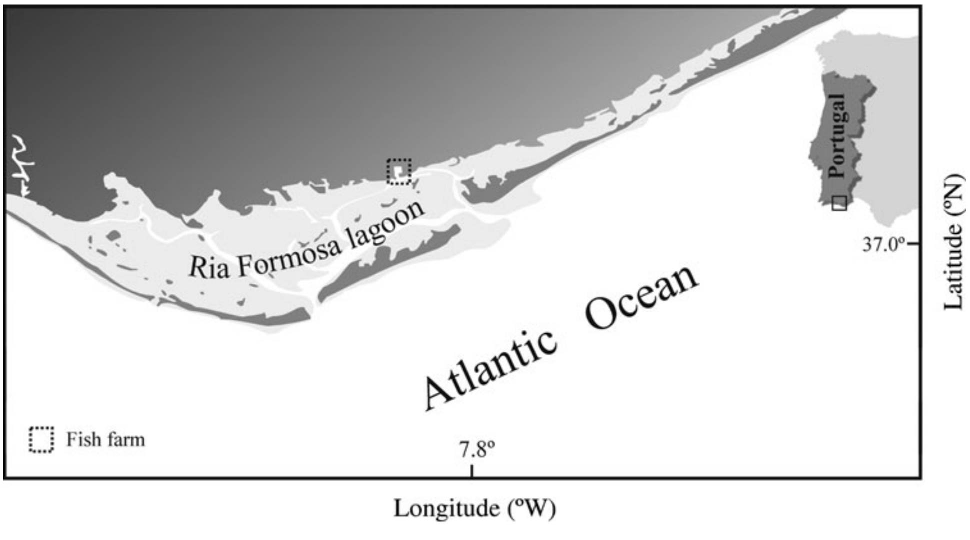

Fig. 1. Location of the Olhão Fish Culture Experimental Station in Ria Formosa lagoon (southern Portugal).

dividing the number of individuals of that taxon by the number of feeding functional groups in which it could be included (Boaventura *et al.*, 1999; Cancela da Fonseca *et al.*, 2001b). Therefore, trophic groups should be regarded as functional groups instead of taxonomic categories (Boaventura *et al.*, 1999). The identified taxa were assigned to at least one of the following feeding functional modes: filter-feeding (F), deposit-feeding (D), carnivory (C) and herbivory (H) (adapted from Sprung, 1994; Gaston *et al.*, 1998; Mancinelli *et al.*, 1998; Roth & Wilson, 1998; Gaudêncio & Cabral, 2007).

The effectiveness of twelve biotic metrics (percentage of amphipods to total faunal abundance (Edgar *et al.*, 2005); percentage of bivalves to total faunal abundance (Edgar *et al.*, 2005); percentage of gastropods to total faunal abundance (Edgar *et al.*, 2005); percentage of polychaetes to total faunal abundance (Edgar *et al.*, 2005); percentage of bivalves to total molluscs abundance (Edgar *et al.*, 2005); percentage of gastropods to total molluscs abundance (Edgar *et al.*, 2005); polychaete/amphipod ratio (e.g. Gesteira & Dauvin, 2000); abundance of *Capitella* spp. (e.g. Webb, 1996); Pielou's equitability index J'; Margalef species richness d'; Shannon–Wiener diversity H'; AMBI (e.g. Muxika *et al.*, 2005; Carvalho *et al.*, 2006a, c)) in the assessment of organic enrichment resulting from fish production in aquaculture earthen ponds was assessed by analysis of variance (ANOVA) using STATISTICA v6.

RESULTS

Environmental parameters

The analysis of the LOI content in the sediment showed that in pond B values were usually higher than in pond A (Figure 2). In both ponds, values were generally higher in F than in WE area but in pond A the opposite trend was evident from January to April. With the increasing temperature and the consequent increase in the food quantity added to the ponds, values become once more higher in the feeding area until the end of the sampling period (Figure 2). In pond B, only in February, where the lower water temperature was observed, the WE area presented higher organic matter content than the F area (Figure 2). Surprisingly, the higher values observed in both ponds were registered during the autumn–winter period, when water temperature was lower. In terms of chl-*a* in the WE area, the concentration generally increased during the sampling period with the higher values being observed from June to September 2005 (Figure 3). In the feeding zone, the pattern was not consistent in both ponds. While in pond A the trend was similar to that described for the WE zone, in pond B values fluctuated with several peaks in December–January, March, June and August (Figure 3). With regards to the phaeo concentration, values tend to increase with the production cycle with a noticeable peak observed in March in the WE areas of both ponds (Figure 3).

Macrobenthic communities

The analysis of univariate biological variables showed that the lowest values of number of taxa, abundance and biomass in both ponds were observed in September 2004 (Figures 4 & 5). Considering the number of taxa and abundance, the general trend that was observed consisted of an initial increase during winter, followed by a decrease in March and increasing again (Figure 4). While the number of taxa tended to stabilize during spring and summer, abundance showed higher

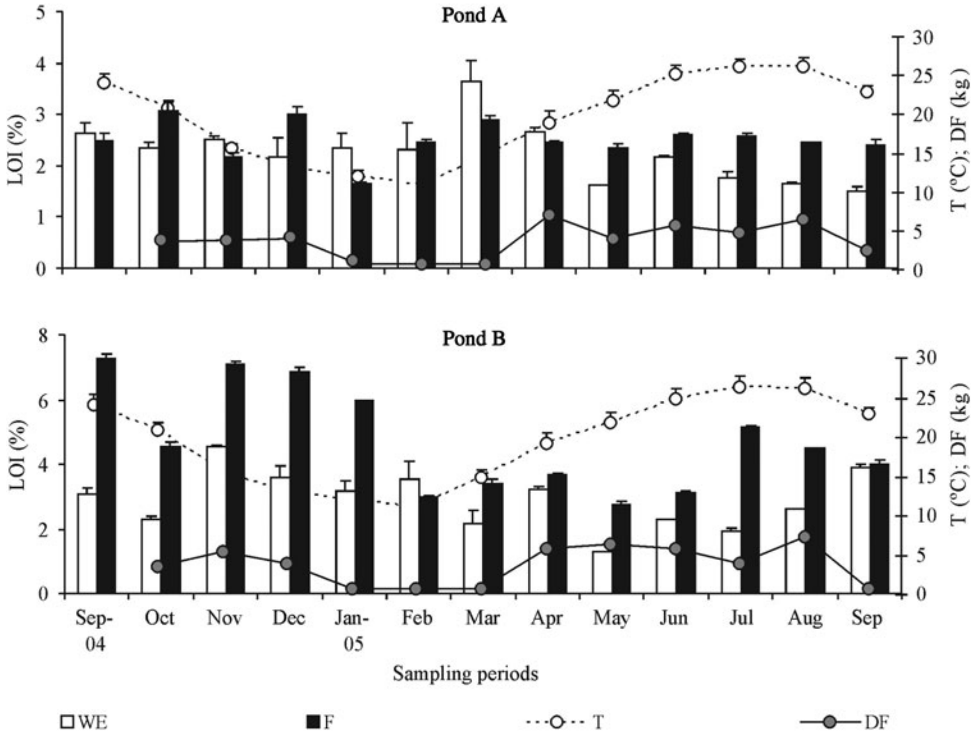

Fig. 2. Variation of the percentage of organic matter content (LOI; +SD), water temperature (T; +SD), and amount of dry food (DF) in the ponds A and B during the sampling period. WE, water entrance area; F, feeding area.

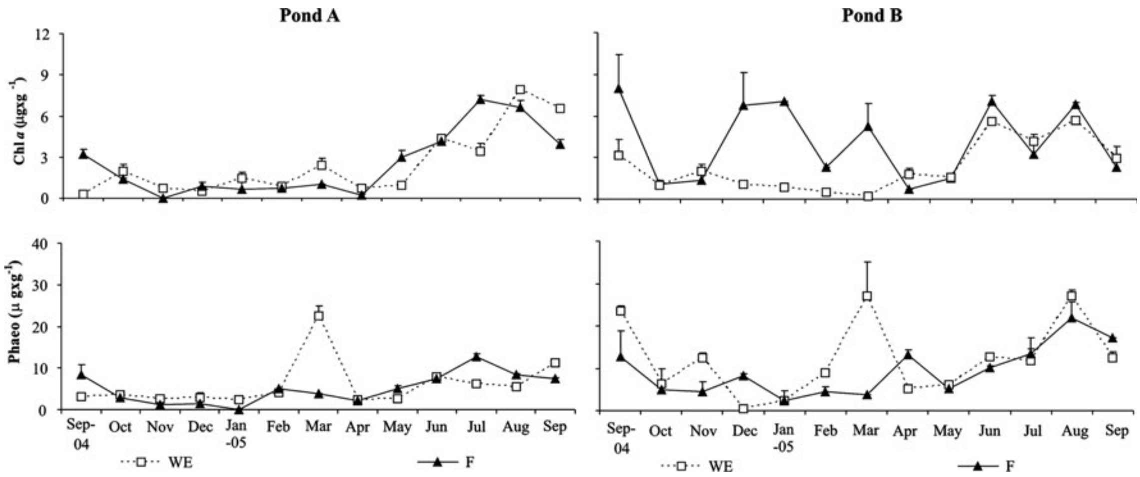

Fig. 3. Variation of the chlorophyll-*a* (chl-*a*; +SD) and phaeopigment (Phaeo; +SD) contents in the ponds A and B during the sampling period. WE, water entrance area; F, feeding area.

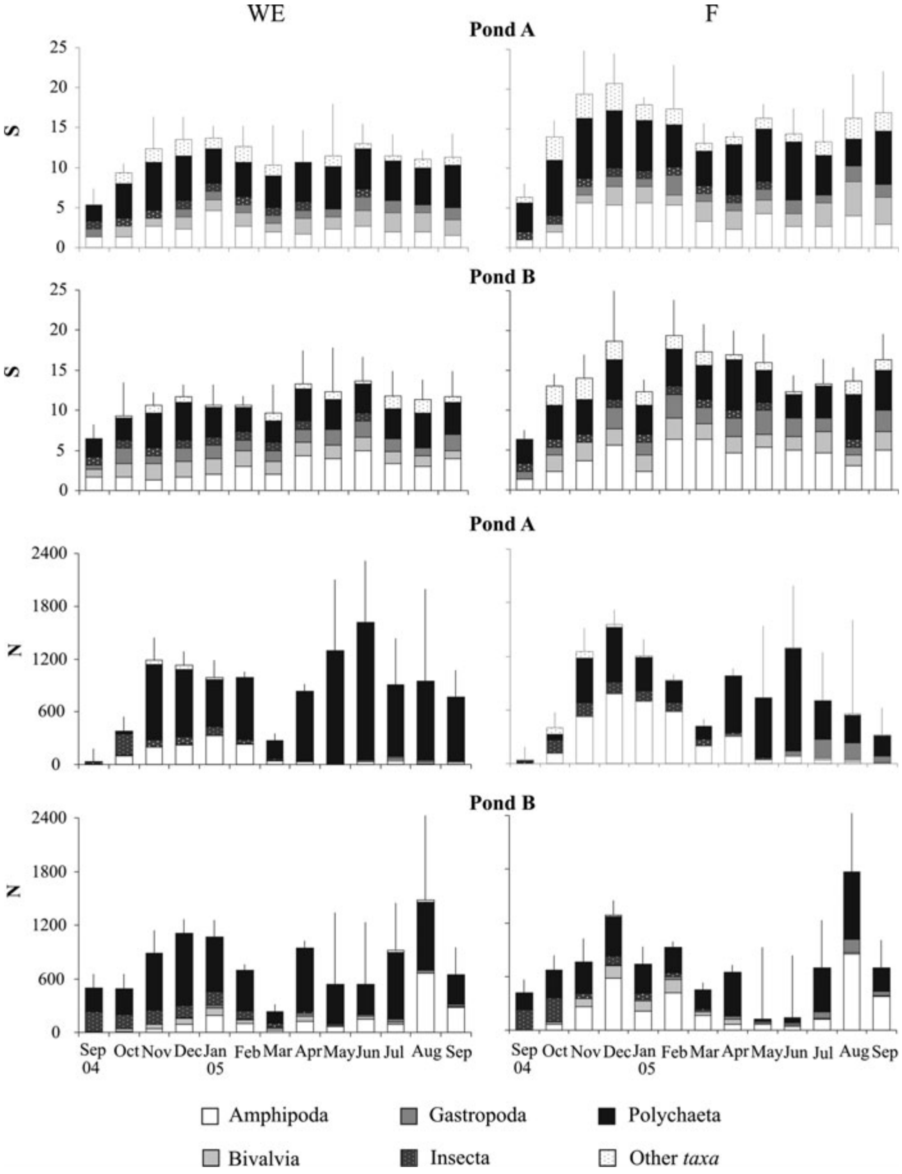

Fig. 4. Variation of the mean values of number of taxa (S, taxa × m^{-2}; +SD), abundance (N, ind. × m^{-2}; +SD) and the relative contribution of the main taxonomic groups during the sampling period for ponds A and B. WE, water entrance area; F, feeding area.

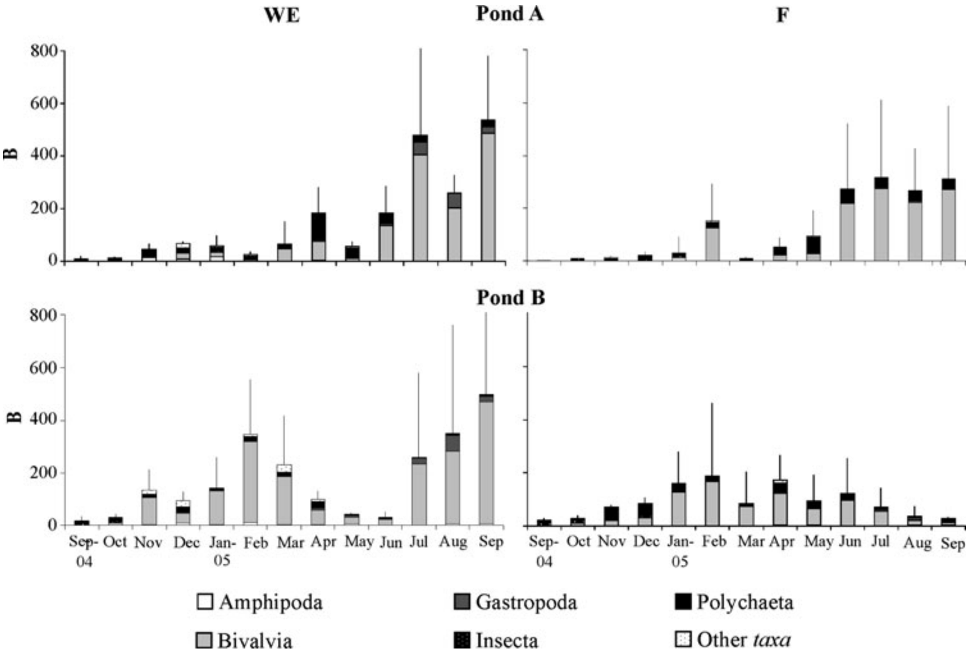

Fig. 5. Variation of the mean biomass (B, AFDW × m^{-2}; +SD) and the relative contribution of the main taxonomic groups in ponds A and B for the months analysed. WE, water entrance area; F, feeding area.

oscillations during this period (Figure 4). Regarding faunal composition, the polychaetes were in general the taxonomic group with the highest contribution for the number of taxa (Figure 4). This dominance was higher in the F than in the WE zone. For the other major taxa it was observed that the contribution for the mean number of taxa was usually higher in the WE than in the F zone (Figure 4). Concerning abundance, the same general pattern was also visible for both ponds, but the dominance of polychaetes was higher (Figure 4). In terms of abundance, this dominance was especially due to the polychaete *Capitella* spp., but also to *Hediste diversicolor*, *Neanthes caudata*, *Pseudopolydora paucibranchiata* and *Desdemona ornata*. Amphipods (mainly *Microdeutopus gryllotalpa, Corophium acherusicum* and *Ampithoe rubricata*) were the second most abundant group. A thorough analysis on the faunal composition of the analysed earthen ponds was presented in a previous paper (Carvalho *et al.*, 2006a).

In terms of biomass, a general increase in pond A both in the WE and the F zones was observed during the sampling period (Figure 5). In pond B, the highest values were registered during July to September 2005 for the WE zone, with a smaller peak in February and March, but for the F area, biomass increased until February decreasing afterwards until the end of the experimental period (Figure 5). Within the analysed system, Bivalvia was the taxonomic group with the highest contribution for the biomass observed (Figure 5).

In general, and except for abundance, the variables analysed showed higher values in the WE than in the F areas in both ponds. Abundance was generally higher in the WE than in the F zone during winter months and early spring (for pond A). In late spring and summer, the trend was inverted or in some cases with both zones showing similar values (Figure 4).

The analysis of the relative abundance of the feeding functional groups for both ponds and areas analysed puts in evidence the dominance of the deposit-feeding mode both in the WE and the F zones, considering the duration of the study (Figure 6). The filter-feeding were the second most abundant feeding mode and usually with higher percentages in the WE than in the F zone. In relation to the herbivory and carnivory, the sum of its relative abundance was higher in the WE than in the F zones and values were never higher than 18.6% in pond A (August) and 24.6% in pond B (June) (Figure 6).

In Figure 7 a set of biological metrics is presented for both zones analysed. The percentage of the main taxonomic groups in both zones did not show any significant difference between the areas (Figure 7). The ratios between Bivalvia and total Mollusca, Gastropoda and total Mollusca, as well as the ratio between Polychaeta and Amphipoda were also unsuitable for the discrimination between the two areas of the ponds (Figure 7). However, the abundance of the opportunistic polychaete *Capitella* spp. and the marine biotic index AMBI were significantly lower in the WE than in the F areas (Figure 7). On the other hand, the Pielou's eveness (J'), Shannon–Wiener diversity (H') and Margalef species richness (d) were significantly lower in the F zones when compared to the WE zones (Figure 7).

DISCUSSION

The macrofaunal patterns in earthen fish ponds of the Ria Formosa lagoon were already shown to be influenced by the input of fish food during the production cycle and the changes in abiotic parameters caused by seasonality and fish production (Carvalho *et al.*, 2007; Serpa *et al.*, 2007).

The distribution of macrobenthic communities is controlled by a variety of environmental (e.g. habitat characteristics, water

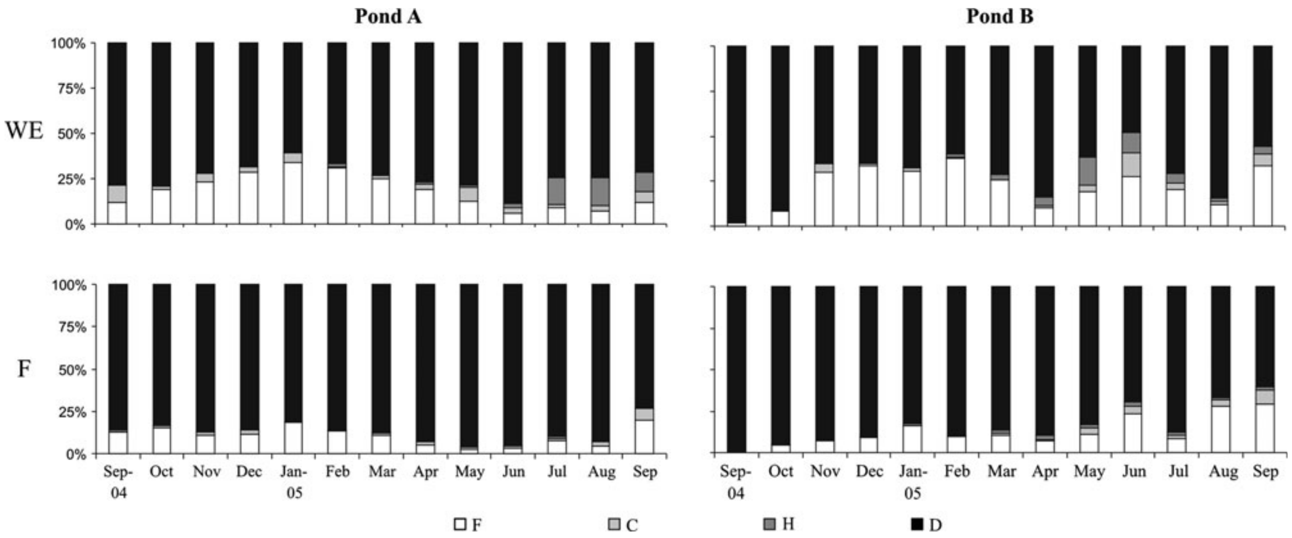

Fig. 6. Variation of the relative contribution of the different functional trophic groups in ponds A and B for the months analysed. C, carnivory; H, herbivory; F, filter-feeding; D, deposit-feeding; WE, water entrance area; F, feeding area.

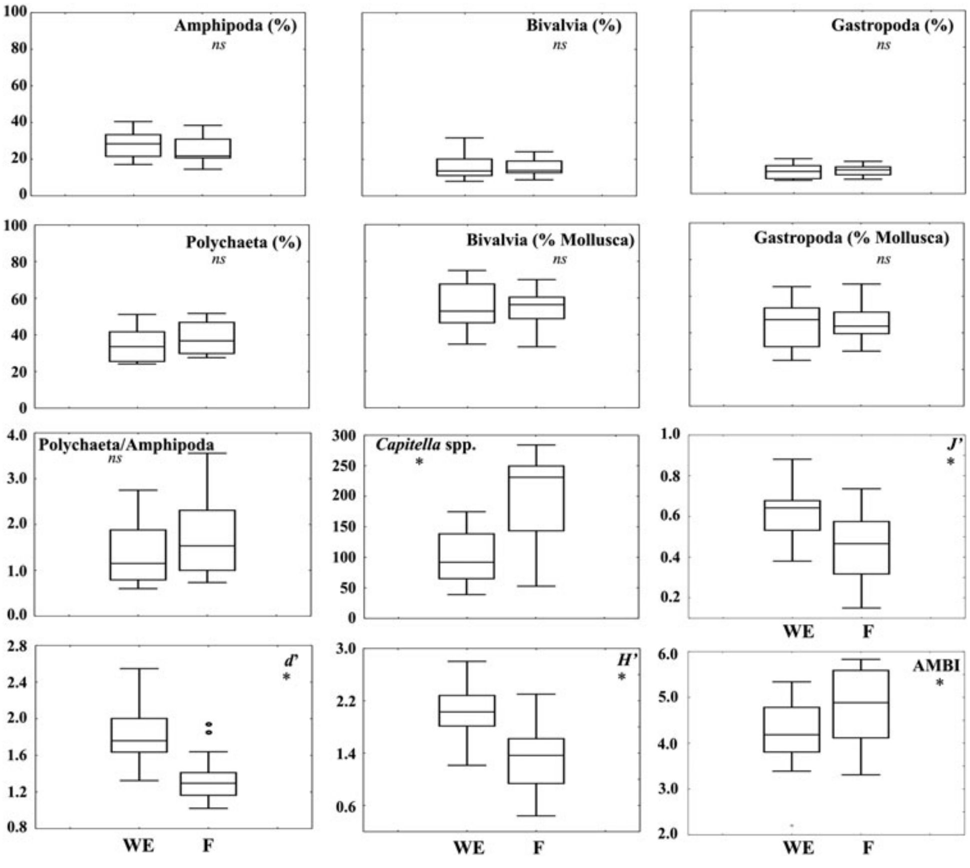

Fig. 7. Box plots showing median, box edges at first and third quartiles, and outlying data points for several biotic metrics. WE, water entrance area; F, feeding area; *, significant differences at $P < 0.05$.

quality, sediment quality and food) and biological factors (e.g. competition and predation) (Peeters *et al.*, 2004 and references therein). Both abiotic and biotic factors play a role in shaping macrobenthic communities (Angermeier & Winston, 1998), the former being often regarded as determinant for large scale patterns and the latter for local scales (Menge & Olson, 1990; Levin, 1992). The macrobenthic communities of the studied systems seem to reflect both factors. The communities were characterized by a small total number of taxa, high variability in abundance, and dominance of a few taxa, which is typical of some Mediterranean and Atlantic lagoon systems (Gamito, 1989; Arias & Drake, 1994; Bachelet *et al.*, 2000; Costa *et al.*, 2003). The isolation of the production areas that do not communicate directly with the source population (water reservoir) cause constraints to the colonization process. Moreover, the high amount of organic matter that is characteristic of these systems is also restrictive to more sensitive species. Polychaetes were generally dominant both in terms of number of taxa and abundance. In terms of abundance, this dominance was especially due to *Capitella* spp., but also to other polychaete species such as *Hediste diversicolor*, *Neanthes caudata*, *Pseudopolydora paucibranchiata* and *Desdemona ornata*. Amphipods (mainly *Microdeutopus gryllotalpa, Corophium acherusicum* and *Ampithoe rubricata*) were the second most abundant group.

The analysis of the benthic environment in fish earthen ponds indicated that the localized introduction of commercial food during the production cycle has clear consequences both in abiotic and biotic parameters. These changes were supported by the differences observed in water entrance and feeding areas both in community structure, biotic metrics and organic matter and pigments in sediment. Severe impacts of fish production have been already documented namely under fish cages (e.g. Johannessen *et al.*, 1994; Karakassis *et al.*, 1999). Organic enrichment has been shown to have a significant effect on diversity and macrobenthic composition (Perus & Bonsdorff, 2004; Carvalho *et al.*, 2006a, 2007). Usually, at the beginning, macrobenthic populations seem to be favoured, reflecting a combination of the nutritional value of moderate amounts of organic matter present in the sediment (Magni, 2003). This was the basis of the intermediate productivity hypothesis (IPH) (Grime, 1973a, b) that predicted maximum species diversity at intermediate level of productivity, due to the reduction of the competition for food, promoting the existence of potentially competing species (Widdicombe & Austen, 2001). A similar model was proposed by Connell (1978) named the intermediate disturbance hypothesis (IDH). However, an excessive disturbance effect (in the present case organic load) exposes the benthic animals to physiological stress (Diaz & Rosenberg, 1995; Gray *et al.*, 2002), and may lead to a decline in the biological variables (number of taxa, abundance and biomass) (Magni, 2003). The oscillations of the number of taxa during the study period indicated a similar pattern for water entrance and feeding areas although with lower values in the latter. Therefore, after one year of production, the benthic environment of the fish ponds did not present signs of high disturbance. This seems to indicate that the amount of food added was appropriate to the amount of produced fish.

In terms of the functional analysis of the benthic communities by trophic guilds, the present systems were dominated by the deposit-feeding functional group, mainly annelids, which is characteristic of fine soft-bottom areas of European and North American estuaries (Boesch, 1973; Gaston *et al.*, 1988; Boaventura *et al.*, 1999; Bachelet *et al.*, 2000; Cardoso *et al.*, 2004; Carvalho *et al.*, 2005; Gaudêncio & Cabral, 2007). Many deposit feeders have an opportunistic behaviour presenting higher growth and reproduction rates as well as survival in favourable environmental conditions, such as an increase of food (Pearson & Rosenberg, 1978; Rossi, 2003). Although there was clear dominance of the deposit-feeding mode in the fish ponds, within water entrance areas there was an increase of suspension-feeding, carnivory and herbivory, when compared to the feeding areas. The decline of suspension-feeding and the increase of deposit-feeding functional groups resulting from the increase of the organic input to the sediment were already described by Pearson & Rosenberg (1978) for macrobenthic communities, regardless of the type of organic material responsible for the enrichment (Widdicombe & Austen, 2001). The higher relevance of the trophic functional groups other than deposit-feeding at the WE agrees with the shift from grazing to detritus pathways from the less to the more eutrophic areas inside brackish estuarine/lagoon systems. As pointed out by Cancela da Fonseca *et al.* (2001b) the existence of gradients of trophic functions in these systems may represent the best way the communities can adapt to exploit the existing resources as a response to physical gradients. The presence of less trophically mixed communities was also indicated by Gaston & Nasci (1988) as being probably related to higher disturbance levels, which is in agreement with the observations in the present study. However, according to Dauer (1984), caution is necessary, namely when using polychaete feeding guilds as biological variables, since in estuarine-like systems, physical and chemical factors along with large biological disturbance may override and obscure the importance of trophic factors. Moreover, the assignment of feeding types to each species is sometimes ambiguous and not consensual (Chardy & Clavier, 1988), making it difficult to use this methodology.

Severe impacts on benthic environment due to organic input in fish production areas are of major concern to farm managers because of its relationship with fish health. Edgar *et al.* (2005) tested the effectiveness of a variety of biological metrics in assessing fish farm impacts at different distances from production cages. These authors indicated the ratio of bivalves to total molluscs as the biological metric that best fitted the results obtained. Significantly lower values of this ratio were observed from reference to compliance sites. The use of molluscs as bioindicators of organic enrichment had been already indicated by Brooks & Mahnken (2003). However, in the present study the bivalve to total molluscs ratio did not allow the discrimination between feeding and water entrance areas. A likely reason for the disagreement of the results of both works is the existence of bivalves that are tolerant to organic enrichment. This is the case of *Abra ovata*, a quite abundant species in the fish ponds, which showed a preference for the feeding zone. In the present study, the most sensitive indicators between feeding and water entrance areas were the abundance of the opportunistic polychaete *Capitella* spp., diversity (both as Margalef species richness and Shannon–Wiener), Pielou's eveness, and AMBI indices. Although the ratio of bivalves to total molluscs had been indicated by Edgar *et al.* (2005) for the south-eastern Tasmania region as a suitable biological metric to be used in monitoring studies, the same authors also stated that the most sensitive univariate indicator of the broad-scale effects of fish farming seems to be the difference between the total density of a set of species positively correlated with farm impacts and the total density of a set of negatively correlated species. Different results may be observed according to the typical fauna of production areas and the analysis of a wide variety of biological indices should be undertaken in order to discriminate the best metrics to be used in monitoring studies for a certain area. Despite the clear dominance of the polychaete *Capitella* spp. in the feeding area, the co-existence of other species indicates that in the present production conditions, the system is not in a high healthy risk.

A common approach to assess risks to ecosystem health is the identification of stressors and their potential effects through the use of indicators (Fisher *et al.*, 2001). A good indicator should be amenable to measurement and preferably easy to measure (Hyland *et al.*, 2005) but also should be meaningful to decision-making with respect to the risk of concern (Fisher *et al.*, 2001). From the biotic indicators that show a differential response to organic input in fish earthen ponds, the abundance of *Capitella* spp. as well as the diversity (Shannon–Wiener and Margalef species richness), evenness (Pielou) and AMBI indices seem to be good indicators to be used in monitoring studies in similar systems. Managers should have particular attention if this polychaete species is the only species observed within the feeding areas. Nevertheless, manipulative experiments are needed in order to test the dominance levels of *Capitella* spp. and the values of those indices that are of concern. Overall, macrobenthic communities, as shown for other production systems or anthropogenic disturbances (e.g. Pohle *et al.*, 2001; Dernie *et al.*, 2003; Sánchez-Moyano *et al.*, 2004; Cardoso *et al.*, 2007) can also have a relevant role in monitoring programmes to assess the environmental quality of aquaculture fish ponds.

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