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# Benthic Recovery after the Cessation of a Gilthead Seabream, *Sparus aurata*, Farm in the Mediterranean Sea

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# Abstract

**An environmental recovery study was done after cessation of a gilthead seabream farm off the Mediterranean coast of Spain. Physico-chemical variables of sediments, *in situ* benthic fluxes of oxygen and nutrients, and benthic macrofauna were measured in the farming area and at a control station. Five sampling campaigns were made, one before the closure, and the others at 1, 3, 9, and 24 months after cessation. Benthic flux of ammonium was the first variable to recover, followed by benthic fluxes of phosphate and dissolved oxygen and % organic matter in the sediments, which 3 months after cessation of farming already showed levels similar to those in the control station. Nine months after cessation the other abiotic variables of the sediments disturbed by the activity had recovered, such as % coarse fraction, total phosphorus concentrations, and redox potential measurements. The recovery of the macrofauna was slower than the abiotic variables. Three months after cessation, *Capitella capitata* abundance had reduced drastically in the farming area, but similar specific richness levels were not observed between the two sampled zones until 2 years after farming cessation.**

More than half of all sea products consumed by the world population are now produced on fish farms. This milestone has been reached after four decades of continuous and intense development (APROMAR 2012). The FAO (2010) estimates by 2030, 65% of aquatic food will come from aquaculture. Ensuring high food standards is an essential goal in the European Union, and ensuring food security, environmental protection, and social welfare of employees are essential principles.

For correct environmental management, it is important to have good knowledge about the processes regulating the effects of aquaculture residues on the ecosystem (Sanz-Lázaro and Marín 2011) and understand the processes of ecosystem recovery when activity ceases or during a fallow period (Aguado et al. 2012).

There is considerable literature regarding the environmental effects of fish farming, and following fish farm expansion, various researchers have studied the impacts on water column, sediments, fauna, and flora in the vicinity of fish farm facilities. These studies – some of which were made in the early days of farming – include: Gowen and Bradbury (1987); Hall et al. (1990); Holby and Hall (1991); Holmer and Kristensen (1992); Diaz and Rosenberg (1995); Karakassis et al.(1998), and these have been supplemented over the years by Mazzola et al. (2000); La Rosa et al. (2002); Cancemi et al. (2003); Porrello et al. (2005); Pitta et al. (2005); Maldonado et al. (2005); Pergent-Martini et al. (2006). The most recent studies were made by: Yucel-Gier et al. (2007); Freitas et al. (2008); Olsen et al. (2008); Ferrón et al. (2009); Matijević et al. (2009); Siokou-Frangou et al. (2010); Sanz-Lázaro and Marín, (2011); Huang et al. (2011); Morata et al. (2012). However, few studies have examined the evolution of the environment after production ceases – despite the fact this information is needed for carrying capacity estimations, fallowing times, and for making future impact predictions.

The biogeochemical processes of recovery are poorly studied, and the time needed to restore an ecosystem has not been determined (McGhie et al. 2000; Pereira et al. 2004; Gray and Elliott 2009). Despite this, recovery has been studied under various settings, including situations of temporary cessation (fallowing between two production periods) (McGhie et al. 2000; Brooks et al. 2003; Macleod et al. 2006, 2007; Vita and Marín 2006; Lin and Bailey-Brock 2008), and after the complete cessation of productive activity (Karakassis et al. 1999; Mazzola et al. 2000; Kraufvelin et al. 2001; Brooks et al. 2004; Macleod et al. 2004; Pereira et al. 2004; Sanz-Lázaro and Marín 2006; Aguado et al. 2012). In the case of fallowing, sediment recovery is understood, from a sustainability point of view, as recovery to the extent of preventing a progressive sediment deterioration which may be sufficient to support long-term farming operations (Macleod et al. 2007).

A complete recovery is not then required or expected after fallowing. Nevertheless, in the context of definitive cessation, sediment recovery is understood as a return to conditions similar to those in adjacent undisturbed sediments (Sanz-Lázaro and Marín 2006). Brooks et al. (2003) observed that a chemical remediation should first occur to allow a subsequent biological remediation. Degrees of recovery are quite variable, depending mainly on the hydrological characteristics of the area, the sediment type, and in the case of fallowing, the duration of fallowing (Pereira et al. 2004; Macleod et al. 2007; Lin and Bailey-Brock 2008). Moreover, benthic response and thus recovery processes are scale-dependent and may vary in terms of the extent of the impact (Villnäs et al. 2011). Additionally, biotic factors, such as community composition and other characteristics (dispersal, recruitment, life stage, etc.), and their relationships (competition, predation, etc.) influence recovery processes (Norkko et al. 2006).

The aim of this study was to analyse the recovery of an area in the Mediterranean affected by a gilthead seabream, *Sparus aurata*, farm after farming cessation. To achieve this, different physicochemical variables of the sediments and macrobenthic community were analysed. Moreover, benthic fluxes of oxygen and nutrients *in situ* were measured – this being the first study to do these incubation experiments *in situ* after farming cessation.

## Materials and Methods

The study area was located in the north-west Mediterranean, in the Gulf of Valencia (Spain), at a site which previously produced gilthead seabream, *Sparus aurata*. The fish farm installation was located in the open sea, about 2 km from the coast at a depth of 19 m. Fish farming began in 1999 and closed in June 2009. Fish production at this farm was 500 t per year during the final seven years of operation. Different variables in sediments and *in situ* measurements of fluxes were studied at two sampling points: one that was affected by the farming activity and located under one of the central cages in the installation (0º 3’ 11.101’’ W; 39º 50’ 19.6243’’N), and the other at a control station (0º 3’ 6.1871’’W; 39º 50’ 21.4126’’N). The control station was used as an indicator of reference conditions that were in place close to the installation (130 m northeast of the fish farm) which had the same sediment characteristics, depth, and up-current from dominant sea currents (Morata et al. 2013). Other nearby sites were not adequate due to having a different benthic habitat which did not allow comparisons.

Samples were collected during five sampling campaigns: the first in early summer, before farming cessation (June 2009); and the remaining ones, 1 month (July 2009), 3 months (September 2009), 9 months (April 2010), and 24 months (July 2011) after farming cessation. In the final sampling campaign, benthic fluxes were not measured.

During each sampling campaign, three samples were taken of unaltered sediment layers from both the fish farm and control station, using corers (with an internal diameter of 6.5 cm), which were taken by scuba divers. The upper most layer (1 cm) was removed to analyse granulometry, porosity, organic matter (OM), and total phosphorus (TP). Granulometry was performed using the Wentworth scale (Shepard 1954). Sediment porosity was calculated following Dell'Anno et al. (2002). OM was analysed using the combustion method (Dell'Anno et al. 2002). To determine sediment TP, digestion was performed following Arocena and Conde (1999). When the corers were brought to the surface, redox potential (Eh) was measured at a depth of 0.5 cm using a Crison PH25 potentiometer.

To measure nutrients and oxygen benthic fluxes *in situ*, six benthic chambers (3 light and 3 dark) were used. The chambers were made of semi-spherical methacrylate (diameter of 40 cm and a volume of 16.7 L) and contained a manual stirrer to minimise concentration gradients (Niencheski and Jahnke 2002). The chambers were placed in the sediment manually by scuba divers, and the total incubation period was around six hours. Water samples were taken from inside the chambers every 2 hours, and the variables analysed were: dissolved oxygen (DO), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$) and phosphate ($PO_4^{3-}$). Benthic fluxes were calculated from the slope of a linear regression of the time series results and the chamber volume (Niencheski and Jahnke 2002), and Equation 1, as used by Nizzoli et al. (2007): (1) $F=(C_t-C_o)\cdot(1/(A\cdot t))\cdot V\cdot 24$. Where F is the calculated flux in mmol/m$^2$*d; $C_t$ and $C_0$ are the final and initial concentrations (mmol) obtained from the linear fit; A is the area of incubation in m$^2$; t is the total incubation time in hours; and V is volume of incubated water in L. A more detailed description of benthic chambers and the calculation to obtain fluxes is available in Morata et al. (2012).

The DO samples were fixed immediately and analysed using the Winkler iodometric method (Baumgarten et al. 1996). Nutrients were analysed using the methods described by Aminot and Chaussepied (1983) and adapted by Baumgarten et al. (1996).

To identify and count benthic macroinvertebrates three additional corers were taken in each area during all the sampling campaigns. These corers were sieved using a 0.5-mm mesh and 7% magnesium chloride was used as an anaesthetic. Organisms were later fixed in 7% formaldehyde solution.

A one-way ANOVA was used to determine the existence of significant differences ($P<0.05$) among variables measured in sediment and benthic fluxes in each sampling campaign between the affected area and the control station. When data did not meet the assumptions for the ANOVA, we applied appropriate transformations.

The effects of benthic environmental variables on the abundances of species in the macrofauna and their spatial variation were analysed using Canonical Correlation Analysis (CCA). Since rare taxa can distort the coordination points, the taxa that were only observed during a sampling campaign at either the fish farm installation or the control station were excluded. The abundance values were converted into log (abundance+1). The analysis was made with the samplings campaign data before cessation and 1, 3, and 9 months after cessation.

## Results

### Sediment Physicochemical Variables

The sediments were sandy with a grain size mode between 0.125 and 0.063 mm and an average grain size corresponding to very fine. Porosity, at both sites, was similar between 0.44-0.50 at the former fish farm and 0.43-0.49 at the control station. In the sampling campaign before cessation and in the first two samplings after cessation (1 and 3 months), significant differences could be observed between the farming area and the control station in the % of sediment coarse fraction (%Cf). This material represents particle sizes greater than 2 mm and is mainly composed of shells. These shells were mostly mussel valves, which in the affected area came from cleaning the submerged structures of the farm while it was operating. The %Cf in this area reached 8.5% (in the sampling 1 month after cessation), 37 times more than the average value found at the control station (0.2 ± 0.1).

Figure 1 shows %OM, TP concentrations, and Eh measurements in sediments from the farming area and the control station during the five sampling campaigns. In the sampling campaigns before and 1 month after cessation, significant differences were observed in the sediments between both areas in %OM, TP concentrations, and Eh measurements. In the sampling campaign 3 months after cessation significant differences in the TP concentrations and Eh measurements were observed, although the %OM no longer showed significant differences. In the other sampling campaigns there were no significant differences between the sampled zones in any measured variables.

## Benthic Fluxes

In general, DO fluxes were negative (Fig. 2a), which indicates DO consumption by the sediment. The exceptions were at the control station in the light chambers, in the sampling campaign before  cessation, and in campaign 9 months after cessation, in which the fluxes were positive. Significant differences were only observed between both station in the sampling campaigns before cessation and 1 month after cessation.

All the chambers showed positive fluxes in $NH_4^+$ from the sediment to the water column (Fig. 2b). Significant differences were only found between the affected area and the control station before cessation, and the largest flux was found under the cages in the dark chambers (13.6 ± 1.0 mmol/m$^2$*d). In the remaining sampling campaigns, the fluxes were similar in both zones and were no greater than 2 mmol/m$^2$*d.

$NO_2^-$ fluxes did not reveal a clear trend and were low compared with the other measured fluxes in sampling campaigns at both sites (Fig. 2c). $NO_3^-$ fluxes were negative, meaning that $NO_3^-$ was consumed by the sediment from the water column (Fig. 2d). No sampling campaigns showed significant differences in $NO_2^-$ and $NO_3^-$ fluxes between both areas.

$PO_4^{3-}$ fluxes were generally positive (Fig. 2e), meaning there was an input of phosphorus from the sediment to the water column. Significant differences were observed between the farming area and the control station in the sampling campaign before cessation and in the campaign 1 month after cessation. The greatest differences were observed before cessation with an average difference of 0.73 mmol/m$^2$*d, and fluxes were higher under the cages. In the remaining sampling campaigns, the $PO_4^{3-}$ fluxes in both areas were low compared to measurements taken before cessation in the farming area.

## Benthic Organisms

Figure 3 shows the results for abundance and specific richness of benthic macrofauna. In the sampling campaigns before and 1 month after cessation, the affected area showed greater total abundance (mostly due to Polychaeta) compared with the control station (Fig. 3a). In the remaining sampling campaigns, the control station showed greater total abundance than the farming area, although in the sampling campaign 2 years after cessation the differences were minimal. For specific richness, the farming area was always considerably less rich than the control station, except for sampling campaign 2 years after cessation when levels were similar (Fig.

3b). Both areas contained very few species of Mollusca and Crustacea in all the sampling campaigns. Among the Crustacea, *Apseudes latreilli* was always present at the control station, nevertheless, in the affected area, this species was only found in the sampling campaigns conducted 9 months and 2 years after cessation.

The number of species of Polychaeta found at the control station was between 6 and 12 belonging to the Eunicidae, Glyceridae, Lumbrineridae, Nephtydae, Pectinariidae, Phyllodocidae, Spionidae, Maldanidae, Paraonidae, and Sabellidae families. In contrast, in the farming area, the number of species ranged from a single species (*Capitella capitata*) in the sampling campaign before cessation to 10 species of Polychaeta in the sampling campaign 2 years after cessation, in which individuals of the Eunicidae, Glyceridae, Lumbrineridae, Spionidae, Maldanidae, Sabellidae and Acoetidae families were found. It is worth noting in the final sampling in the farming area, organisms belonging to the Nematomorpha, Equinodermata and Sipuncula groups were found for the first time, some of which had been found at the control station in some previous sampling campaigns.

Environmental factors included in the CCA were those showing differences between the area that was affected by farming and the control station, namely: benthic fluxes of $NH_4^+$, $PO_4^{3-}$ and DO, %OM, TP concentrations, Eh, and %Cf, with the aim of detecting those that may be associated with the distribution of the benthic macrofauna.

Table 1 shows the results of CCA performed on macrofauna abundance and environmental variables. Analysis showed the first axis accounted for 37.8% of the total variance contained in the data for the species in the benthic community, the second 18.7%, and the third 13.5%. All the environmental variables correlated better in Axis 1 (Table 1). The variables that best correlated with Axis 1 were TP (0.89), %Cf (0.83), Eh (-0.79), DO fluxes (-0.71) and %OM (0.65). The Pearson correlation between the species and the environmental variables was 1 for the three axes, and the Monte Carlo permutation test ($P < 0.05$) gave $P = 0.004$ for the correlation between the environmental variables and the macrofauna – meaning that the observed correlations were significant. The factors diagram (Fig. 4) for Axis 1, showed a clear differentiation in the two sampled zones, given that in the four sampling campaigns (before and 1, 3, and 9 months after cessation), there was a negative correlation for the control stations, while the farming area was positively correlated.

## Discussion

The effects observed at the fish farm before cessation were consistent with other studies on the effects of farming on the physicochemical properties of sediments, benthic fluxes, and macrofauna (Karakassis et al.1998; Karakassis et al. 2000; Aguado et al. 2004; Maldonado et al. 2005; Nizzoli et al. 2007; Freitas et al. 2008; Ferrón et al. 2009). Morata et al. (2012, 2013) analysed these abiotic and biotic variables during a yearly cycle demonstrating the maximum difference between the installation and the control station were produced in summer as a consequence of increased food supply grown the higher water temperature and metabolic activity of fish. However, variables such as $NO_2^-$ and $NO_3^-$ fluxes, did not follow this pattern, showing the biggest differences in autumn as they were mainly related to high $NO_3^-$ levels in the water column (Morata et al. 2012). For that reason, after ceasing farming which took place in summer, no differences were found in $NO_2^-$ and $NO_3^-$ fluxes between the farming area and the control station in any sampling campaigns.

Of the environmental variables measured in the farming area before cessation, the $NH_4^+$ flux was the first to recover by showing levels similar to those measured at the control station in sampling campaign 1 month after cessation. This result suggests most nitrogenous organic matter provided by the farm, which mainly came from uneaten food, was rapidly degraded biochemically and reincorporated into the water column (Cromey et al. 2002).

The next variables to recover were %OM, $PO_4^{3-}$ and DO fluxes, as there were no differences between the farming area and the control in the sampling campaign taken 3 months after cessation. These results show when the supply of OM from the farm stopped, the OM accumulated in the sediment was quickly mineralized. This rapid mineralization of organic matter may have been accelerated by high temperatures (Pereira et al. 2004) reached during summer when water temperature at the bottom was around 24C. Moreover, the great abundance of *Capitella capitata* in this area before and 1 month after cessation (31,151 and 17,122 ind/$m^2$, respectively) may have contributed to the consumption of organic material. According to Banta et al. (1999), this phenomenon may account for up to 15% of the total respiration of sediments. The $PO_4^{3-}$ and DO fluxes are directly related to the diagenesis of organic matter (Vink et al. 1997). The highest consumption of DO by sediment was found under the cages before cessation of farming, coinciding with the highest OM content in the sediment (1.8%). Pereira et al. (2004) also measured the fluxes of DO, but *ex situ,* after the cessation of a fish farm, and saw it as an early variable of benthic recovery.

In the sampling campaign 9 months after cessation, Eh, TP, and %Cf were the next to recover, showing values similar to those measured at the control station. The recovery in the Eh measurements could be interpreted as a decrease in geochemical anaerobic processes, which is another sign of chemical recovery. However, Aguado et al. (2012) observed 8 months after cessation of a fish farm, Eh measurements were still significantly different from those measured at the control sites. The decrease in TP levels in sediments in the farming area during the first 9 months was nearly constant at about 3.955 mg TP/kg*d (Fig. 5). However, the measured benthic fluxes of $PO_4^{3-}$ did not follow this tendency (Fig. 2e). To compare the TP losses in the sediment with the benthic $PO_4^{3-}$ fluxes measured, it is necessary to transform the mg TP/kg*d in mmol TP/m$^2$*d (flux units). We estimated an outer layer (1 cm deep) of 1 m$^2$ sediment, had an average dry weight of 19 kg (data estimated from the weight of the first cm of the 6.5 cm diameter corers with an average humidity of 25%). The TP losses were 2.42 mmol/m$^2$*d. We also assumed the $PO_4^{3-}$ fluxes came from the first centimetre of sediment, and between two consecutive sampling campaigns, the $PO_4^{3-}$ fluxes had the same levels as the first of the two sampling campaigns. We see between the first and second sampling campaign, fluxes of $PO_4^{3-}$ could represent a maximum of 30.2% of TP losses. However, between the second and third sampling campaign, $PO_4^{3-}$ fluxes explained 5.4%; and between the third and fourth sampling campaign only 1.2% of TP losses. The remaining TP losses could be partially related to the decrease of the opportunistic polychaete *Capitella capitata* observed in the affected area; as well as the burial of sediment and/or dispersal of waste due to the hydrodynamics of the area and passing storms. The decrease in %Cf in the affected area could also be explained by burial and/or the hydrodynamics of the area.

The differences in macrofauna, found in the farming area and the control station before cessation, were largely as a consequence of organic enrichment occurring in sediments under the cages (Morata et al. 2013). *Capitella capitata* was found under the cages before the cessation in densities of 31,151 ind/m$^2$ and it is thought to be an indicator par excellence of anoxic conditions (Rosenberg 2001) and is classified as an opportunistic species (Pinedo et al. 2007).

Unlike the other variables measured in this study, the macrofauna showed a slow recovery. In the sampling campaigns taken 3 and 9 months after cessation, the situation in the farming area showed a significant improvement, although differences with the control station could still be observed. The abundance of *Capitella capitata* decreased significantly at 3 and 9 months (with levels of 552 and 221 ind/m$^2$ respectively), while *Apseudes latreilli* could be found 9 months after cessation. Since these two species are surface deposit feeding (Borja et al. 2000), the replacement could be explained by the physicochemical changes associated with the mineralization of organic matter and not only by the quantity of organic matter. The decline of *Capitella* was also accompanied by an increase in mobile carnivorous species such as *Glycera* and *Nephtys* (Fauchald and Jumars 1979) and sessile burrowers such as Maldanidae (Borja et al. 2000). The CCA results indicate benthic environmental variables were responsible for a significant percentage of the total variance of the species.

Axis 1 of the CCA was related to % coarse fraction (% Cf), total phosphorus (%TP), organic matter (%OM) and fluxes of ammonium (F-$NH_4^+$) and phosphate (F-$PO_4^{3-}$) (positive coefficients). The higher the content of organic matter in the sediment, the lower the Eh values were and the higher the oxygen consumption (negative coefficients), due to aerobic mineralization of organic matter. Few benthic species occurred where accumulation and decomposition of the organic matter were higher and many species where these were low. This axis accounted for 38% of the variability of the data. Whereas in axes 2 and 3 no relationship was found between the environmental variables and the species, nor in the sampling campaigns.

These results showed that the development of the whole structure of the macrofauna is associated with improved abiotic conditions, which were potentially less aggressive for the growth of biota. However, it was not until 2 years after cessation that we found an absence of *Capitella capitata* in the farming area. It was only then the specific richness was similar to the control station. Our results are consistent with what several authors have suggested (Brooks et al. 2003) on the recovery of the soft bottom affected by organic discharge from fish farms, which suggest the chemical recovery of the sediments is the first to occur and is necessary for a subsequent biological recovery. The recovery rate of an impacted system is difficult to compare with other locations, because it depends, among other things, on the characteristics of the area and the ecological processes taking place (Dernie et al. 2003). However, we have reviewed other studies that have analysed the macrobenthic recovery after a fallow period or a fish farm cessation (Johannessen et al. 1994; Pohle et al. 2001; Pereira et al. 2004; Villnäs et al. 2011; Aguado et al. 2012). None of these studies observed a full recovery of the macrofauna, although, in most of them, the study period was less than 24 months. Only Villnäs et al. (2011) continued their study 2 years after the cessation of two fish farms in Finland, but they only observed a partial recovery in benthic macrofauna.

Studies of recovery after ceasing farming are less frequent than studies about the impact of working farms, given it is necessary to work both with an installation that has ceased farming and to have data from the functioning period to make comparisons. Furthermore, it would have been interesting to have had more reference points to validate the comparisons made, however we had to compromise between what was desirable and what was possible, as there were no other similar habitats available. Moreover, increasing the number of control sites would have meant a reduction in the measured variables and number of incubation experiments in sediments, as it would not have been possible to carry out with only one team of divers.

## Conclusions

Before farming cessation, the abiotic and biotic conditions of the sediment under the cages showed differences when compared with the control station, mainly due to the continuous discharge of organic matter generated by the fish farm. Although it is difficult to establish when there is a complete recovery of a stressed benthic environment, this study observed signs at various time scales that can be considered as a partial recovery. These changes were attributed mainly to farming cessation. The $NH_4^+$ benthic flux was the first variable to recover, just 1 month after cessation. This was followed by fluxes of $PO_4^{3-}$, DO, and %OM in the sediments, which showed levels similar to the control station just 3 months after cessation. Nine months after cessation the remaining abiotic variables of sediments disturbed by farming (%Cf, TP, and Eh) had recovered.

Three months after cessation the abundance of *Capitella capitata* had fallen drastically in the farming area, but the recolonisation of species tolerant of lower levels of contamination in unaffected nearby areas was slower, and similar levels of specific richness in the two sampling areas were not observed until 2 years after cessation.

In our study, the role of benthic fluxes in recovery after farming cessation was limited to a maximum of 3 months, as these are associated with the diagenesis of organic matter. In our case, a complete recovery was only observed after 2 years.

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TABLE 1. Results of CCA performed on macrofauna abundance and environmental variables.

| AXES | 1 | 2 | 3 |
|---|---|---|---|
| Correlations of environmental variables | | | |
| Fluxes $NH_4^+$ | 0.421 | -0.334 | 0.288 |
| Fluxes $PO_4^{3-}$ | 0.507 | -0.377 | 0.215 |
| Fluxes DO | -0.705 | 0.273 | 0.098 |
| % Organic matter | 0.648 | -0.081 | 0.440 |
| Total phosphorus | 0.885 | 0.265 | 0.177 |
| Redox potencial | -0.790 | -0.355 | -0.089 |
| % of sediment coarse fraction | 0.834 | 0.280 | -0.144 |
| Summary statistics for ordination axes | | | |
| Eigenvalue | 0.549 | 0.273 | 0.197 |
| Variance in species data | | | |
| % of variance explained | 37.8 | 18.7 | 13.5 |
| Cumulative % explained | 37.8 | 56.5 | 70 |
| Pearson Correlation, Species–Environment | 1.000 | 1.000 | 1.000 |

# Figure Legend

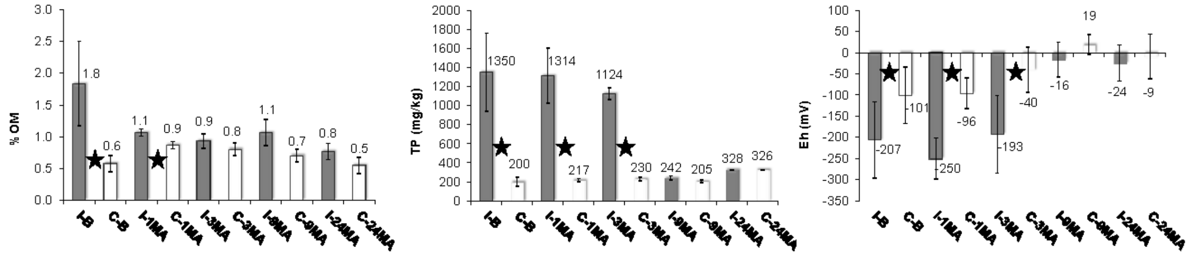

FIGURE 1. Percentage organic matter (%OM), total phosphorus (TP), and redox potential (Eh) in sediments in the farming area (I) and the control station (C) in the five sampling campaigns: before farming cessation (B), and 1, 3, 9, and 24 months after farming cessation (1MA, 3MA, 9MA, and 24MA, respectively).

Significant differences (ANOVA, *P*<0.05) between the farming area and the control are indicated by a star.

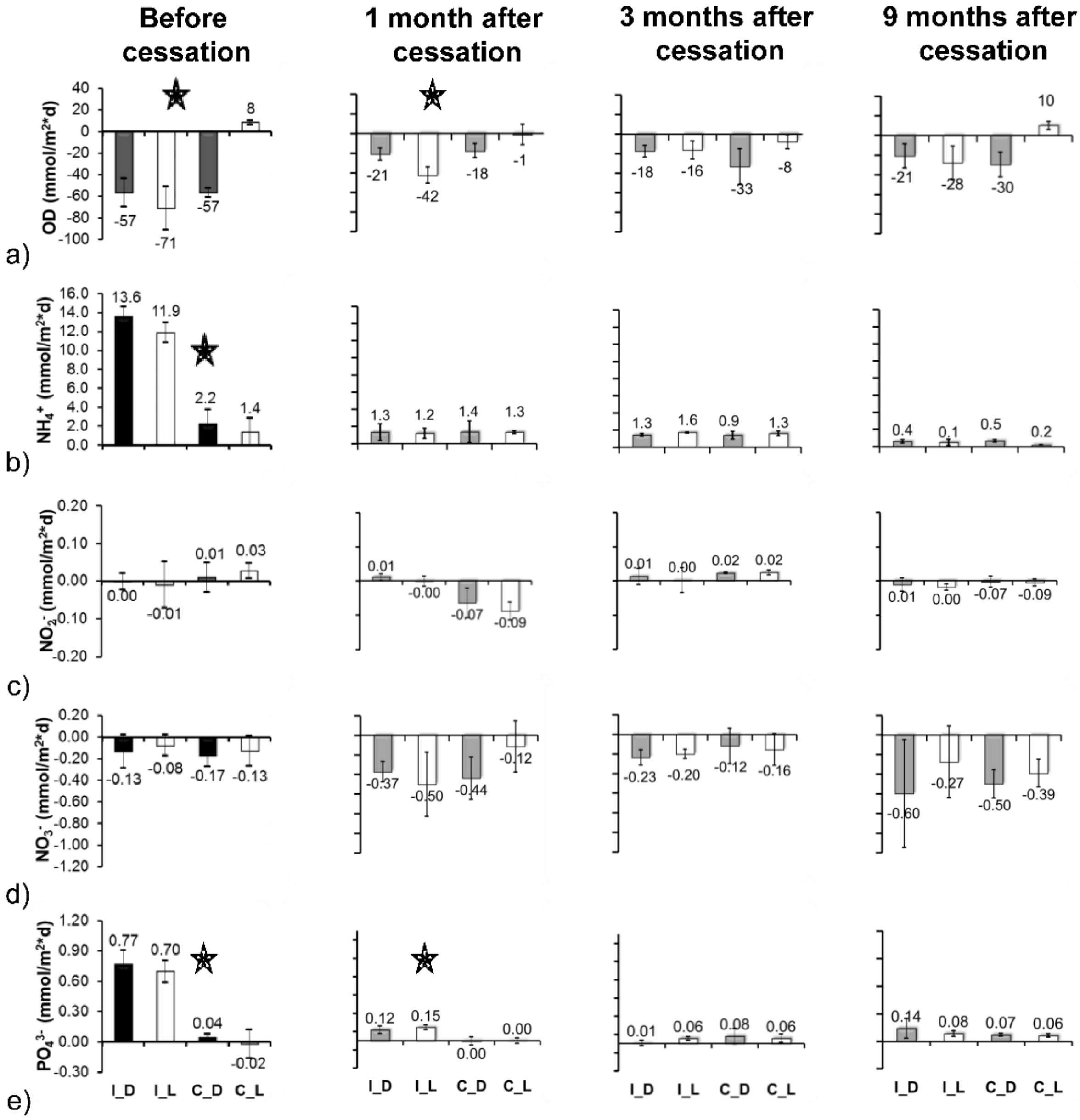

FIGURE 2. Benthic fluxes of DO, $NH_4^+$, $NO_2^-$, $NO_3^-$, and $PO_4^{3-}$ in dark (D) and light (L) chambers in the farming area (I), and the control station (C), before farming cessation and 1, 3, and 9 months after farming cessation.

Significant differences (ANOVA, $P<0.05$) between the farming area and the control are indicated by a star.

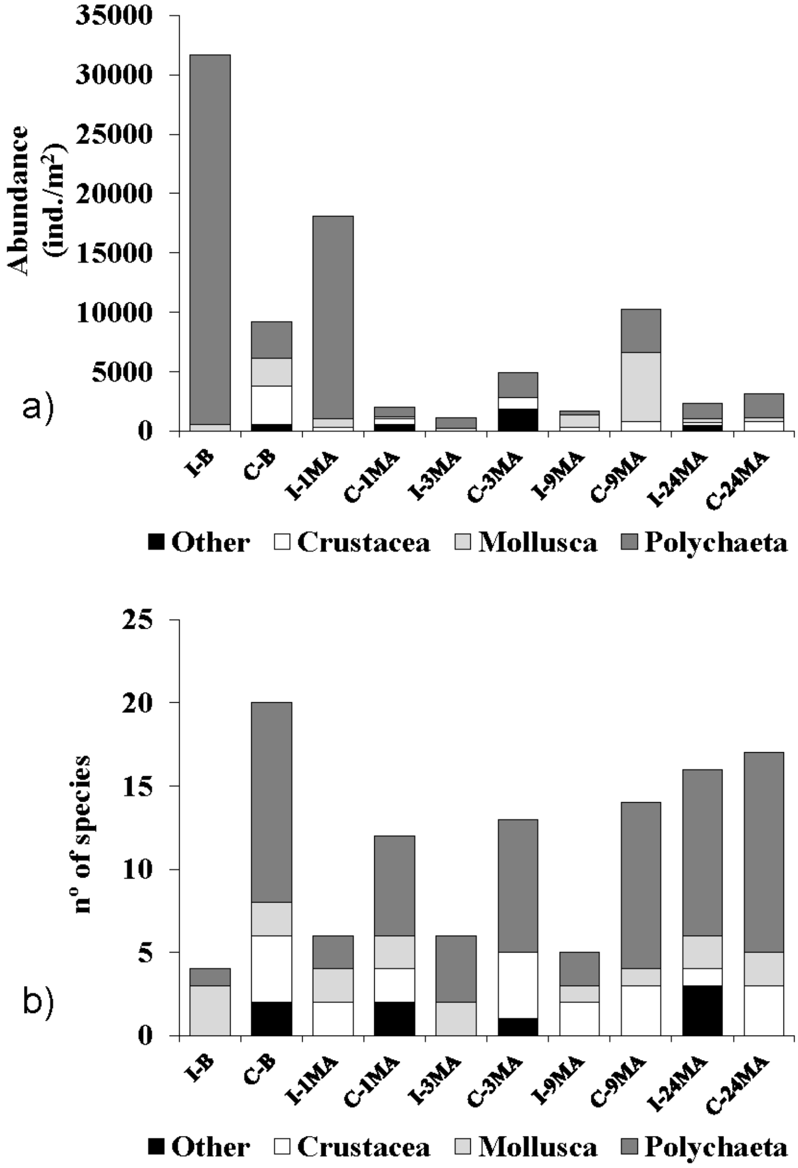

FIGURE 3. a) Density of individuals, and b) number of species in the farming area (I), and the control station (C) in the five sampling campaigns: before farming cessation (B) and 1, 3, 9, and 24 months after farming cessation (1MA, 3MA, 9MA, and 24MA, respectively).

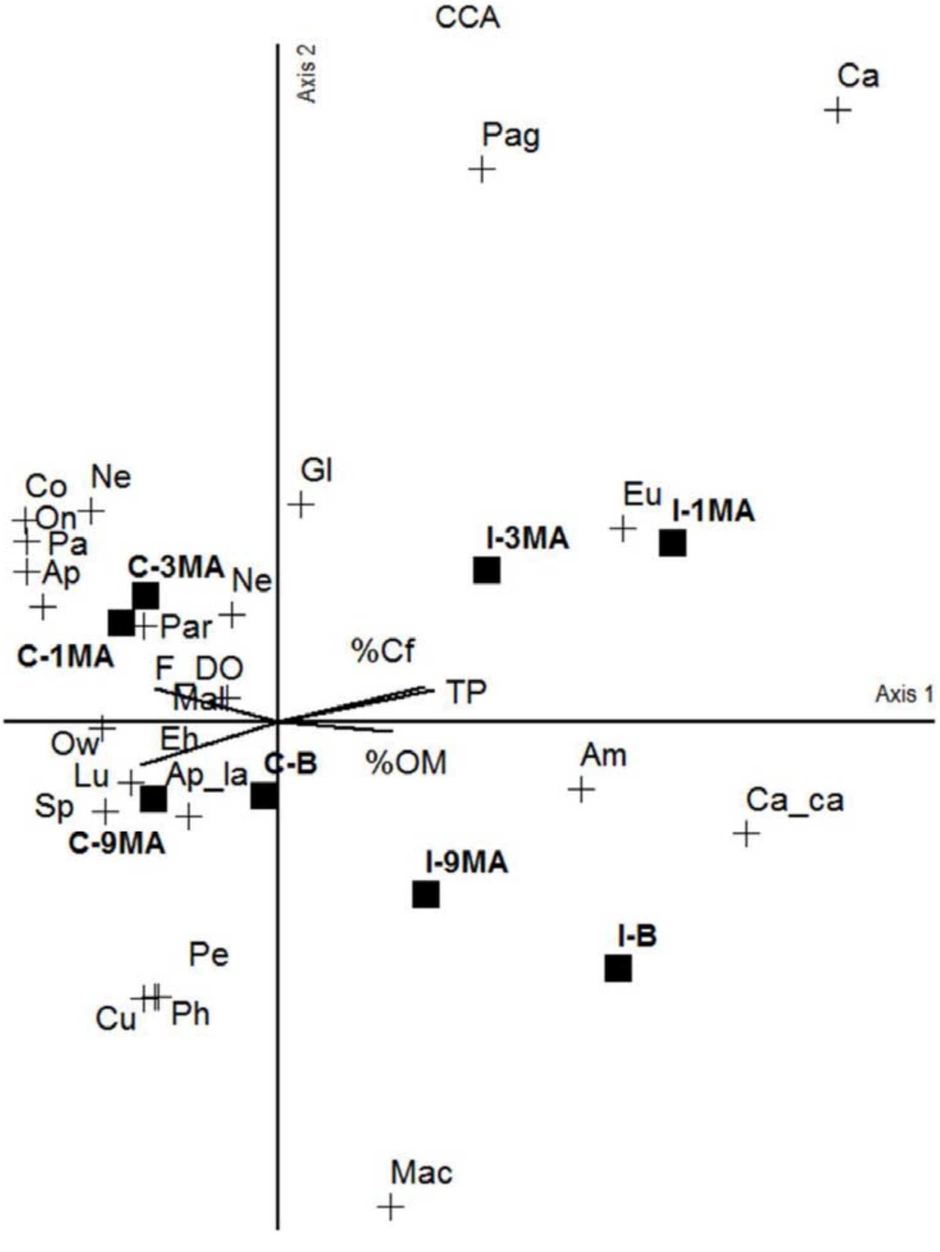

FIGURE 4. CCA ordination diagram showing the study sites positions: farming area (I), and control station (C), in four sampling campaign (square symbol), before farming cessation (B), and 1, 3, and 9 after farming cessation (1MA, 3MA, and 9MA, respectively); and distribution of species (sum symbol) in relation to predictor variables: flux of dissolved oxygen (F-DO), % organic matter (%OM), total phosphorus (TP), redox potential (Eh), and % coarse fraction (%Cf). Cu (Cumacea), Par (Pariambidae), Ap_la (*Apseudes latreilli*), Co (Corophiidae), Am

(Ampeliscidae), Pag (Paguridae), Ca (Cardiidae), Mac (Mactridae) Ap (Apistobranchidae), Eu (Eunicidae), Gl (Glyceridae), Lu (Lumbrineridae), Ca_ca (*Capitella capitata*), Mal (Maldanidae), Ne (Nephtydae), On (Onuphidae), Ow (Oweniidae), Pa (Paraoniodae), Pe (Pectinariidae), Ph (Phyllodocidae), Sp (Spionidae), Ne (Nematomorpha).

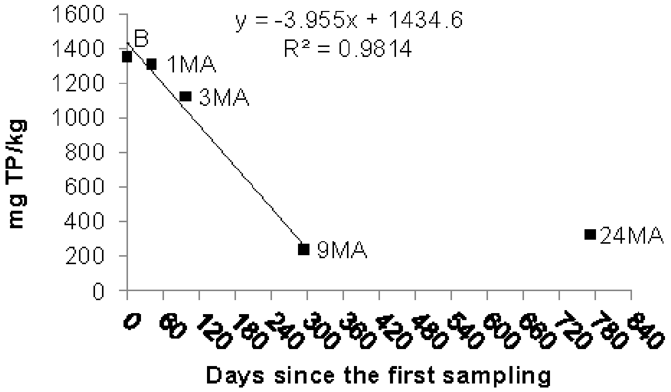

FIGURE 5. Temporal variation of the concentration of TP in the sediments in the farming area in the five sampling campaigns: before farming cessation (B) and 1, 3, 9, and 24 months after farming cessation (1MA, 3MA, 9MA, and 24MA, respectively).