Relationship between sedimentation rates and benthic impact on Maërl beds derived from fish farming in the Mediterranean

Running title: Relationship between sedimentation rates and benthic impact

Carlos Sanz-Lázaro[1]*, María Dolores Belando[1], Lázaro Marín-Guirao[2], Francisco Navarrete-Mier[1] & Arnaldo Marín[1]

Names and middle names

Surnames

[1]Departamento de Ecología e Hidrología, Facultad de Biología, Universidad de Murcia, 30100 Murcia

SPAIN

Phone: (+34)868884977

Fax: (+34)868883963

[2]Grupo de Ecología de Angiospermas Marinas, Centro Oceanográfico de Murcia, Instituto Español de Oceanografía, C/ Varadero, 1, San Pedro del Pinatar 30740, Murcia

SPAIN

Phone: +34 968 180500

Fax:+34 968 184441

*Corresponding author

E-mail of the corresponding author: carsanz@um.es, carsanzla@gmail.com

Eliminado: ¶
E-mail of the rest of the authors (listed respectively) : m.doloresbelando@um.es, arnaldo@um.es, lazaro.marin@mu.ieo.es, kfrancisco.navarrete@um.es.¶

Abstract

The aim of this work was to study the dispersion of particulate wastes derived from marine fish farming and correlate the data with the impact on the seabed. Carbon and nutrients were correlated with the physico-chemical parameters of the sediment and the benthic community structure. The sedimentation rates in the benthic system were 1.09, 0.09 and 0.13 g m^{-2} day^{-1} for particulate organic carbon (POC), particulate organic nitrogen (PON) and total phosphorus (TP), respectively. TP was a reliable parameter for establishing the spatial extent of the fish farm particulate wastes. Fish farming was seen to influence not only physico-chemical and biological parameters but also the functioning of the ecosystem from a trophic point of view, particularly affecting the grazers and the balance among the trophic groups. POC, PON and TP sedimentation dynamics reflected the physico-chemical status of the sediment along the distance gradient studied, while their impact on the benthic community extended further. Therefore, the level of fish farm impact on the benthic community might be underestimated if it is assessed by merely taking into account data obtained from waste dispersion rates. The benthic habitat beneath the fish farm, Maërl bed, was seen to be very sensitive to aquaculture impact compared with other unvegetated benthic habitats, with an estimated POC-carrying capacity to maintain current diversity of 0.087 g C m^{-2} day^{-1} (only 36% greater than the basal POC input). Environmental protection agencies should define different aquaculture waste load thresholds for different benthic communities affected by finfish farming, according to their particular degree of sensitivity, in order to maintain natural ecosystem functions.

Keywords: Mediterranean, particulate wastes, finfish aquaculture, trophic groups.

1. INTRODUCTION

In recent decades, seafood production from marine aquaculture has undergone almost exponential growth worldwide in terms of cultured biomass and is expected to follow the same trend in the future (FAO 2007). A combination of factors, including production levels, feed characteristics and feeding efficiency, influence the quantity and quality of the wastes released by fish farming (Islam 2005). The main negative impact of finfish aquaculture is the resulting organic enrichment derived from these wastes, which mainly consist of fish faeces and uneaten food and which may spread from tens to hundreds metres from the fish farm (Brown et al. 1987, Hall et al. 1990, Iwama 1991). Such wastes take two forms: particulate and dissolved. In the water column, the levels of dissolved wastes rapidly reach background levels, whereas particulate wastes tend to sink and accumulate on the seabed. This process may produce important changes in sediment geochemistry and in the benthic communities (Brown et al. 1987, Weston 1990, Karakassis et al. 2000, Holmer et al. 2005).

The spatial dispersion and potential effect of aquaculture particulate wastes on the ecological benthic status is site-specific and influenced by local physical-chemical and biological parameters (Karakassis et al. 1999, Sanz-Lázaro and Marin 2006). Benthic impact due to organic enrichment has been extensively studied in unvegetated beds (e.g. Black 1998, Karakassis et al. 2000, Brooks and Mahnken 2003, Aguado-Gimenez and Garcia-Garcia 2004, Kutti et al. 2007), identifying a well defined gradient (both in distance and time) from the source of contamination (Pearson and Rosenberg 1978). In contrast, the impact of aquaculture has been less studied in vegetated beds (Sanz-Lázaro and Marin 2008). The influence of finfish farming among vegetated beds, finfish farming influence has been studied to some extent (e.g. Ruiz et al. 2001, Holmer et al. 2004, Apostolaki et al. 2010), while, as regards Maërl beds, to the best of our knowledge, only one study, performed in Scotland, that has looked at the impact of aquaculture in this type of habitat (Hall-Spencer et al. 2006).

Research into the impact of fish farming on the benthic system has focused on two separate but closely related topics. Some studies have focused on predicting the reach of aquaculture particulate wastes by means of particulate waste dispersion modelling, while others have directly assessed the benthic impact of aquaculture by studying chemical and biological changes in the seabed. However, many of the studies carried out to date, with some exceptions (e. g. Pusceddu et al. 2007, Díaz-Almela et al. 2008, Kutti et al. 2008, Hargrave et al. 2008), cannot be considered integrative since they study isolated processes (i.e. either the benthic impact or the aquaculture particulate wastes in the water column) and make no attempt to merge them.

There is continuing pressure for aquaculture to become a more ecologically sound activity (Naylor et al. 2000, Lazard et al. 2009). Consequently, studies linking aquaculture waste dispersion rates with ecological benthic status must be performed to define thresholds below which finfish farm-derived organic matter deposition does not substantially affect benthic communities. In this way, protection agencies will be able to ensure the sustainability of aquacultural practices (Sanz-Lázaro and Marin 2008).

A great number of waste dispersion models have been developed to estimate the reach of organic residues derived from aquaculture (e.g. Cromey et al. 2002, Perez 2002) . Nevertheless, these models are frequently deficient as regards their accuracy for forecasting (Chamberlain and Stucchi 2007). This is partly due to the fact that robust and defensible information is not available for some of the key model parameters, such as waste dispersion and sedimentation rates (Islam 2005), for which the accuracy of the different models varies greatly (Chamberlain and Stucchi 2007). Field measurements associated with finfish aquaculture-derived particulate wastes are scarce and reliable replication over time is lacking, especially in the case of the Mediterranean (Holmer et al. 2007). Hence, more data related with aquacultural waste sedimentation dynamics are needed in order to refine models and improve their forecasting capacity.

'The aim of this work was to: (1) quantify fish farm particulate organic carbon, particulate organic nitrogen and total phosphorus and (2) correlate them with physico-chemical parameters of the sediment and the benthic community structure at a fish farm in the Mediterranean.

2. METHODS

2.1 Study area

The study was conducted at a marine fish farm located in Águilas, SE Spain (Western Mediterranean; 37º 24' 56.2" N, 1º 32' 4.0" W), which produces gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*). The fish farm consisted of two groups of 12 fish cages with an annual production of 1000 tonnes. Each fish cage had a diameter of 25 m and the bottom of the cage reached a depth of 19 m. The fish cage studied was located at the edge of the fish farm facility, "up-current" to the current flow of the other cages, and contained only gilthead sea bream. The water depth at this point was 31 m. During the sampling period (September and October 2006) an average of 83000 kg of fish were cultured in the studied fish cage and the daily feed input varied greatly: 994 ± 70 kg day^{-1} (mean ± SE; Fig. 1).

The computerized feeding system of the fish farm automatically distributes feed from the silo among the fish cages at an optimal rate and frequency according to satiety, which is controlled by the fish farm staff through the use of underwater cameras. The feed pellets supplied to the fish were cylindrical, with a diameter of 6 mm, and contained 45.8, 7.3 and 0.9 % of particulate organic carbon (POC), particulate organic nitrogen (PON) and total phosphorus (TP), respectively. During the study time the residual current direction was NE with a mean value of 0.08 m s^{-1} (Valeport 106 current meter, Valeport Limited, Dartmouth, UK; located in the fish farm ~30 m from the studied fish cage at a depth of 15 m; Fig. 2). The water temperature, at a depth of 6 m, during the sampling period ranged between 21 and 26 ºC. The seabed consisted of carbonate, coarse and medium sand, with an unattached Maërl bed habitat.

2.2 Particulate sedimentation sampling

POC, PON and TP sedimentation rates were measured by means of sedimentation traps composed of four attached cylinders (100 cm height and 12 cm diameter). Each cylinder had a funnel at the bottom, which guided the particulate matter into a 250 ml polyethylene tube. The stem of the funnel was too narrow to allow the passage of fish. Five to seven samples of the collected material from the sedimentation traps, taken every 5 days, were obtained within each sampling station placed along the environmental gradient influenced by the fish farm during the seven weeks that the experiment lasted (September and October of 2006). At each sampling station the sedimentation rates for each parameter were calculated as the mean values obtained out of the respective samples.

Particulate waste dispersion was analyzed by measuring the POC, PON and TP sedimentation rates using sedimentation traps placed along a spatial gradient upstream of the prevailing water-current, at increasing distances from the fish cage [0 (directly under the fish cage), 20, 120 and 600 m]. By means of a mooring system the sedimentation traps were installed 3 m above the seabed to avoid resuspension.

Sedimentation trap samples were stored frozen for no longer than two months at -20 ºC and thawed for analysis. After centrifuging at 4°C for 10 min at 1000 *g*, the overlying water was carefully removed (Vita et al. 2004). The settled particles were dried in an oven at 60 ºC to constant weight before being finely ground. POC (after a pre-treatment consisting of adding 1:1 HCl), and PON were determined using a Carlo Erba Inst. EA 1108 Elemental Analyser (Carlo Erba Strumentazione, Milan, Italy). TP was determined following the 4500-PE Ascorbic acid method (APHA 1995).

2.3 Sediment sampling

Sampling stations were located close to the mooring system of the sediment traps (~2 m) used for measuring waste dispersion (0, 20, 120 and 600 m). All distances were sampled once during the period that the sediment traps were deployed, taking four replicates at each sampling station. At each station physico-chemical sediment parameters were sampled by divers using cylindrical hand-operated corers within an area of ~0.5 m. The samples were immediately transported to the laboratory. The top 2 cm of the sediment were used for the physico-chemical analyses. Macrofaunal samples were taken using a hand grab (400 cm^2) that penetrated to a depth of ~10 cm.

From the cores taken at each sampling station the following parameters were measured. Four cores were used to measure the total ammonia nitrogen (TAN) content of the interstitial water, which was first extracted and then analysed by means of an Orion 95-12 ammonia selective electrode, according to the protocols described in APHA (1995). Four cores were used to measure the redox potential with an Orion ORP 91-80 electrode that was previously calibrated using a redox buffer solution (220 mV at 25 ºC). The same cores were used to measure the rest fo the physico-chemical parameters. Sediment grain size was assessed by dry-sieving with a mechanical shaker through a series of sieves (2, 1, 0.5, 0.25 and 0.064 mm mesh), in accordance with the Wentworth scale (Wentworth 1922). The fine fraction of the sediment (i.e. silt/clay) was taken as the sediment percentage that passed through the 0.064 mm mesh. The organic matter content was measured by weight difference after heating the dried sediment at 450 ºC for 5 hours. POC, PON and TP percentage of the sediment were determined following the same protocol as used for the samples from the sedimentation traps. The POC/PON (C/N) and PON/TP (N/P) ratios were determined in the sedimented material collected from the sedimentation traps as well as in the sediment.

Macrofauna was used as a surrogate of the ecological benthic status. For the macrofaunal analysis, sediment samples were washed through a 1 mm sieve with sea water. The remaining sediment was fixed in a 4% formalin buffered solution, separated into major faunal groups and stored in a 70% alcohol solution for later identification. The determination of benthic groups was made to the lowest possible taxonomic level using a binocular dissecting lens.

Macrofauna ash-free dry biomass was determined separately for each of the four sediment samples taken for the macrofaunal analysis at each station by weight difference after drying to constant weight at 60 ºC and subsequently heating at 450 ºC for 5 h.

2.4 Data analysis

The POC, PON and TP sedimentation rates and their respective concentrations in sediment at increasing distances from the fish farm were correlated using a linear Pearson correlation, after verifying that underlying statistical assumptions were fulfilled (Statistica, v6). Macrofaunal data (abundance and species richness) were used to calculate the Shannon-Wiener diversity index ($\log_2$ base; *H'*). As descriptors of the community structure, macrofaunal abundance, species richness, biomass and *H'* were plotted vs distance from the fish farm. Species richness and *H'* were used as indicators of the ecological benthic community status. In order to study the changes in the macrofaunal community from a functional perspective, the species were arranged according to their feeding habits. We gathered species into their respective trophic group, following the recommendations of Pearson (2001) and Fauchald and Jumars (1979). The abundance and biomass of each trophic group was plotted vs distance from the fish farm. The trophic group abundance ratio has been found to be more even and stable in non-polluted areas than in areas influenced by fish farming (Sanz-Lázaro and Marin 2006). Since *H'* is a metric that considers the number of species and the evenness within them, it was applied to compare the diversity and structure of the trophic group abundance. To calculate the *H'* of the trophic groups, each trophic group was considered as a species in the formula of *H'*.

Regression analyses were performed among POC, PON and TP sedimentation rates, as well as the community descriptors (abundance, biomass, species richness and *H'*) and distance from the fish farms. Regressions were performed using a non-linear curve fitting by means of an iterative process using the Marquardt-Levenberg algorithm (Marquardt 1963). The best-fit equation to the data was a single exponential decay, three-parameter equation, $f=y_0+a\ exp^{-b\ x}$ (SigmaPlot v10), where f=sedimentation rate/community descriptor, x=distance and a and b are constants.

A one-way ANOVA was performed individually for each parameter to identify significant differences among stations with respect to the POC, PON and TP sedimentation rates and concentrations in the sediment, as well as the community descriptors. In all these parameters, the levels of the factor (station) were treated as fixed levels. If the data did not meet the parametric assumptions, a log(x+1) transformation was applied. When significant differences were found among the treatments a Tukey *post-hoc* analysis was performed. If, after transformation, the data still did not meet ANOVA assumptions, a non-parametric Kruskal-Wallis test was performed. When significant differences were found in this test, a non-parametric multiple comparison Dunn test was applied to assess treatment differences (Dunn 1964) (GraphPad Prism v5). All data were reported as mean ± standard error (SE) and all statistical tests were conducted with a significance level of $\alpha = 0.05$.

Multivariate analyses were performed with the software package Primer (v6). Principal component analysis (PCA) was employed to show the relationship among the stations (samples) according to the physico-chemical parameters (variables: POC, PON, TP, TAN, organic matter, redox potential, fine fraction of the sediment, C/N and N/P). As a previous step to avoid skewness, a log(x+1) transformation was applied to the variables, which were then normalized (subtracting the mean and dividing by the standard deviation) for the scales to be comparable. Following this, a Pearson correlation analysis between the physico-chemical parameters and the scores of the first two axes of the PCA analysis was carried out in order to ascertain the extent to which the physico-chemical parameters correlated with the two main axes of the PCA. These axes were respectively multiplied by the variance explained by each axis, and were used in the group-average clustering procedure based on Euclidean distance. SIMPROF was used to find significant differences in the classification among the samples (Clarke and Gorley 2006).

A non-parametric multidimensional scaling (nMDS) ordination analysis (Clarke and Warwick 1994) based on the abundance matrix of the macrofaunal data was performed to examine differences in the assemblages of taxa and to represent the similarity among the samples. The index of dispersion (*D*) was applied to all data showing significant evidence of clumping, for which the data were dispersion-weighted following the recommendations of Clarke et al. (2006). A Bray–Curtis similarity matrix (Bray and Curtis 1957) was calculated after a mild transformation (square root) following Clarke and Gorley’s (2006) recommendations. After nMDS, a group-average clustering procedure based on Bray-Curtis similarity was carried out.

As with the PCA analysis, SIMPROF was applied to identify differences in the classification among the samples.

As another tool to assess the macrofaunal ecological benthic status, the Azti Marine Biotic Index (AMBI) was calculated using the February 2010 AMBI species list, which assigns species to a determined group according to their sensitivity to stress. Based on the AMBI values, the ecological benthic status of each location is qualitatively assessed as undisturbed (AMBI $\leq$ 1.2), slightly disturbed (1.2 < AMBI $\leq$ 3.3), moderately disturbed (3.3 < AMBI $\leq$ 5.0), heavily disturbed (5.0 < AMBI $\leq$ 6.0) or extremely disturbed (6.0 < AMBI $\leq$ 7) (Borja et al. 2000).

3. RESULTS

3.1 Waste dispersion

The POC sedimentation rate reaching the seabed below the fish farm was 1.09 ± 0.22 g m^{-2} day^{-1}, while nutrient sedimentation rates were 0.09 ± 0.02 and 0.13 ± 0.04 g m^{-2} day^{-1} for PON and TP, respectively (Fig. 3). The regression models of the POC, PON and TP sedimentation rates in relation to distance from the fish farm were significant (Table 1). These sediment rates were one or two orders of magnitude greater below the fish cage than at the furthest station.

All sedimentation rates fell exponentially as distance from the fish farm increased. POC, PON and TP sedimentation rates exhibited great variability among replicates taken from beneath the fish cage. This variability decreased as distance from the fish farm increased (Fig. 3).

There was a positive correlation between the sedimentation rates and sediment content of the measured parameters along the spatial gradient. Pearson correlation coefficients between sedimentation traps and sediment samples were 0.62, 0.69 and 0.90 for POC, PON and TP, respectively.

3.2 Physico-chemical parameters

TP gradually decreased with distance from the fish farm, while N/P increased. The redox potential showed a non-uniform increasing tendency. All three parameters showed significant differences among samples at 0 and 600 m from the fish farm. POC, PON, Organic matter, TAN, silt/clay and C/N showed changes with distance from the fish farm, although there were no significant differences among samples at 0 and 600 m (Table 2).

In the PCA analysis, principal components one (PC1) and two (PC2) accounted for 44.3% and 21.7% of the variability, respectively. A cluster analysis of PC1 and PC2 using SIMPROF arranged the variables into two groups that showed significant differences between them. One group included the stations that were situated 0 and 20 m from the fish farm and the other group comprised the 120 and 600 m stations (Fig. 4). In the group comprising the stations situated 0 and 20 m from the fish farm, samples were more dispersed compared with the samples of the group comprising the furthest stations from the fish farm. The Pearson correlation analysis among the physico-chemical parameters measured and PC1 and PC2 showed that PON (0.82), organic matter (0.80) and TP (0.73) were highly correlated with PC1, while redox potential (-0.83), C/N (-0.82) and N/P(-0.70) were negatively correlated. TAN (0.14) was poorly correlated with PC1. In the case of PC2, POC (0.82) was highly correlated, while the fine granulometric fraction (-0.79) was negatively correlated (Fig. 4).

3.3 Benthic structure

During the sampling and the sorting of the macrofauna it was found that coralline algae in the stations located 0 and 20 m from the fish farm had a greyish colour (a sign of stress; Wilson et al. 2004) and were noticeably smaller than at the most distant stations. At 120 m from the fish farm the coralline algae had a typical yellowish and pinkish pigmentation, but algal density was visibly lower than in the station located 600 m from the fish farm. This fact was reflected in the abundance of grazers. At 0 and 20 m from the fish cage there was no grazers, while at 120 m from the fish cage the densitiy of grazers was much lower 50 ± 14 individuals m^{-2} compared with the density at 600 m (306 ± 71 individuals m^{-2}).

The regression models of all the community structure descriptors were significant, except in the case of biomass. Macrofaunal abundance, biomass, species richness and *H'* showed a very similar trend with distance, greatly increasing close the fish farm and stabilizing further from the fish farm. This trend was opposite to that observed for POC, PON and TP sedimentation rates along the spatial gradient from the fish farm. Even so, the regression model showed that the community structure descriptors reached their asymptotic point further from the fish farm than the POC, PON and TP sedimentation rates (Fig. 5).

From the nMDS plot, SIMPROF significantly grouped samples of each sampling station. The nMDS values showed that the within-station similarity among samples increased with distance from the fish farm. Only one sample from below the fish cage stations was not grouped within any other group (Fig. 6).

The AMBI values were 2.2, 2.3, 1.4 and 0.6 at 0, 20, 120 and 600 m from the fish farm, respectively. According to these values the stations located at 0, 20 and 120 m were classified as slightly disturbed, while the station at 600 m from the fish farm was classified as undisturbed. The AMBI assessment agreed to some extent with the rest of the parameters measured, indicating that no influence of the fish farm at 600 m, while at 120 m the influence was still noticeable.

According to the trophic groups of the macrofauna, there were no grazers 0 and 20 m from the fish cage. Predator abundance peaked at 120 m from the fish cage, where this parameter was significantly different from the rest of the distances. Surface deposit feeder and grazer abundance significantly increased with distance from the fish cage, while suspension and sub-surface deposit feeder abundance did not differ significantly within the distance gradient. As regards biomass, surface deposit feeders and grazers showed a significantly greater biomass 600 m from the fish cage than below the fish cage, while biomass did not significantly differ for the rest of the trophic groups within the distance gradient (Fig. 7). The *H'* based on trophic group abundance was 0.89 ± 0.24, 0.92 ± 0.26, 1.44 ± 0.10 and 1.86 ± 0.04 bits for 0, 20, 120 and 600 m from the fish cage, respectively. The *H'* based on the trophic group abundance was significantly higher 600 m from the fish cage than at 0 and 20 m.

4. DISCUSSION

Particulate output derived from fish farming is a transient process in cage culture and depends on the particular physical, biological and feeding characteristics occurring at the farm site (Corner et al. 2006). The output of particulate wastes in the studied fish farm was low (1.09 ± 0.22, 0.09± 0.02 and 0.13 ± 0.04 g m^{-2} day^{-1} of POC, PON and TP, respectively), considering the time of the year when the study was performed (the higher the temperature, the greater the amount of feed supplied) compared with other fish farms in the Mediterranean that culture the same species: 1.00 – 7.00 g C m^{-2} day^{-1}, 0.05 – 1.20 g N m^{-2} day^{-1} and 0.14 - 0.80 g P m^{-2} day^{-1} (Holmer et al. 2007).

TP and redox potential values in the sediment were significantly different at 600 m and at 0 m from the fish cage and followed a decreasing and increasing trend, respectively, indicating that they were influenced by fish farming, which agrees with Karakassis et al. (1998) and Edgar et al. (2010). Furthermore, out of POC, PON and TP, the last parameter was best for establishing the spatial extent of fish farm particulate wastes, since it showed the highest correlation ($r = 0.90$) between sedimentation rate and sediment concentration. Among the parameters analyzed in the samples of material collected from the sedimentation traps, TP was the parameter that showed the highest correlation ($r = 0.90$) between sedimentation rates and sediment concentration. This fact indicates that TP can be considered suitable for indicating the spatial extent of fish farm particulate wastes. This finding was in agreement with Karakassis et al. (1998) and Holmer et al. (2007), who found that TP is a sensitive indicator of fish farm wastes.

The POC, PON and TP sedimentation dispersal pattern reflected the physico-chemical status of the sediment along the distance gradient studied. According to the physico-chemical parameters measured, the impact of the fish farm was not noticeable 120 m from the fish farm (Fig. 4). However, according to the biological parameters, such as the Shannon-Wiener diversity index and the nMDS, the impact on the benthic community due to fish farming extended further (Figs. 5 and 6). The AMBI classification agreed with the other biological parameters, indicating that the extent of the impact of the fish farm was still noticeable 120 m from the fish farm, this station being classified as slightly disturbed. Furthermore, the AMBI values also showed that the ecological benthic status was less disturbed 120 m from the fish farm than at 0 and 20 m. These findings suggest that the level of fish farm impact on the benthic community might be underestimated if it is only assessed by taking into account data of particulate waste dispersion rates or by only measuring physico-chemical parameters.

The ecological succession of macrofaunal communities due to organic enrichment has been widely studied in many parts of the world and similar community patterns have been found (Pearson and Rosenberg 1978). Briefly, along an organic enrichment gradient, macrofaunal abundance, biomass and species richness peak at intermediate levels of organic enrichment, and then, there is a sharp decrease at higher levels of organic enrichment up to a point where the sediment becomes azoic. In this work however, macrofaunal community parameters such as abundance and species richness did not peak at an intermediate impact level. As other studies have previously suggested, the macrofaunal community response under the influence of marine aquaculture in the Mediterranean may not always follow the pattern described by Pearson and Rosenberg (1978) (Karakassis et al. 2000, Apostolaki et al. 2007). This fact may be due to the differences in the physico-chemical parameters of the Mediterranean, such as higher temperatures and the different type of sediment (carbonate sediments), compared with areas culturing salmon where most studies have been performed (e.g. Brown et al. 1987, Morrisey et al. 2000, Brooks and Mahnken 2003, Pereira et al. 2004, Edgar et al. 2005).

From a functional perspective of feeding habits, grazers were not found 0 and 20 m from the fish cage. Surface deposit feeders and grazers significantly increased both in abundance and biomass within the fish farm gradient. For the rest of the trophic groups, there were no significant differences in abundance or biomass between 0 and 600 m from the fish cage. The observation that deposit feeders were more abundant in sites uninfluenced by the fish farm does not agree with other studies (Pearson and Rosenberg 1978, Sanz-Lázaro and Marin 2006) since the abundance and biomass of surface deposit feeders, such as capitelids, is expected to increase as the organic matter load increases. This could be due to the fact that the level of organic enrichment below the fish cage was not sufficient to produce a peak of surface deposit feeders. Anyway, the *H'* of the trophic groups showed an increasing trend as distance from the fish cage increased, indicating that not only the number of trophic groups but also the evenness among them increased as the influence of the fish farm diminished. This agrees with Sanz-Lázaro and Marín (2006), who found trophic groups to be more even and stable in non-polluted areas than in areas influenced by fish farming. According to the above, the present study indicates that fish farming influences not only physico-chemical and biological parameters of the sediment but also altered the functioning of the benthic system from a trophic point of view.

The information gap linking data for aquaculture particulate waste dispersion and community ecological status must be resolved for different areas and habitats, since such knowledge is necessary for predicting the level and spatial extent of fish farming impact and for enabling protection agencies to adequately manage this activity. Cromey et al. (2002) created a waste dispersion model and established semi-empirical quantitative relationships between the predicted accumulation of solids and observed faunal benthic indices. Even so, it was difficult to describe a relationship for the suite of benthic indices except for the total individual abundance and the Infaunal Trophic Index (ITI), whose reliability for assessing community ecological status is arguable (Maurer et al. 1999).

H' has proved to be amongst the best community ecological status indicators (Giles 2008). According to the diversity regression model obtained in this study, the increase in diversity starts to stabilize around 200 m from the fish farm. From the regression model equation, the corresponding POC sedimentation rate at 200 m for the studied fish farm was 0.087 g C m^{-2} day^{-1}, a value that can be taken as the carrying capacity to maintain current H' for the habitat, above which diversity starts to be decreased. The studied fish farm showed a relatively low release of organic matter to the environment, even though the impact was noticeable up to a considerable distance from the fish farm (200 m). Therefore, this habitat seems to be very sensitive to fish farming, and shows low resistance to increases in organic matter input (the carrying capacity for POC to maintain current H' corresponds to an increase of 36% of the basal POC input, which was calculated as the POC sedimentation rate at 600 m from the fish farm). This high sensitivity of Maërl beds agrees with Wilson et al. (2004), who found that Maërl species are not affected by industrial effluents containing high concentrations of pollutants, such as metals, but the presence of fine sediment with a high organic load such as fish farm wastes is highly lethal.

Conservation of the fauna and flora of benthic habitats is of primary importance, since soft bottom ecosystems are fundamental for the functioning of marine systems (Olsgard et al.

2008). For example, the macrofauna inhabiting marine benthic habitats play an important role in benthic-pelagic coupling through enhancing microbial carbon oxidation and nutrient recycling (Kristensen 2001). Most studies related with the benthic impact of finfish farming have been performed on unvegetated beds. As the present work shows with unattached coralline algae beds, different habitats may have different degrees of sensitivity to contamination. Very similar impact levels and their spatial extent were observed in Scottish Maërl beds as regards *H'*, in open water sites (Hall-Spencer et al. 2006). Consequently, the assessment of any impact of aquaculture on the benthos has to take into account the fact that the chemical and biological changes produced in the sediment may be highly dependent on the communities inhabiting the affected ecosystems.

The main contributions of the present study are explained next. First, new data for POC, PON and TP sedimentation rates reaching the seabed below an open water fish farm were obtained, the data indicating that the sedimentation rates are low compared with other fish farms in the Mediterranean (Holmer et al. 2007). This may be due to the fact that the fish farm is located in an open area where the depth of the water column is considerable (31 m), which would help the dispersion of fish farm wastes, and/or to the computerized system of the fish farm, which may help to reduce the fish farm waste load. Second, the regression models of the POC, PON and TP sedimentation rates in relation to distance from the fish farm were significant, although TP was the best parameter for establishing the spatial extent of fish farm particulate wastes. Other works have also found that TP is a sensitive indicator of fish farm loadings (Karakassis et al. 1998, Holmer et al. 2007). Third, POC, PON and TP sedimentation dynamics matched the physico-chemical status of the sediment along the distance gradient studied, while their impact on the benthic community extended further. This fact should be taken into consideration since the data obtained to predict benthic impacts derived from sedimentation rates could underestimate fish farm impact. Fourth, fish farming modified the benthos not only in a physico-chemical and biological way, but also functionally, lowering the number of trophic groups and the evenness among them. And fifth, the estimated carrying capacity to maintain current H' of the studied Maërl bed habitat for POC was 0.087 g C m^{-2} day^{-1},. To the best of our knowledge this is the first estimation of the carrying capacity for an unattached coralline algae habitat related with fish farming. Thus, environmental protection agencies should define specific aquaculture waste load thresholds for each benthic habitat according to its degree of sensitivity, in order to maintain an good ecological status and the functions of the different benthic communities in the vicinity of finfish farms.

5. CONCLUSIONS

This work confirms that TP is a reliable parameter for establishing the spatial extent of fish farm particulate wastes. The present study demonstrates that fish farming not only influences physico-chemical and biological parameters but also alters the functioning of the ecosystem from a trophic point of view, affecting mainly the grazers and the evenness among the trophic groups. This work shows that the level of fish farm impact on the benthic community might be underestimated if it is assessed by only taking into account data obtained from waste dispersion rates. The unattached coralline algae habitat studied seems to be very sensitive to fish farming compared with other unvegetated benthic habitats. Its POC carrying capacity to maintain current H' for the unattached coralline algae habitat is 36% over basal POC input.

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Tables

Table 1. Non-linear regressions among different variables and the distance gradient from the fish farm. For all regressions, the best-fit equation to the data was a single exponential decay, three-parameter equation ($f=y_0+a\ exp^{-b\ x}$), where f=variable, x=distance and a and b are constants. POC, PON and TP correspond to particulate organic carbon, particulate organic nitrogen and total phosphorus, respectively.

		Parameter					
		y_0		a		b	
Variable	ANOVA of regression (*P*)	Coefficient	Significance (*P*)	Coefficient	Significance (*P*)	Coefficient	Significance (*P*)
POC sedimentation rate	<0.0001	0.0868	0.1443	1.0056	<0.0001	0.094	0.0108
PON sedimentation rate	<0.0001	0.0088	0.1169	0.0853	<0.0001	0.1124	0.0486
TP sedimentation rate	<0.0001	0.0015	0.8492	0.1299	<0.0001	0.1624	0.2848
Abundance	0.0019	2386.4	<0.0001	-1797.1	0.0005	0.0194	0.1863
Biomass	0.0464	4.2407	0.1557	-3.1943	0.0174	0.0509	0.4387
Species richness	<0.0001	31.544	<0.0001	-22.177	<0.0001	0.0229	0.0111
H'	<0.0001	4.2718	<0.0001	-1.4345	<0.0001	0.034	0.0124

Table 2. Organic matter, POC, PON, TP, TAN (total ammonia nitrogen), redox potential, silt/clay (i.e. <0.064 mm), C/N and N/P along the environmental transect (mean ± SE; n=4).

Distance from fish farm (m)	Organic matter (%)	POC (%)	PON (%)	TP (%)	TAN (mg l^{-1})	Redox potential (Eh)	Silt/clay (%)	C/N	N/P
0	4.03 ± 0.20	7.06 ± 0.36	0.112 ± 0.006	0.211 ± 0.041	3.06 ± 0.20	-311 ± 20	2.28 ± 0.46	64.18 ± 6.91	0.59 ± 0.09
20	4.29 ± 0.12	5.54 ± 0.46	0.11 ± 0.007	0.12 ± 0.013	2.91 ± 0.14	-331 ± 8	5.14 ± 0.86	51.84 ± 7.41	0.96 ± 0.15
120	3.01 ± 0.12	6.42 ± 0.27	0.074 ± 0.004	0.058 ± 0.007	4.32 ± 0.4	-36 ± 20	3.74 ± 0.39	86.65 ± 4.54	1.33 ± 0.13
600	3.22 ± 0.77	6.88 ± 0.19	0.084 ± 0.013	0.028 ± 0.002	2.13 ± 0.53	-25 ± 33	2.75 ± 1.13	77.96 ± 7.58	2.88 ± 0.52

Figure captions

Figure 1. Feeding rate of the studied fish cage during the time span that the sedimentation traps were deployed. During that the same period of time the fish cage contained an average of 83000 kg of cultured fish

Figure 2. Current intensity (m s^{-1}) measured every 20 min in the fish farm during the time that the sedimentation traps were deployed. Data was obtained from a current meter located ~30 m from the studied fish cage at a depth of 15 m.

Figure 3. Carbon, nitrogen and phosphorus sedimentation rates (open circles; mean ± SE; n=5-7) and their sediment concentrations, dry weight %, (0 - 2 cm; filled circles; mean ± SE; n=4) along the distance gradient. Solid curves show non-linear curve fitting for sedimentation rates. Overall model fit and its coefficients are shown in the upper right of each graph according to the formula $f=y_0+ae^{(-b\ x)}$, where *f*=sedimentation rate, *x*=distance and *a* and *b* are constants.

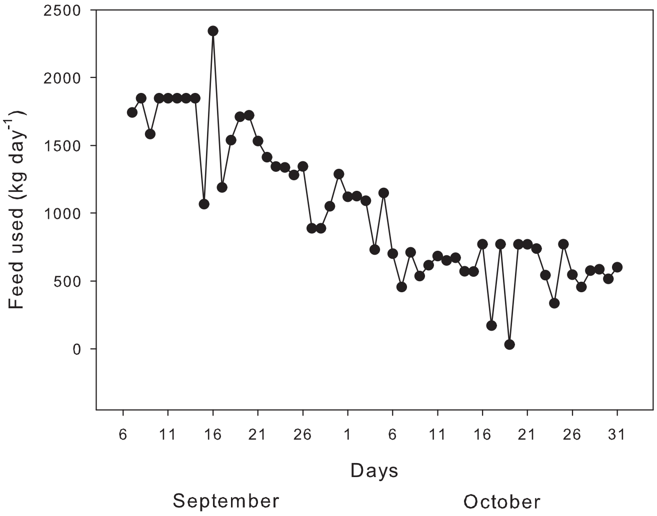
Figure 4. Principal components analysis of the percentage of silt/clay (i.e. <0.064 mm; fines), redox potential (redox), total ammonia nitrogen (TAN), organic matter (OM), POC, PON, TP and the atomic ratios of nutrients (C/N and N/P). Results are grouped according to SIMPROF (dotted lines; significance level 5%).

Figure 5. Descriptors of the community structure (mean ± SE; n=4) along the distance gradient. A) abundance, B) biomass, c) number of species and d) Shannon-Wiener diversity. Solid curves show non-linear curve fitting. Overall model fit and coefficients are shown in the lower right of each graph according to the formula $f=y_0+ae^{(-b\,x)}$, where *f*=descriptor, *x*=distance and *a* and *b* are constants.

Figure 6. Non-parametric multi-dimensional scaling plot of macrofaunal community abundance along the distance gradient from the fish farm (indicated by the numbers). Results are grouped according to SIMPROF (significance level 5%).

Figure 7. Trophic group abundance (A) and biomass (B; mean ± SE; n=4) along the distance gradient from the fish farm.

Figure 1.



2500
2000
1500
1000
500
0
Feed used (kg day^{-1})
6
11
16
21
26
1
6
11
16
21
26
31
Days
September
October

Figure 2.

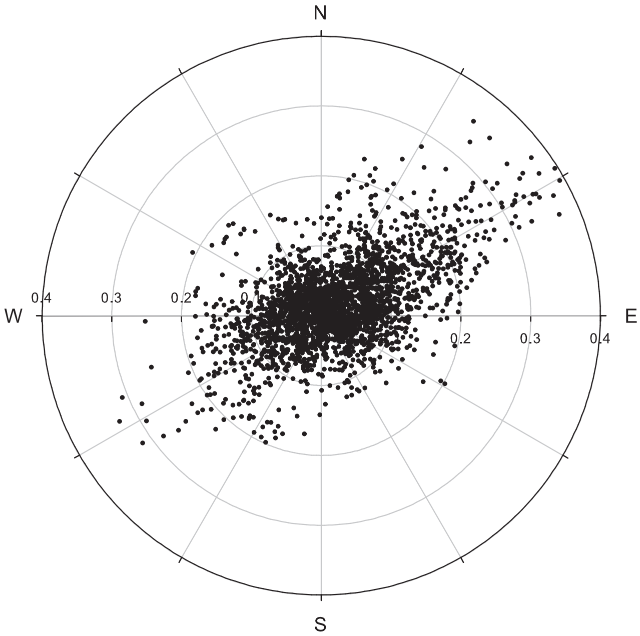


Figure 3.

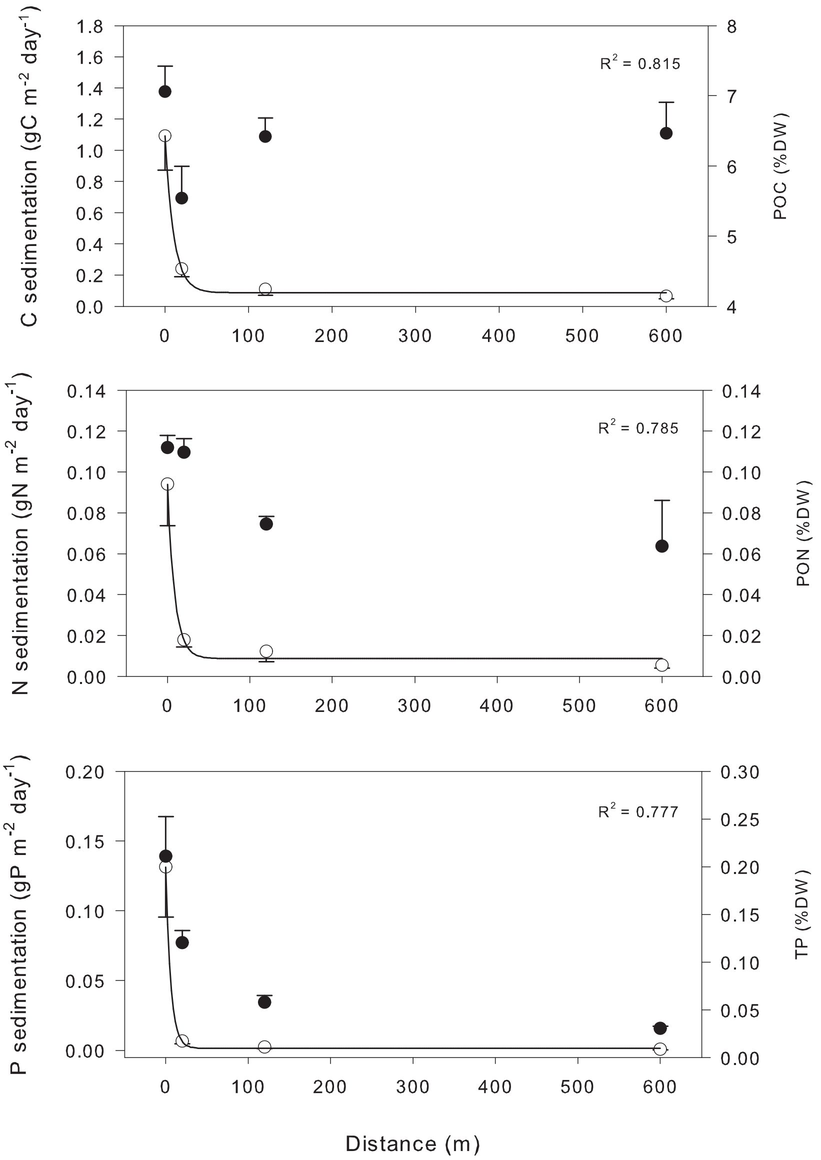
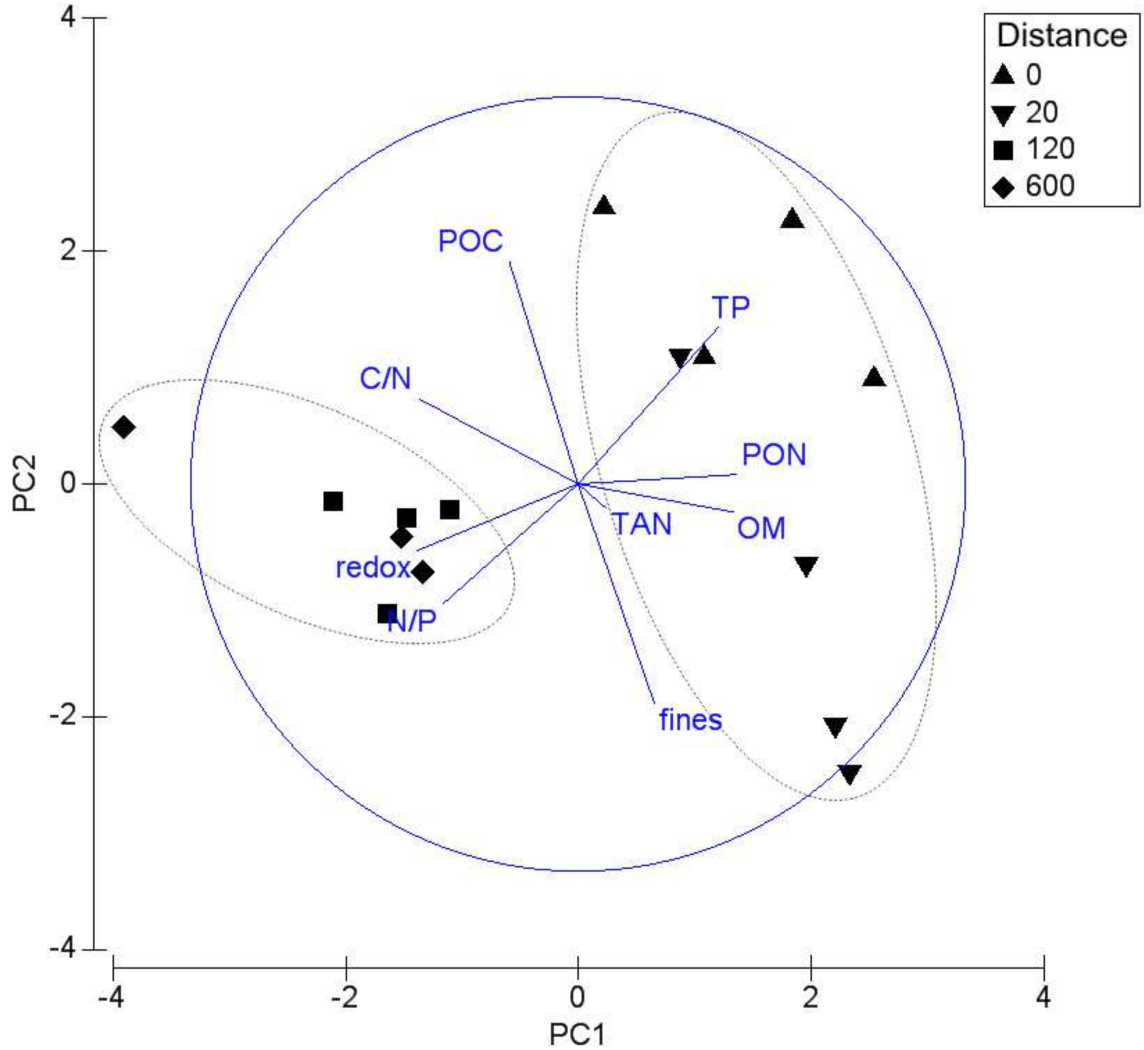
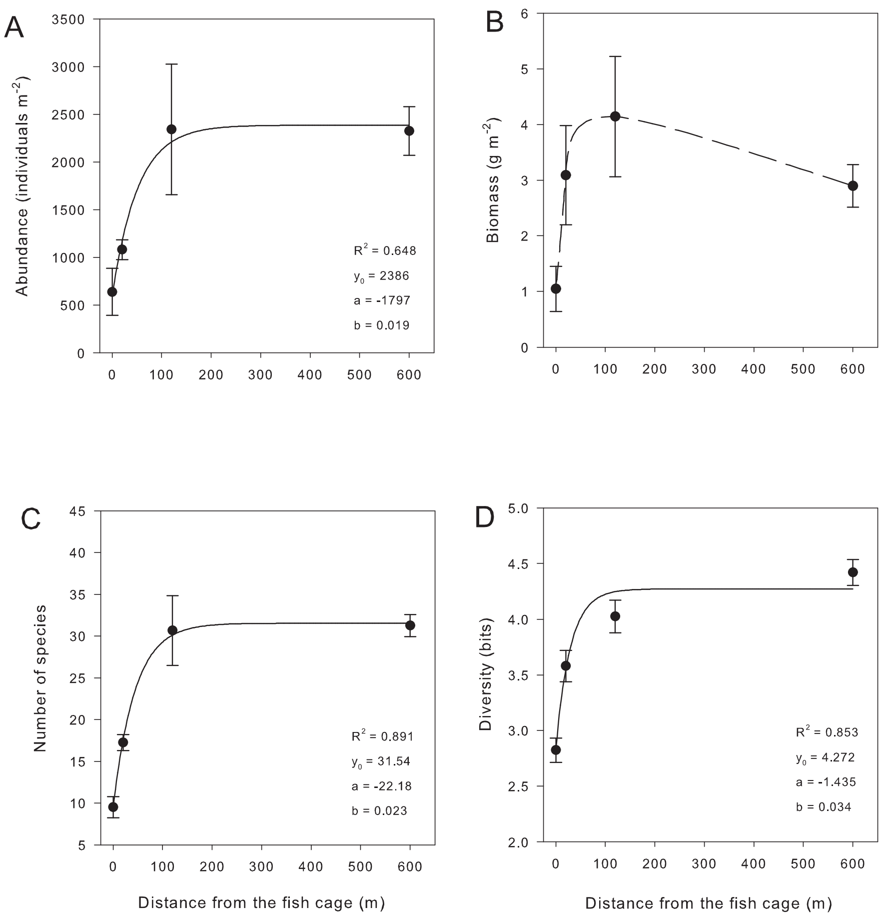

C sedimentation (gC m-2 day-1)
POC (%DW)
R2 = 0.815
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
8
7
6
5
4
0
100
200
300
400
500
600
N sedimentation (gN m-2 day-1)
PON (%DW)
R2 = 0.785
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
P sedimentation (gP m-2 day-1)
TP (%DW)
R2 = 0.777
0.20
0.15
0.10
0.05
0.00
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Distance (m)

Figure 4.



Distance
0
20
120
600
POC
TP
C/N
PON
TAN
OM
redox
N/P
fines
PC2
PC1
4
2
0
-2
-4
-4
-2
0
2
4

Figure 5.



A
Abundance (individuals m-2)
R2 = 0.648
y0 = 2386
a = -1797
b = 0.019
B
Biomass (g m-2)
C
Number of species
R2 = 0.891
y0 = 31.54
a = -22.18
b = 0.023
D
Diversity (bits)
R2 = 0.853
y0 = 4.272
a = -1.435
b = 0.034
Distance from the fish cage (m)
Distance from the fish cage (m)

Figure 6.

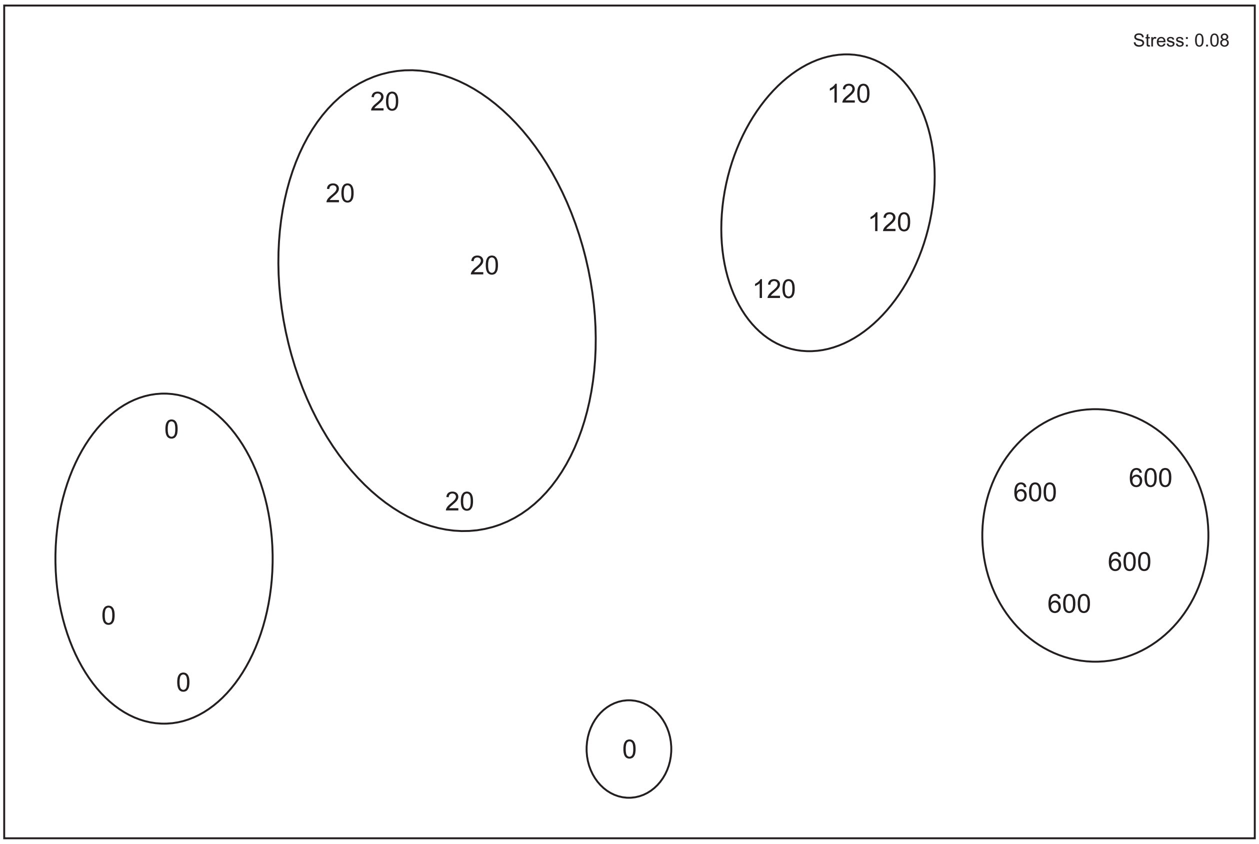
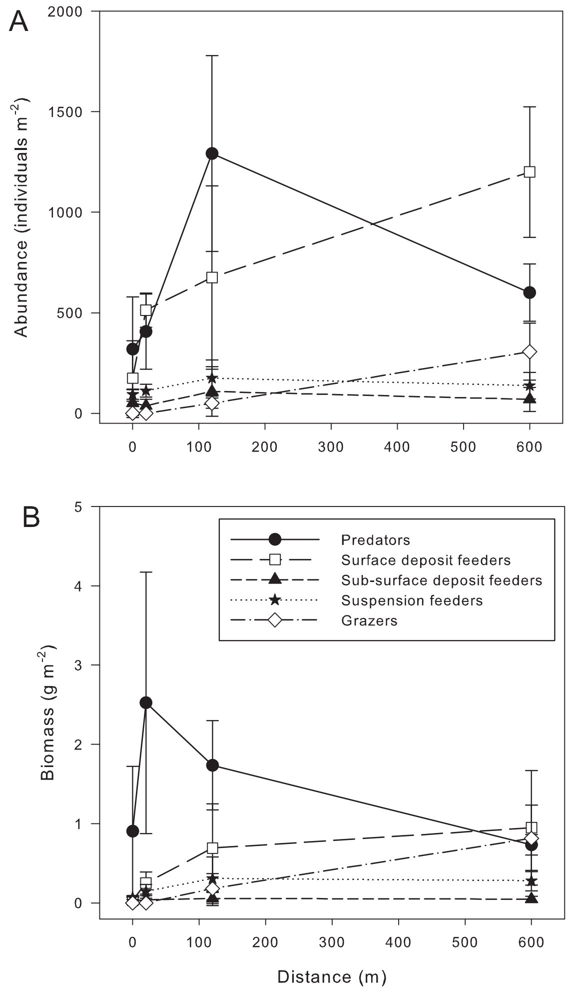


Figure 7.


A
2000
1500
1000
500
0
Abundance (individuals m-2)
0
100
200
300
400
500
600
B
5
4
3
2
1
0
Biomass (g m-2)
Predators
Surface deposit feeders
Sub-surface deposit feeders
Suspension feeders
Grazers
Distance (m)