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Do benthic biofilters contribute to sustainability and restoration of the benthic environment impacted by offshore cage finfish aquaculture?

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ABSTRACT

Benthic biofilters were deployed under a cage fish farm and in two reference locations to assess the influence of the farm on the biofilters and the surroundings, as well as to verify the usefulness of this technology as a mitigation tool. The biofilters underneath the farm recruited a fouling community practically identical to that of the control biofilters, which included a variety of trophic strategies. The former showed a higher ^{15}N enrichment, indicating that fouling beneath the farm was benefiting from the farm waste. The waste retention efficiency was low (0.02 g N m^{-2} $month^{-1}$) beneath the farm. Benthic biofilters aggregated demersal wild fish around and within them. Pelagic wild fish also frequently used the biofilters beneath the farm, forming compact shoals around them. The increased complexity of the habitat below the fish farm enhanced biodiversity, but this improvement did not lead to the recovery of the sediments around the biofilters.

1. Introduction

The influence of organic waste derived from marine fish cage aquaculture on the benthic environment has always been considered as the main concern in aquaculture–environment interactions (Brooks et al., 2002). Several attempts have been made to prevent, ameliorate or remedy these effects, e.g. air-lift systems to collect waste, sediment dredging, the dispersal of waste by mean of submersed mixers, harrowing the seafloor, etc. (Beveridge, 1987 and references therein), but none of these methods can normally be considered acceptable under offshore conditions (Angel and Spanier, 2002).

Increased nutrient and food availability in cage aquaculture areas stimulate the proliferation of high diversity hard-substrata epibenthic communities attached to infrastructures such as cages, nets, ropes and buoys (Bongiorni et al., 2003; Sarà et al., 2007). Also, wild fish aggregate around fish farms due to food and shelter availability, which has been postulated as an "ecosystem service" (Dempster et al., 2009), since both benthic organisms and wild fish consume dissolved nutrients, surplus feed and feces, recycling waste and reducing particulate sedimentation around farms. In view of these facts, the use of artificial structures as biofilters in the pelagic (Cook et al., 2006; Tsemel et al., 2006) and benthic (Angel et al., 2002; Gao et al., 2008) environments has been investigated for the purpose of mitigating a farm's environmental impact.

The deployment of artificial structures on the seafloor can stimulate biological productivity around them (Bohnsack and Sutherland, 1985; Bombace, 1989), even in the surrounding soft bottom infaunal communities (Ambrose and Anderson, 1990; Barros et al., 2001; Danovaro et al., 2002). The application of artificial reefs to cage aquaculture for the purpose of mitigating the environmental impact has been evaluated in the Red Sea (Angel et al., 2002) and in the China Sea (Gao et al., 2008). Angel et al. (2002) found a greater wild fish aggregation around artificial reefs deployed under the cages as compared to control reefs. They also found a huge fouling biomass attached to the reefs, but the differences between farm and control reefs were inconsistent. In addition, these authors did not find significant changes in the organic matter content of the sediments around the reefs. Conversely, Gao et al. (2008) reported a slight but significant improvement in the sediment biotic and abiotic conditions around artificial reefs deployed beneath a fish farm.

In light of these findings, we planned the present work under the assumption that the presence of a fish farm will influence the aggregation of fauna on benthic artificial structures deployed underneath. Increased structural and trophic complexity of the benthic habitat around an offshore finfish aquaculture facility should favor the colonization of benthic and nektonic organisms, which could participate in the reutilization of fish culture-derived waste, thereby improving the benthic environment and mitigating the environmental impact. To this end, benthic biofilter-like artificial reefs were deployed in a Mediterranean fish farming area. The

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aim was to ascertain the influence of cage fish farming on these benthic biofilters and their immediate surroundings, and to assess whether this technology could be effective in reducing seabed degradation, enhancing the recycling of waste as a result of increased biodiversity.

2. Material and methods

2.1. Manufacture of benthic biofilters

The design of the benthic biofilters (hereafter BBs) was closely based on those of Angel et al. (2002). Each BB was formed by 28 cylinders (40 cm diameter, 210 cm length) arranged in a triangular pyramid formation. Each cylinder was made from a roll of 5 mm-thick 50 mm black mesh high-density polyethylene (HDPE), reinforced with 6 polyvinyl chloride (PVC) rings. The cylinders were held together with plastic tie-wraps and 1.5 mm cotton string. The pyramid was placed on a reinforced-concrete base ($250 \times 250 \times 20$ cm) so that the final dimensions were $250 \times 250 \times 240$ cm (Fig. 1). Six BBs were constructed in our workshop.

2.2. Study area and fish farm facilities

The study area is located off the coast of San Pedro del Pinatar (Murcia, SE Spain). The seabed consists of a detrital sedimentary floor with a very low slope (<2%) and 37–38 m depth. A cage fish farm (Fig. 1) was located 4.8 km east of the coast (37°48.941′ N; 00°41.731′ W), a site with a high degree of exposure to dominant wind and wave regimes. It consisted of 18 offshore sea cages, with a diameter of 16 m and a net depth of 15 m (approximately 3000 m^3 per cage) and a maximum authorized production of 810 tons of gilthead seabream (*Sparus aurata*) and meagre (*Argyrosomus regius*) per year. Fig. 2 shows the evolution of fish biomass and food supplied during the study period.

2.3. Experimental and sampling design

An asymmetrical design (Underwood, 1993, 1997; Glasby, 1997) was used with one impacted and two control locations. Two BBs were deployed in May 2006 in each sampling location: under the seacages (hereafter BI: BI-1 and BI-2) and in two control locations (hereafter BC: BC1 and BC2) situated 1 and 1.3 km downstream from the fish farm. BBs within a given pair were placed 150 m apart (Fig. 1), a distance considered sufficient for them to be independent from one another. Sampling was restricted by financial resources and as a result, only one BB in each pair was monitored (always the same one: BI-1, BC-1.1 and BC-2.1, as shown in Fig. 1), except for in the case of wild fish assemblage monitoring, for which both BBs in each location were sampled. We planned for a three-year study, but in autumn 2007, the company changed ownership and the facilities were progressively remodeled and the cages emptied; as a result, the study finished earlier than expected (summer 2007). All samples were taken by scuba divers. The following four aspects were studied in each area.

2.3.1. Particulate matter

Starting in the summer of 2006, and then at six-month intervals, four sediment traps were suspended from one BB in each study area. Each sediment trap consisted of four vertical PVC pipes

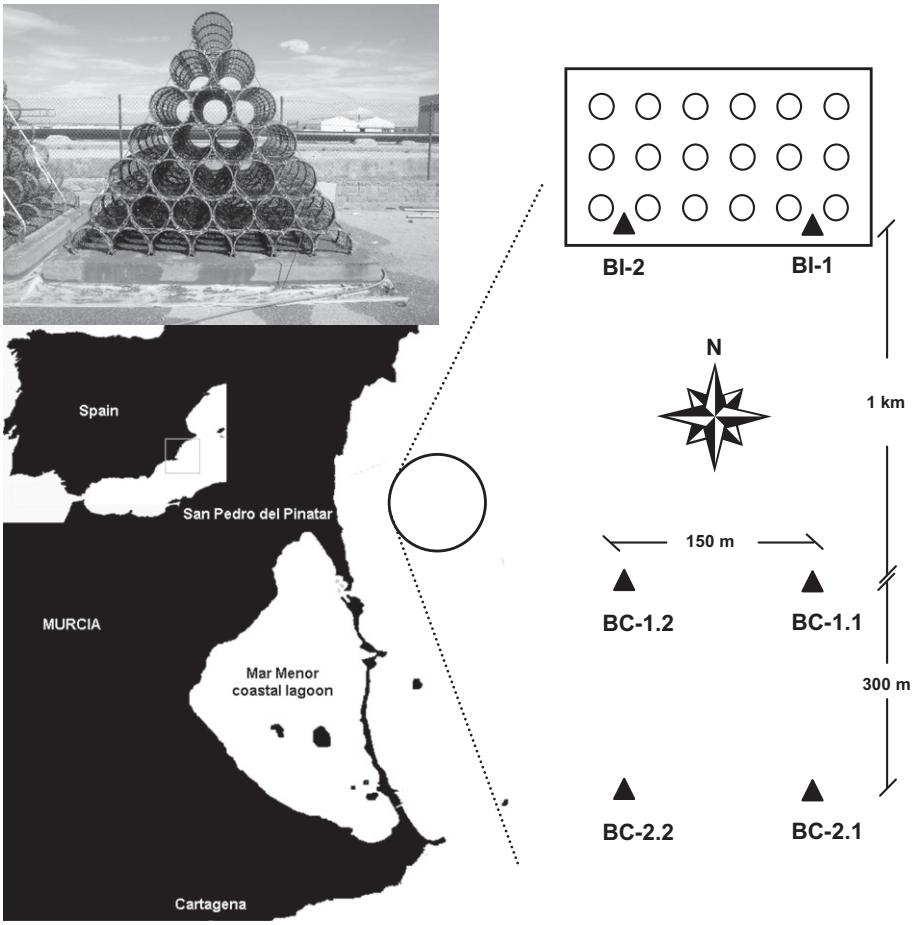

Fig. 1. Benthic biofilter design, localization of the study area and layout of the sampling stations.

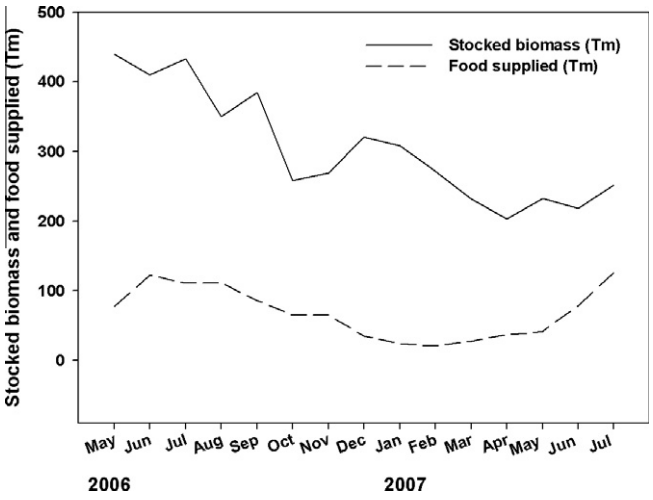

Fig. 2. Stocked fish biomass and food supplied through the study.

measuring 140 mm in diameter and 1 m in length, open at the top and funnel-shaped at the bottom, below which sample containers (glass test tubes) were attached with a rubber band. The traps remained suspended 1.5 m over the BBs for 48 h after which time the samples from each trap (four tubes) were pooled and treated as one replicate. Total particulate dry weight matter flux (TPM: $g\,m^{-2}\,d^{-1}$), total nitrogen (TN; considered only to estimate the farm-derived N retention) (elemental autoanalyzer LECO 932), and ^{15}N isotopic composition (Finnigan MAT Delta Plus mass spectrometer) were analyzed. The ^{15}N isotopic composition was expressed as:

$$\delta^{15}N(‰) = \left[(R_{sample}/R_{standard}) - 1\right] \times 10^3$$

where $R = {}^{15}N/{}^{14}N$ and atmospheric N_2 was the standard, with an analytic precision of 0.1‰. The ^{15}N isotopic composition in the fish food used in the farm throughout the study was determined in summer 2006 and 2007.

2.3.2. Fouling community

Every BB was provided with sample units consisting of 30×30 cm squares made of the same HDPE mesh as the BBs, and attached to the external sides of the BBs using plastic tie-wraps. The approximate surface of the sample units was 0.113 m^2. Each season, starting in summer 2006, four sample units were randomly taken from one (always the same) of each pair of BBs at each location. The macrozoobenthos were carefully removed from the sample units and identified at the family level. Colonial taxa (sponges, bryozoans and some cnidarians and polychaetes) were not included in this analysis. Total fouling biomass per surface unit ($g\,m^{-2}$ d.w.) was also considered, and $\delta^{15}N$ isotope composition of the fouling was analyzed and expressed as above.

2.3.2.1. Estimate of farm-derived N retention by fouling. Assuming that the control locations were not influenced by fish farm waste, the TN in TPM and fouling would represent the natural N background. By knowing the TN concentration and the stable isotope ^{15}N signature ($\delta^{15}N$) for TPM and the fouling community in the impacted and control locations, it is possible to estimate farm-derived N retention by fouling in BI. Considering that the isotopic partition between TPM and the fouling community was the same at BI and at BCs, the proportion of TN in fouling derived from TPM in BI was estimated according to the model proposed by Phillips (2001):

$$\delta^{15}N_{fouling} = B \cdot \delta^{15}N_{TPMb} + F \cdot \delta^{15}N_{TPMf}; \quad B + F = 1;$$

where B and F are the fractions of background and farm-derived TPM, respectively, and $\delta^{15}N_{TPMb}$ and $\delta^{15}N_{TPMf}$ are their respective nitrogen isotopic signatures.

2.3.3. Wild ichthyofauna

The wild fish population associated with the BBs was assessed by means of a visual census according to the methodology described by Harmelin (1987), assigning the observed fishes to log_2-abundance classes. The sampling volume considered around the BBs measured 5 m in diameter and 10 m over the seabed (approximately 785 m^3). Fishes were identified to the species level. Visual censuses were conducted every season from summer 2006 on by the same scuba diver for all the BBs, with two replicates being recorded for each sampling location and campaign.

2.3.4. Sediment

For sediment physical–chemical characterization, samples were taken with methacrylate hand corers (53 mm in diameter, 300 mm in height), using the top 5 cm for the analyses. Grain size distribution (Buchanan, 1984) and acid volatile sulfides (AVS-S; Allen et al., 1993) were determined. For the former, the finest fraction (clay and mud (CM) <0.064 mm) was selected as the descriptor. To investigate the sediment macrobenthic infauna, samples were taken with a hand grab (stainless steel box; $20 \times 20 \times 10$ cm) and then placed directly into a 500 μm mesh sieve sack and closed with a tie-wrap so that the sample was sieved as the divers ascended. The material retained was preserved in a buffered 5% formalin-sea water solution. In the laboratory, the macrofauna from the preserved samples were sorted. Polychaete assemblage was selected because of its sensitivity to organic enrichment in soft bottoms (Salas, 1996), and the taxonomic resolution used was the family level, in accordance with Lampadariou et al. (2005). Upon deployment of BBs in May 2006 (pre-operational: PO) and then every six months from summer 2006 on, four replicates were randomly taken inside a 5 m radius circular area around one of each pair of BBs for physical–chemical and biological sediment variables.

2.4. Statistical procedures

2.4.1. Univariate analysis

An asymmetrical analysis of variance (asymm-ANOVA) (Underwood, 1993, 1997; Glasby, 1997) was run for univariate data (particulate matter flux and its $\delta^{15}N$ isotope composition, fouling biomass and its $\delta^{15}N$ isotope composition, and sediment geochemical variables) after $\ln(x+1)$ transformation to minimize heterogeneity of variances (Levene's test) when necessary. The model took into consideration two factors: Time of sampling campaigns (SC: 3 levels for particulate matter variables; 4 levels for sediment variables; 5 levels for fouling variables; random) and Location (L: asymmetric with 2 levels, 1 impacted and 2 control locations; fixed and crossed with SC), with $n = 4$ observations. Location variability was decomposed into impacted *vs.* controls (BI *vs.* BCs) and among the controls (BCs).

2.4.2. Multivariate analyses

All multivariate analyses were based on the respective Bray–Curtis similarity matrixes (Bray and Curtis, 1957). To visualize multivariate patterns, data were subjected to non-metric multidimensional scaling (n-MDS) ordination for the mean abundance values of each location (BBs) and sampling campaign (SC) combination, and then were 2D plotted. Asymmetrical permutational multivariable analyses of variance (PERMANOVA, Anderson, 2001) were performed for multivariate data. A fourth-root transformation was applied to fouling community and sediment polychaetes data, and $\ln(x+1)$ to ichthyofauna data (fish abundance was estimated on a log basis; Harmelin, 1987), in order to

downweight the contribution of dominant species to the similarities calculated between samples (Clarke and Warwick, 2001). An asymmetrical PERMANOVA was run in the same way as described above for the univariate tests ($n = 2$ replicates for ichthyofauna). If any significant difference with respect to multivariate structure was detected, then the SIMPER procedure (Clarke, 1993) was performed to identify the most significant taxa for differentiating among locations and sampling campaigns. All multivariate data were analyzed using PRIMER 6 (PRIMER, 2006) and PERMANOVA v1.6 (Anderson, 2005) computer software packages and Excel worksheets.

3. Results

3.1. Particulate matter

The results for particulate matter are shown in Fig. 3A and B, and the statistical results in Table 1. TPM flux (Fig. 3A) was always greater in BI, although no significant differences were detected. In BI, the highest TPM fluxes were observed in the summer campaigns, especially in summer 2007. This could be due to the relative excess feed supplied with respect to the biomass of the stocked fish, as the amount of feed supplied in the summer of 2006 and 2007 was more or less the same (around 120 Tm), even though there were about 200 Tm less fish in 2007 (see Fig. 2). With regard to TPM flux, the asymm-ANOVA only detected statistical differences between BCs over time ($P < 0.05$) due to a contrasted peak in BC1 in summer 2007. The ^{15}N signature (Fig. 3B) was also always higher in BI. The asymm-ANOVA showed significant differences between BI and BCs over time ($P < 0.001$). While $\delta^{15}N$ was almost constant in BCs, it tended to increase in BI prior to the summer campaign of 2007.

Table 1
Results of the asymmetrical ANOVA of particulate matter variables with locations (BI *vs.* BCs) and BCs, and sampling campaigns (SC). All variables were Log($x + 1$) transformed prior to analysis.

Variable	Source of variation	d.f.	F_{denom}	MS	F	P
TPM flux	L	2	SC × L	0.1155		
	BI *vs.* BCs	1	[a]BCs	0.2026	7.11	n.s.
	BCs	1	SC × BCs	0.0284	0.68	n.s.
	SC	2	SC × L	0.1416	4.03	n.s.
	SC × L	4	Resid. L	0.0351		
	SC × (BI *vs.* BCs)	2	SC × BCs	0.1273	3.04	n.s.
	SC × BCs	2	Resid. BCs	0.0418	12.18	***
	Resid. L	27		0.0038		
	Resid. BCs	18		0.0034		
	Total	35				
$\delta^{15}N$	L	2	SC × L	0.0135		
	BI *vs.* BCs	1	[a]BCs		No test	
	BCs	1	SC × BCs	0.0004	0.00	n.s.
	SC	2	SC × L	0.0206	24.28	**
	SC × L	4	Resid. L	0.0008		
	SC × (BI *vs.* BCs)	2	SC × BCs	0.0206	79.00	***
	SC × BCs	2	Resid. BCs	0.0002	0.00	n.s.
	Resid. L	27		0.0010		
	Resid. BCs	18		0.0106		
	Total	35				

$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[a] No test unless SC × (BI *vs.* BCs) was not significant ($P > 0.25$). If so, F_{denom} would be BCs.

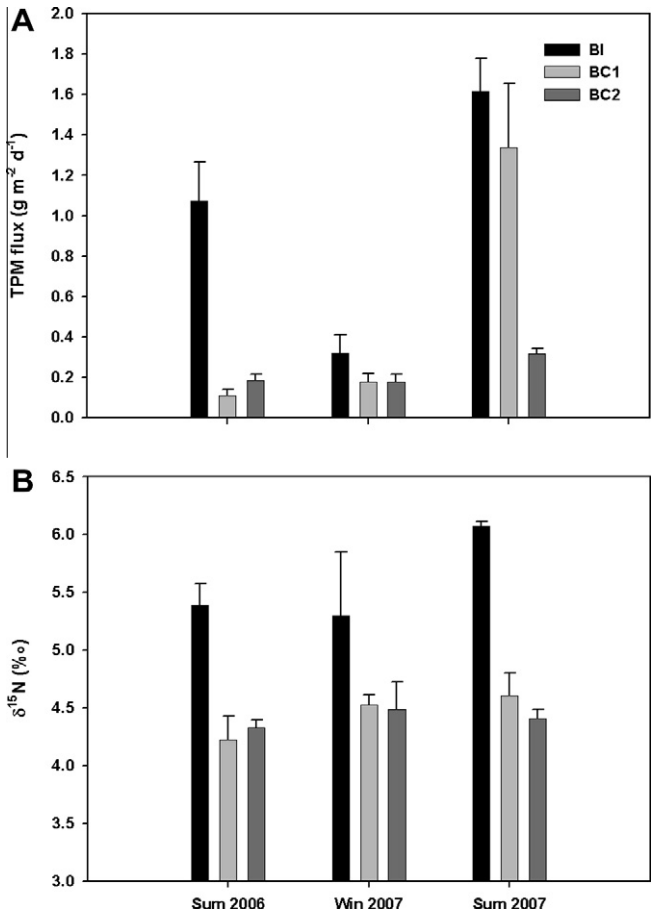

Fig. 3. Total particulate matter (TPM) variables. (A) TPM flux; (B) nitrogen stable isotopic ($\delta^{15}N$) composition of TPM.

3.2. Fouling

The evolution of fouling biomass is shown in Fig. 4A, and Table 2 shows the results of the statistical analysis. In general, the fouling biomass increased significantly ($P < 0.001$) during the first four sampling campaigns. In summer 2007, the biomass showed a drop in all BBs. The amount of biomass varied among the locations without any consistent pattern. The fouling biomass over time was different between BI and BCs ($P < 0.001$). However, an asymm-ANOVA did not ascertain significant differences ($P > 0.05$) between BCs over time with regard to fouling biomass, despite the fact that each BC showed a distinct behavior from the others. In fact, the data suggest that the fouling biomass in each BB established its own pattern. The average fouling biomass for all sampling campaigns was lower in BI (216.28 g m^{-2}) than in BC1 (244.73 g m^{-2}) and BC2 (235.01 g m^{-2}), but this difference was not statistically testable (Table 2).

The fouling $\delta^{15}N$ signature (Fig. 4B) was higher in BI and the difference over time between BI and BCs was statistically significant ($P < 0.001$). In BI, $\delta^{15}N$ increased until spring 2007 and then decreased during the last sampling campaign, while BCs showed an erratic pattern. The mean $\delta^{15}N$ composition of the fish food pellets supplied during the study changed slightly; it averaged 7.41 ± 0.01‰. Table 3 shows the input values and results estimating the farm-derived N fraction retained by the fouling community in BI. Of the fouling total nitrogen content, 16% was derived from the farm, which reflects a retention rate of 0.02 g N m^{-2} $month^{-1}$.

A total of 67 non-colonial macrozoobenthic families were recorded: 23 mollusks, 22 crustaceans, 18 polychaetes, 1 echinoderm, 1 cnidarian, 1 sipunculid and 1 tunicate. Appendix A shows the average abundance of fouling fauna. The n-MDS plot (Fig. 5A) shows that the structure of the fouling community varied over time in all BBs due to changes in abundance and the progressive incorporation and disappearance of families. However, a PERMANOVA (Table 2) failed to show any significant differences for any source

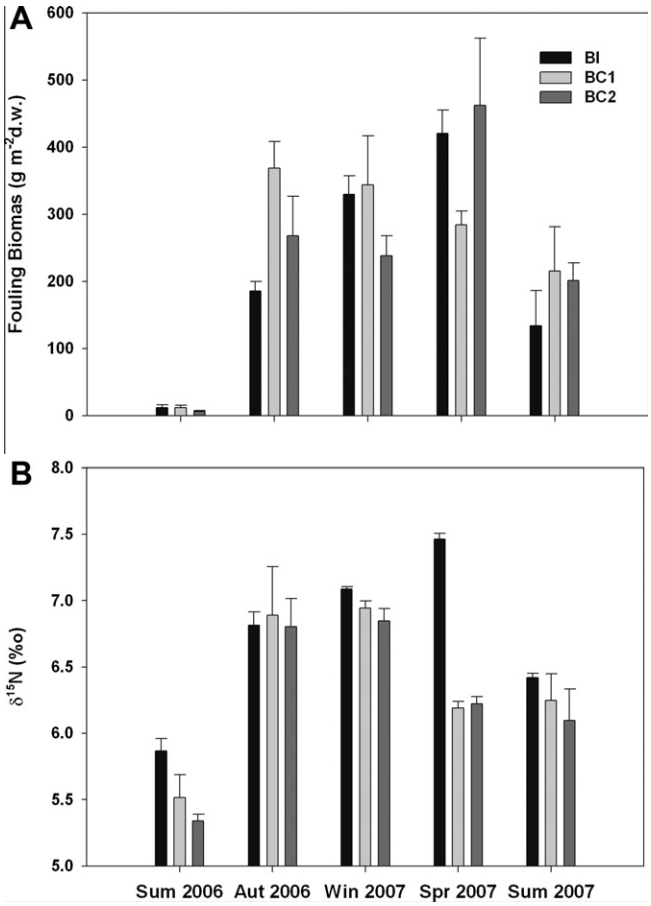

Fig. 4. Fouling physic-chemical variables. (A) Fouling biomass evolution; (B) nitrogen stable isotopic (δ^{15}N) composition of fouling.

of variation. The fouling community was very similar in all BBs, with *Balanidae* and *Mytilidae* being the dominant families.

3.3. Wild ichthyofauna

Thirteen families with a total of 22 species were observed, with *Sparidae* (7 species), *Scorpaenidae* (3 species) and *Serranidae* (3 species) being the most widely represented. Appendix B shows the average abundance of ichthyofauna. The results of the statistical analysis are shown in Table 4. Differences with regard to the fish assemblage structure were significant between BI and BCs over time ($P < 0.05$), and also in general among sampling campaigns ($P < 0.01$). The n-MDS plot (Fig. 5B) shows the spatial ordination. The BI of all sampling campaigns and the BCs in summer 2006 and autumn 2006 were close to each other and separated from the BCs of the remaining campaigns. A SIMPER test (Table 5) shows the contribution of different species to the dissimilarities among locations. Differences between BI and BCs are mainly due to a higher abundance of the pelagic species *Trachurus mediterraneus* and *Boops boops* in BI, and differences over time attributable to the seasonal variations in abundance of both species, since the species richness and abundance fluctuations of demersal and benthic fish was very similar among locations. Nekton-feeders such as *Spicara maena* and *Diplodus vulgaris* were more abundant in BI, and *Chromis chromis* in the BCs.

3.4. Sediment

Table 6 shows the results of the statistical contrast for all sediment variables. Fig. 6A shows the evolution of the CM sediment fraction in the study areas during the sampling campaigns. An asymmetrical ANOVA only showed significant differences between BCs over time ($P < 0.01$) with respect to CM. However, CM was always higher in BI than in BCs, but not to a statistically significant extent ($P > 0.05$). AVS-S (Fig. 6B) was always significantly more concentrated in BI than in BC ($P < 0.05$) sediments, but no significant changes were detected over time ($P > 0.05$). However, differences over time between BCs were detected ($P < 0.05$), although values barely exceeded 50 ppm. During the winter 2007 sampling campaign, AVS-S values were the lowest for all the BBs. We found that the CM content of the sediment followed a trend similar to the biomass of farmed fish during the study period, while AVS-S demonstrated a similar trend to that of the amount of feed provided (see Figs. 2, 6A and B).

A total of 36 polychaete families were identified (Appendix C shows their average abundance). A PERMANOVA (Table 6) showed a statistically significant difference between BI and BCs over time ($P < 0.05$). This means that the structure of the polychaete assemblage changed in BI in a way different to how it did in BCs. The n-MDS plot (Fig. 5C) shows that the polychaete assemblage structure and evolution in BI and in BCs was different before BBs deployment. A tendency for BI to resemble BCs was evident after the first sampling campaign, but it then progressively returned to a state similar to that before the deployment of the BBs. On the other hand, the polychaete assemblage structure in BCs did not seem to follow a clear trend. A SIMPER test (Table 7) showed that the polychaete families contributed to the differences among locations. These differences are mainly due to a higher abundance of opportunistic and tolerant families, such as *Capitellidae* and *Dorvilleidae* in BI, and to a greater abundance of sensitive or indifferent families such as *Onuphidae*, *Syllidae*, *Maldanidae* or *Eunicidae* in BCs.

4. Discussion

The main purpose of the use of artificial structures around fish farms is to convert waste into biomass, thus enhancing biodiversity, and in the case of benthic biofilters, to improve the surrounding sediment quality. However, the greater nutrient availability derived from fish farming that we observed close to the bottom in BI did not result in a fouling biomass that was greater than in the BCs. The biomass in question fluctuated, and was usually slightly higher in any of the BCs than in BI. Angel et al. (2002) also found this type of inconsistent fluctuation in fouling biomass between fish farm and reference sites. Conversely, the fouling biomass pattern was more consistent in the pelagic biofilters deployed by Lojen et al. (2005) and Cook et al. (2006), who always obtained a greater fouling biomass in suspended biofilters close to fish farms than in reference sites. The fouling biomass measured in BI was considerably lower than that observed by Lojen et al. (2005) for suspended biofilters (average biomass of 216 *vs.* approx. 1200 g m^{-2}, respectively) during a similar permanence time. A comparison of our biomass data with those of Cook et al. (2006) for a biofilter permanence time of 5–6 months produced similar results (100–400 g m^{-2}). Whatever the case, differences would arise from the inherent dissimilarity between locations, and also as a result of the differences in light and nutrient availability between mid and deep water columns around fish farms.

Monitoring of nitrogen stable isotope composition (δ15N) confirmed that the fouling community under the aquaculture facilities was benefiting at least partially from the increased availability of food resources derived from the fish farm. Lojen et al. (2005) also showed that fouling attached to artificial structures suspended in the water column close to a fish farm demonstrated a ^{15}N enrichment as compared to reference locations. However, the retention efficiency of farm-derived N by biofilters both in the Lojen et al.

Table 2
Results of the asymmetrical ANOVA of fouling variables with locations (BI *vs.* BCs) and BCs, and sampling campaigns (SC). All variables were Log(x + 1) transformed prior to analysis.

Variable	Source of variation	d.f.	F_{denom}	MS	F	P
Biomass	L	2	SC × L	0.1520		
	BI *vs.* BCs	1	[a]BCs	No test		
	BCs	1	SC × BCs	0.2160	0.74	n.s.
	SC	4	SC × L	27.20	71.73	***
	SC × L	8	Resid. L	0.3793		
	SC × (BI *vs.* BCs)	4	SC × BCs	27.15	93.27	***
	SC × BCs	4	Resid. BCs	0.2911	1.69	n.s.
	Resid. L	45		0.1802		
	Resid. BCs	30		0.1721		
	Total	59				
δ^{15}N	L	2	SC × L	0.0225		
	BI *vs.* BCs	1	[a]BCs	No test		
	BCs	1	SC × BCs	0.0016	0.00	n.s.
	SC	4	SC × L	0.0800	78.03	***
	SC × L	8	Resid. L	0.0010		
	SC × (BI *vs.* BCs)	4	SC × BCs	0.0796	278	***
	SC × BCs	4	Resid. BCs	0.0003	0.11	n.s.
	Resid. L	45		0.0017		
	Resid. BCs	30		0.0025		
	Total	59				
PERMANOVA	L	2	SC × L	617		
	BI *vs.* BCs	1	[a]BCs	8863	4.43	n.s.
	BCs	1	SC × BCs	1997	2.67	n.s.
	SC	4	SC × L	2715	0.72	n.s.
	SC × L	8	Resid. L	3743		
	SC × (BI *vs.* BCs)	4	SC × BCs	2215	2.97	n.s.
	SC × BCs	4	Resid. BCs	746	0.64	n.s.
	Resid. L	45		295		
	Resid. BCs	30		290		
	Total	59				

$P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.
[a] No test unless SC × (BI *vs.* BCs) was not significant ($P > 0.25$). If so, F_{denom} would be BCs.

Table 3
Fouling biomass, nitrogen content and nitrogen stable isotopic (δ^{15}N) composition in total particulate matter (TPM) and fouling, and overall retention efficiency and retention rate of fish farm-derived N.

Average BI fouling biomass (d.w.) ($g\ m^{-2}$)	202.4
Average N in BI Fouling (d.w.) (%)	0.96
($g\ N\ m^{-2}$)	1.94
Average TPM background (δ^{15}N ‰)	4.42
Average δ^{15}N in BI TPM (δ^{15}N ‰)	5.58
Average δ^{15}N in BI fouling (δ^{15}N %)	6.73
Farm-derived N retained (%)	16
($g\ N\ m^{-2}$)	0.31
Farm-derived N retention rate ($g\ N\ m^{-2}\ month^{-1}$)	0.02

(2005) study (1.3 $g\ N\ m^{-2}\ month^{-1}$) and the present study (0.02 $g\ N\ m^{-2}\ month^{-1}$) was very low.

The observed fouling biomass in all BBs was mainly attributable to the great abundance of *Mytilidae* and *Balanidae*. Both families have been shown to act as pioneer settlers in both Mediterranean (Bombace et al., 1994) and Baltic (Laihonen et al., 1996; Antsulevich et al., 2000) artificial reefs. Mussels are typical filter-feeders and barnacles are euryphagous suspension-feeders able to trap particles up to several millimeters in size (Antsulevich et al., 2000), making them potential consumers of particulate matter derived from fish farms. Many other abundant potential direct consumers of particulate organic matter were also counted, such as cnidarians belonging to the family *Actiniidae*, bivalve molluscs belonging to the families *Hiatellidae* and *Ostreidae*, polychaetes belonging to the families *Terebellidae*, *Serpulidae* and *Sabellidae*, and amphipod crustaceans belonging to the families *Gammaridae* and *Caprellidae*. Cook et al. (2010) studied the fatty acid profile of the omnivorous caprellid *Caprella mutica* and reported its ability to feed directly from fish farm-derived waste. Nevertheless, the potential retention of particles derived from fish farming by filter- and suspension-feeders is limited (Troell and Norberg, 1998; Cheshuk et al., 2003; Navarrete-Mier et al., 2010). A high spatial variation in the recruitment of benthic assemblages to artificial substrata has been corroborated, even with small spatial scales (Rule and Smith, 2005). In our study, the fouling community structure did not differ significantly among locations, and any dissimilarity could be attributed to random recruitment and natural variability as opposed to any influence from the farm.

The aggregation of wild fish around fish farms is well documented (Carss, 1990; Dempster et al., 2002; Boyra et al., 2004; Machias et al., 2005; Sudirman et al., 2009). Waste feed is not the only reason explaining the attraction of wild fish to fish farms, since facilities provide other resources such as shadow areas which make zooplankton and particulate matter more easily detectable by consumers, spatial reference for orientation and resting, shelter from predators and substrate for settlement of benthic organisms (Beveridge, 1984; Sudirman et al., 2009). The main effect of the fish farm on the benthic biofilters with regard to wild fish assemblage was that the pelagic fish gathered around the cages were also closely associated with the biofilters. Beneath the farm, pelagic species such as *B. boops* and *T. mediterraneus*, the most abundant fish species, used the biofilter as a resting area. In every sampling visit to BIs, these species formed compact schools just above the biofilters. Both species feed actively on fish farm feed waste, so it

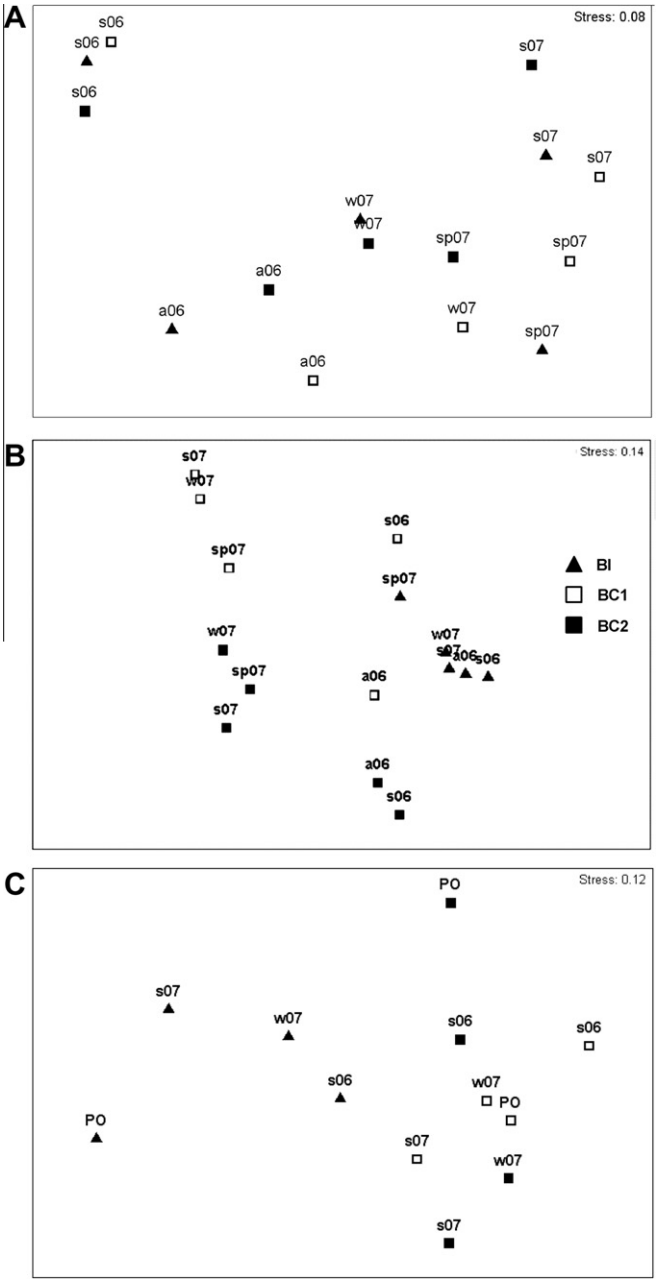

Fig. 5. Non-parametric multidimensional scaling (n-MDS) plots. (A) Fouling community; (B) wild ichthyofauna; (C) polychaete assemblage. Sampling campaigns: PO – pre-operational; S06 – summer 2006; A06 – autumn 2006; W07 – winter 2007; Sp07 – spring 2007; S07 – summer 2007.

would seem that they are mainly attracted to the farm for trophic reasons. However, the presence of elements such as biofilters, which increase habitat complexity, offers the possibility of using the farm milieu for activities other than feeding, and presumably would increase the residence time of these species in the vicinity of the farm. Differences between BI and BCs over time were caused by the much higher abundance and temporal variability of pelagic fish in BI. However, during the initial colonization stages, *B. boops* and *T. mediterraneus* also visited the BCs, albeit in smaller numbers, but after a few months, both species practically disappeared. Other nekton-feeder species, such as *S. maena* and *Diplodus vulgaris* were also more abundant in BIs, not only around them, but also inside the biofilters. The latter species was never observed feeding around the cages, so their presence in the biofilters may be explained by the availability of both food and shelter.

Dempster et al. (2005) showed that most of the wild fish aggregated around fish farms are found between the surface and the floor of the net cages, and are mainly planktivorous species and pelagic predators, while demersal species (linked to the substratum) were less abundant. The presence and variety of demersal fish species around fish farms depends largely on the proximity to other habitats from which some species are exported (Dempster et al., 2002). Our study area was located within a very large, uniform detritic area with low fish abundance. The only different habitat in the vicinity is a vast *Posidonia oceanica* meadow located 2.3 km to the west. Most of the demersal species sighted in BBs are frequently observed in *P. oceanica* beds, so it is reasonable to conclude that they may have migrated from there. Demersal fish species were almost the same in all the BBs, with no particular differences in abundance or permanence between them. The only study with artificial reefs under a fish farm in which wild ichthyofauna was considered was conducted by Angel et al. (2002) in the Red Sea. These authors showed that planktivorous species such as *Neopomacentrus miryae* and *Pseudanthias squamipinnis* were the most abundant fish surrounding not only the fish farm reefs, but also the reference sites, and the composition of the rest of the fish community (mainly demersal species) at the fish farm site was more diverse than in the control reef. We did not observe such a difference and, according to Dempster et al. (2002), this could be explained by the low habitat diversity in the basin where our BBs were deployed, so demersal fish, as well as fouling organisms, colonized the reefs simply because a new habitat was available, independently of the presence of the fish farm. However, in the case of Angel et al. (2002) other nearby habitats (natural reefs, phanerogam meadows, etc.) were abundant, so demersal fish should be recruited differently to the impacted and control reefs according to habitat preference rather than spatial availability.

The aggregation of fish around the cages and the reefs increases the potential reutilization of particulate fish farm waste and zooplankton (Angel et al., 2002), favoring waste dispersal and

Table 4
Results of the asymmetrical ANOVA of ichthyofauna variables with locations (BI *vs.* BCs) and BCs, and sampling campaigns (SC). All variables were Log(x + 1) transformed prior to analysis.

Variable	Source of variation	d.f.	F_{denom}	MS	F	P
PERMANOVA	L	2	SC × L	10,220		
	BI *vs.* BCs		[a]BCs		No test	
	BCs	1	SC × BCs	5434	4.01	n.s.
	SC	4	SC × L	11,511	10.56	**
	SC × L	8	Resid. L	1125		
	SC × (BI *vs.* BC)	4	SC × BCs	10,153	7.49	*
	SC × BCs	4	Resid. BCs	1355	1.11	n.s.
	Resid. L	15		1031		
	Resid. BCs	10		1220		
	Total	29				

$P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

[a] No test unless SC × (BI *vs.* BCs) was not significant ($P > 0.25$). If so, F_{denom} would be BCs.

Table 5
Results of SIMPER test (dissimilarity percentages and species contributions) of ichthyofauna abundance between sampling locations. Cut-off: 90%.

Fish species	BI	BC1	Av. dissimilarity: 88.74			
	Av. abund.	Av. abund.	Av. diss.	Diss./SD	Contrib. %	Cum. contrib. %
Trachurus mediterraneus	168.90	14.20	41.17	1.28	46.40	46.40
Boops boops	503.80	45.90	40.89	1.36	46.08	92.47
	BI	BC2	Av. dissimilarity: 85.10			
T. mediterraneus	168.90	0.00	41.79	1.40	49.10	49.10
B. boops	503.80	77.60	38.09	1.41	44.76	93.86
	BC1	BC2	Av. dissimilarity: 63.67			
Spicara maena	7.00	3.00	11.45	0.74	17.99	17.99
Sciaena umbra	3.50	0.20	10.73	1.07	16.85	34.84
B. boops	45.90	77.60	9.64	0.64	15.14	49.98
Diplodus vulgaris	5.50	5.30	8.70	0.83	13.66	63.64
T. mediterraneus	14.20	0.00	6.88	0.45	10.80	74.44
Serranus hepatus	0.00	1.30	3.45	1.00	5.42	79.86
Sparus aurata	0.00	7.10	3.44	0.45	5.40	85.26
Serranus cabrilla	3.10	1.80	3.24	0.97	5.08	90.34

Table 6
Results of the asymmetrical ANOVA of sediment variables with locations (BI *vs.* BCs) and BCs, and sampling campaigns (SC). All variables were Log(x + 1) transformed prior to analysis.

Variable	Source of variation	d.f.	F_{denom}	MS	F	P
Clay and mud (CM)	L	2	SC × L	0.5118		
	BI *vs.* BCs	1	[a]BCs	1.005	54.34	n.s.
	BCs	1	SC × BCs	0.0185	0.09	n.s.
	SC	3	SC × L	0.0920	0.64	n.s.
	SC × L	6	Resid. L	0.1420		
	SC × (BI *vs.* BCs)	3	SC × BCs	0.0857	0.42	n.s.
	SC × BCs	3	Resid. BCs	0.2035	5.49	**
	Resid. L	36		0.0290		
	Resid. BCs	24		0.0370		
	Total	47				
AVS-S	L	2	SC × L	99.28		
	BI *vs.* BCs	1	[a]BCs	197	176	*
	BCs	1	SC × BCs	1.1199	1.04	n.s.
	SC	3	SC × L	0.4033	1.46	n.s.
	SC × L	6	Resid. L	0.6044		
	SC × (BI *vs.* BCs)	3	SC × BCs	0.0299	0.02	n.s.
	SC × BCs	3	Resid. BCs	1.0686	3.73	*
	Resid. L	36		0.2759		
	Resid. BCs	24		0.2857		
	Total	47				
PERMANOVA	L	2	SC × L	9518		
	BI *vs.* BCs	1	[a]BCs		No test	
	BCs	1	SC × BCs	2282	1.30	n.s.
	SC	3	SC × L	2898	2.72	n.s.
	SC × L	6	Resid. L	1978		
	SC × (BI *vs.* BCs)	3	SC × BCs	2191	10.03	*
	SC × BCs	3	Resid. BCs	1751	1.94	n.s.
	Resid. L	36		1063		
	Resid. BCs	24		901		
	Total	47				

$P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.
[a] No test unless SC × (BI *vs.* BCs) was not significant ($P > 0.25$). If so, F_{denom} would be BCs.

environmental impact mitigation. It has been estimated that wild fish aggregated around fish farms can consume between 40% and 80% of uneaten feed (Vita et al., 2004; Felsing et al., 2005). Wild fish transform the supplied, but uneaten feed into fecal pellets. The settling velocity of fish feces is lower than that of feed pellets, so waste dispersion is wider (Chen et al., 2003; Piedecausa et al., 2009). In addition, nutrient leaching from fish feces is more pronounced than from feed pellets (Fernández-Jover et al., 2007; Piedecausa et al., 2009, 2010), so the net nutrient load reaching the seabed around fish farms should be diminished by the action of wild fish. Therefore, it seems that greater biofiltration is carried out by wild fish and that increasing their diversity and residence time around the farms would mitigate any impact on the seabed.

The influence of rocky reefs on the surrounding soft-sediments can be highly variable as a result of biological (organic content, availability of food, recruitment, predation, etc.) and physical (alteration of water movements, stability of substratum, erosion, sedimentation, changes in particle size distribution, etc.) factors which vary with distance from the reef (Ambrose and Anderson, 1990; Barros et al., 2001). Several relationships between nearby habitats exist, and a greater or lesser degree of dependence between them can be established over the course of time. Some studies have shown this spatio-temporal variability in natural or artificial rocky reefs and surrounding environments along distance gradients (Davis et al., 1982; Ambrose and Anderson, 1990; Posey and Ambrose, 1994; Barros et al., 2001; Danovaro et al., 2002;

Table 7
Results of SIMPER test (dissimilarity percentages and species contributions) of polychaete assemblage abundance between sampling locations. Cut-off: 60%.

Polychaete family	BI	BC1	Av. dissimilarity: 52.05			
	Av. abund.	Av. abund.	Av. diss.	Diss./SD	Contrib. %	Cum. contrib. %
Onuphidae	3.13	22.75	16.23	2.29	31.17	31.17
Dorvilleidae	8.88	4.94	5.53	0.74	10.63	41.80
Capitellidae	6.44	5.88	4.09	2.85	7.85	49.65
Syllidae	1.31	5.63	3.80	1.29	7.30	56.95
Eunicidae	1.75	4.19	2.18	0.88	4.19	61.14
	BI	BC2	Av. dissimilarity: 53.12			
Onuphidae	3.13	17.75	14.40	5.01	27.11	27.11
Dorvilleidae	8.88	1.56	5.54	0.82	10.43	37.55
Maldanidae	1.00	4.81	3.76	2.20	7.08	44.63
Capitellidae	6.44	2.69	3.54	1.14	6.67	51.30
Eunicidae	1.75	4.81	3.22	2.61	6.06	57.36
Lumbrineridae	7.56	4.69	3.17	1.03	5.96	63.32
	BC1	BC2	Av. dissimilarity: 35.54			
Onuphidae	22.75	17.75	7.93	2.17	22.31	22.31
Syllidae	5.63	4.50	3.22	1.54	9.06	31.36
Dorvilleidae	4.94	1.56	2.63	1.07	7.40	38.77
Lumbrineridae	6.13	4.69	2.37	2.95	6.67	45.43
Capitellidae	5.88	2.69	2.29	1.93	6.43	51.87
Eunicidae	4.19	4.81	1.82	2.35	5.12	56.98
Cirratulidae	2.50	4.56	1.71	1.18	4.82	61.81

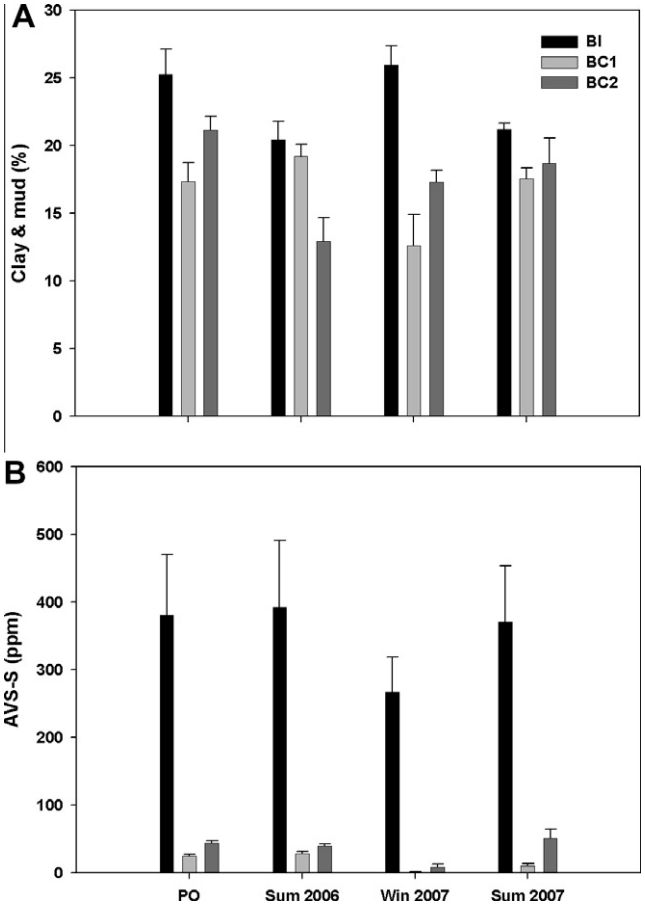

Fig. 6. Sediment physical–chemical variables. (A) Clay and mud fraction; (B) acid volatile sulfide concentration.

Barros, 2005; Gray and Elliott, 2009). Given that our sediment samples were taken randomly within a 5 m-radius of the reefs and the imposed short-term of our study, the observed response of the sediment can hardly reflect the models described in the previously cited literature. This would lead us to conclude that the results must be interpreted as a general reaction in the studied areas. Sediment adjacent to the benthic biofilters below the fish farm did not show any physical–chemical response in terms of recovery as compared to the controls. The effect of the fish farm on the sediments around BI seemed to be the same, regardless of the presence of the BBs. Sulfide accumulation under the cages and around BI remained much higher than in BCs throughout the study, and only minor differences among BCs over time were noted in terms of granulometry. Angel and Spanier (2002) did not show any noticeable change in the organic content of sediments adjacent to an artificial reef deployed under a fish farm in the Red Sea. Gao et al. (2008) mentioned that sediments close to artificial reefs deployed under a fish farm in Hong Kong appeared to show some significant degree of reduction in total Kjeldahl nitrogen content, but no significant reduction in total carbon or total phosphorus was reported.

Normally, the first response that is observed on a biological system experiencing an alteration of its status is biological stimulation: the organisms with a higher capacity for adaptation to the new conditions increase their abundance and biomass. Gradually, the system evolves towards a new state of equilibrium which may be very similar or even identical to the state prior to the alteration (Margalef, 1998). This is what seemed to happen to the infaunal polychaetes in BI (see Fig. 5C): the polychaete assemblage structure in BI and in BCs was different before BB deployment. A few months after deployment, the polychaete assemblage structure in BI changed, tending to resemble that of BCs. However, the assemblage gradually returned to its previous state. In contrast, the polychaete assemblage structure in BCs only experienced some minor changes during the study, which must be considered as natural fluctuations. Beneath the fish farm, the seabed is stressed by the continuous settlement of organic material. The addition of the biofilter implied a change, but the magnitude of the effect of the organic load exceeded that of the biofilter, and the initial transformation was overridden by an impact of greater magnitude.

In general, this work shows that the deployment of BBs presented some benefits for the environment, increasing benthic and wild fish biodiversity in spite of the presence of a fish farm, and in this case enhancing recycling and the opportunity for waste dispersal. However, these obvious environmental benefits did not lead to an improvement (or deterioration) in sediment quality

around BI. The polychaete assemblage responded early to fish farm biofilter installation, but the farm seemed to have an even greater influence on them. The short duration of the study did not allow for achieving more conclusive results regarding the influence of the fish farm on the biofilter or the response of the sediment to the presence of the biofilter. Furthermore, it failed to produce definitive answers in terms of any possible spatio-temporal scale effects, which leaves a number of questions unanswered. Future studies are needed to examine the effects of biofilter design and size (relative to the farm size) in relation to fish farm sediment quality, and different study durations should also be planned. These are some of the questions that need to be answered before recommending BBs as an immediately applicable mitigation tool. However, in our study, what was noticeable was the improvement in the fish farm benthic environment after increasing the habitat complexity (although this does not mean that more complex habitats better withstand the impact of fish farming). This mitigation contributes to aquaculture sustainability. Furthermore, given the potential of benthic communities and wild fish assemblages as waste recyclers, artificial structures with a more appropriate design (shape, size, materials, etc.) and an improved deployment strategy (number of units, spatial arrangement, etc.) may make the application of this technology viable, but obviously more efforts in terms of research and economic sustainability are required.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.marpolbul.2011.05.028.

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