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Influence of fish food and faecal pellets on short-term oxygen uptake, ammonium flux and acid volatile sulphide accumulation in sediments impacted by fish farming and non-impacted sediments

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Abstract

Sediment cores were taken from impacted and non-impacted areas and subjected to different incubations: (i) uninoculated, (ii) inoculated with fish feed and (iii) inoculated with gilthead seabream (*Sparus aurata*) faeces. After inoculation (or not), the cores were incubated for 8 h and the following biogeochemical fluxes were determined: sediment oxygen uptake (SOU), total ammonia nitrogen flux (TAN_f) and the production of acid volatile sulphides (AVS-S_p). The results showed that the impacted sediments had a more pronounced benthic metabolism than non-impacted sediments. Correlations between the variables and factorial analysis showed that oxygen consumption caused by the organic enrichment appeared as the trigger for subsequent biogeochemical alterations. The addition of faeces led to proportionally higher benthic rates of SOU, TAN_f and AVS-S_p than those obtained in the feed incubations. Although the feed is relatively sterile and does not create an oxygen demand until colonized by bacteria, the faeces are already richly colonized with fish gut bacteria and could start to consume oxygen without the lag phase experienced in the incubations with feed. The TAN_f values measured after the addition of feed or faeces seem to be more related to the leaching velocity of TAN than with the benthic flux, given the short incubation time.

Keywords: aquaculture, water-sediment flux, total ammonia nitrogen, acid volatile sulphides, sediment oxygen uptake, wastes

Introduction

One of the most important environmental impacts of marine cage fish farming involves the deposition of particulate waste products, mainly uneaten food and fish faeces below the cages and in the vicinity of the farm. Some of this particulate organic matter (OM) is mineralized or incorporated in the pelagic food web as it sinks and disperses (Piedecausa, Aguado-Giménez, García-García, Ballester & Telfer 2009), but a substantial fraction settles on the seabed (Hall, Anderson, Holby, Kollberg & Samuelsson 1990; Sampou & Oviatt 1991; Holmer & Kristensen 1992; Karakassis & Hatziyanni 2000; Kutti, Ervik & Hoisaeter 2008). Once settled, the OM is rapidly mineralized, releasing nutrients to the water column in the process. This input of waste products from fish farms stimulates the total metabolism of the sediments (Hall, Holby, Kollberg & Samuelsson 1992; Valdemarsen, Kristensen & Holmer 2009), and as a result, oxygen may be used up and sulphate reduction may be stimulated, leading to the increased production of sulphides (Holmer & Kristensen 1994), anoxia and environmental toxicity.

The OM in sediments that is not consumed by the macrofauna is degraded to inorganic compounds through bacterial processes involving different acceptors as oxidants (Canfield, Jorgensen, Fossing, Glud, Gundersen, Ramsing,Thamdrup, Hansen, Nielsen & Hall 1993). The most efficient process of OM mineralization is aerobic oxidation. In sediments with a high organic load, the oxygen contained in the top millimetres of the sediment is rapidly con-

sumed, and microorganisms that use other electron acceptors as oxidants (such as NO_3^-, SO_4^{2-}, Fe oxides and Mn) dominate. Sulphate reduction quickly becomes the predominant respiration (Sloth, Blackburn, Hansen, Risgaard-Petersen & Lomstein 1995; Christensen, Rysgaard, Sloth, Dalsgaard & Schwaerter 2000; Thamdrup 2000) and the equilibrium existing between aerobic and anaerobic metabolism is displaced towards the anaerobic.

Feed and faeces have very different macronutrient contents (Reid, Liutkus, Robinson, Chopin, Blair, Lander, Mullen, Page & Moccia 2008), the former having a higher proportion of proteins and lipids, while the latter has a higher moisture content and more carbohydrates and fibre (Guillaume & Choubert 1999), also carrying gut bacteria. Albertelli, Covazzi-Harriague, Danovaro, Fabiano, Fraschetti and Pusceddu (1999) observed that the benthic response to the input of OM depends more on the quality than on the quantity of the OM. Kristensen (2000) suggested that the rate of OM decomposition depends not only on its quality but also on the stage of the decomposition process and temperature. Obviously, the benthic mineralization of fish farm wastes also depends on how available they are for degradation, so that labile substrates are more easily and more rapidly mineralizable than more refractory material (Van Nugteren, Moodley, Brummer, Heip, Herman & Middleburg 2009). Moreover, the metabolic rates of sediment receiving a pulse of OM at a given moment will depend on the OM already accumulated as these organic compounds also participate in the benthic metabolism (Kristensen & Blackburn 1987).

Several studies have focused on water-sediment fluxes and the biogeochemistry of marine sediments subjected to organic enrichment derived from fish farming (Holmer & Kristensen 1994; Findlay & Watling 1997; Morrisey, Gibbs, Pickmere & Cole 2000; Holmer, Marbá, Terrados, Duarte & Fortes 2002; Holmer, Wildish & Hargrave 2005; Sakami, Yokoyama & Ishihi 2005; Magill, Thetmeyer & Cromey 2006; Chamberlain & Stucchi 2007; Kutti *et al.* 2008; Brigolin, Maschio, Rampazzo, Giani & Pastres 2009), but very few studies deal with the effects of organic enrichment by fish farm-derived wastes (feed and faeces) on the biogeochemical fluxes (Papageorgiou, Kalanzi & Karakassis 2010). This study, then, relates the short-term response of the benthic system to the type of additional organic load and attempts to detect differences between the different sediments used. For this purpose, impacted and non-impacted sediment cores (inoculated with feed or faeces) were incubated, for studying how these organic wastes from fish farms influence some benthic biogeochemical processes, such as sediment oxygen uptake and nitrogen ammonia total fluxes and the production of sulphides in the sediment.

Materials and methods

Sediment cores and water sampling

Sediment and water samples were taken from two sites: an offshore area impacted by a gilthead seabream (*Sparus aurata*) farm (250–300 tonnes year^{-1}), and a non-impacted area approximately 500 m upstream of the fish farm (Murcia, SE Spain: 37°48.941′N; 00°41.731′W). The seabed in both sites was a detritic sedimentary floor with a very low slope (< 2%) and 37 m deep. The mean current velocity was 8.7 cm s^{-1} (Aguado-Giménez, Marín, Montoya, Marín-Guirao, Piedecausa & García-García 2007).

Intact sediment cores were carefully collected by scuba divers using methacrylate hand corers (internal diameter 53 mm; length 300 mm). The sediment cores (height 50 mm) were brought to the surface in special containers to keep them vertical and prevent their disturbance (Wildish, Akagiu, Hamilton & Hargrave 1999). Water for incubation experiments was taken near the seafloor at the same sites as the sediment samples, using a 10 L Niskin water sampler (KC - Research Equipment, Silkeborg, Denmark). The sediment samples were taken randomly during the summers of 2006 and 2007. Bottom water temperature varied from 13 to 20 °C. Once on board, the sediment cores were stored under cold conditions until they reached the laboratory.

Core incubations

Incubation of the sediment cores followed the method described by Dalsgaard, Nielsen, Brotas, Viaroli, Underwood, Nedwell, Sundbäck, Rysgaard, Mites, Bartoli, Dong, Thornton, Ottosen, Castadelli and Risgaard-Petersen (2000) with modifications. The cores were placed in an incubator made from 20-mm-thick high-density polyethylene and comprising two concentric cylinders (Fig. 1). The physically isolated inner cylinder contained the stirring system. The outer cylinder (90 L volume) was divided into four parts to keep the corers vertical and was filled with water from the study zone. The incubation temperature (T) was maintained constant at the temperature of the site and the time of sampling by means of a water recirculating and cooling system.

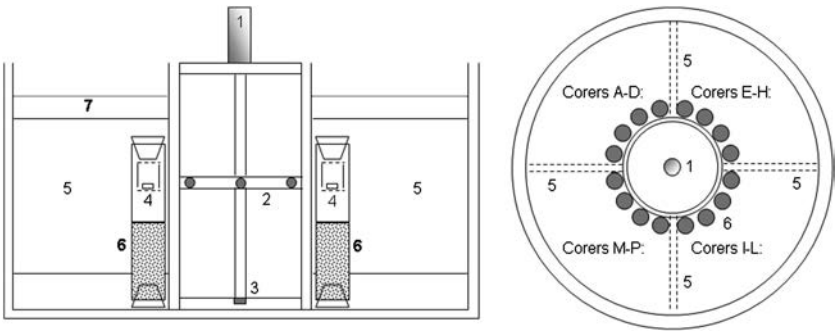

Figure 1 Diagram of the incubation system. 1: stirring motor (adjustable 12 V DC); 2: magnets; 3: ball bearing; 4: magnetic rods; 5: semi-partitioned walls; 6: sediment cores; 7: water level. Corers A–D: after pre-incubation and just before incubation for FF and *p* determination in sediments, and DO and TAN determination in water column (initial incubation conditions). Corers E–H: after pre-incubation and just before incubation for AVS-S determination in sediments (initial incubation conditions). Corers I–L: after incubation (8 h) for DO and TAN determination in water column (final incubation conditions). Corers M–P: after incubation (8 h) for AVS-S determination in sediments (final incubation conditions). AVS-S, acid volatile sulphides; DO, dissolved oxygen; FF, finest fraction; TAN, total ammonia nitrogen.

The above was subsequent to a pre-incubation step, which was carried out upon reception of the cores in the laboratory to homogenize the water of the incubator and corers. This step consisted of maintaining the cores in the incubator with the top of the corers open for 16–18 h (Christensen *et al.* 2000). A PVC disc was inserted into each corer to hold a magnetic stirrer in place, while gentle stirring prevented stagnation without resuspension during both the pre-incubation and incubation steps. After pre-incubation, initial samples of water and sediment were taken and the rest of the cores were covered.

The incubation phase lasted 8 h, a time established previously so that the water of the cores did not fall below 50–60% oxygen saturation (Dalsgaard *et al.* 2000; Karle, Hall & Dallhöf 2007). Both pre-incubation and incubation were carried out under simulated light conditions. The irradiance at the sampling sites was measured with a spherical *quantum* sensor (LICOR LI-1400, Lincoln, NE, USA). At 37 m depth, PAR was equivalent to 2–3% of the surface radiation (15–35 $\mu S\ m^{-2}\ s^{-1}$). These light conditions were simulated in a room by adjusting the light accordingly. The cores taken from the impacted and non-impacted sites were incubated without and with inoculations of feed (moisture: 7.4%, protein: 48.6% dry weight (d.w.), fat: 21.5% d.w.; carbohydrates: 23.1% d.w.) or seabream (*Sparus aurata*) faeces (moisture: 89.3%, protein: 21.1% d.w., fat: 4.3% d.w.; carbohydrates: 71.1% d.w.). The feed (0.15 ± 0.20 g with 51% C content, equivalent to the addition of 5.95 mmol C d.w. = 8.19 mol C $m^{-2}\ day^{-1}$ d.w.; and an N content of 7.5%, equivalent to the addition of 0.77 mmol N d.w. = 1.06 mol N $m^{-2}\ day^{-1}$ d.w.) was triturated and inoculated into the core immediately before incubation. The faeces (0.18 ± 0.10 g, containing 28.6% C, equivalent to the addition of 0.40 mmol C d.w. = 0.56 mol C $m^{-2}\ day^{-1}$; and an N content of 3.4%, equivalent to the addition of 0.04 mmol N d.w. = 0.06 mol N $m^{-2}\ day^{-1}$ d.w.) were obtained by dissecting the distal 4 cm of the intestine (Chen, Beveridge & Telfer 1999). The strong difference with respect to the C and N content between food and faecal pellet additions is motivated by the fact that fish faeces leach N and C compounds much faster than fish food (Fernández-Jover, Sánchez-Jérez, Bayle-Sempere, Carratala & León 2007; Piedecausa *et al.* 2009) and a compromise between gross weight and nutrient content needed to be set up in order to avoid over- or underloading. Combinations of sampling sites (SS, impacted and non-impacted) and inoculum type (IT, non-inoculation and inoculation with feed or faeces) were replicated four times.

Sediment and water variables and analytical methods

Sixteen sediment cores were used in each incubation treatment and four replicates within each treatment. Of the 16 cores, four (cores A–D in Fig. 1) were sampled before incubation (a) to characterize the sediment, determining the finest fraction (FF < 0.063 mm) using granulometric analysis, sieving the dry sediment through a tower (2, 1, 0.5, 0.25 and 0.063 mm) using a mechanical shaker classifying the sediments on the Wentworth scale (Buchanan 1984) as composed of gravel–sand–clay and mud

and also determining the porosity (p) from the weight and volume of the sample [p = (wet weight − dry weight/volume)] (Dalsgaard *et al.* 2000); and (b) to measure dissolved oxygen (DO, luminescent electrode of dissolved oxygen: LDO101-03 Probe HACH LANGE, HACH-LANGE Company, Düsseldorf, Germany) and total ammonia nitrogen (TAN, selective ion electrode: ORION 9512 BN (Thermo Scientific, Fort Collins, CO, USA); American Public Health Association 1995) in the water column. In this way, we obtained the initial conditions before beginning the incubations. Four other cores (cores E–H in Fig. 1) were used to determine the final conditions of DO and TAN after incubation. Another four cores (cores I–L in Fig. 1) were used to measure acid volatile sulphides (AVS-S) with an ion selective electrode (ORION 9616 BN; Allen, Fu & Deng 1993) in the top 5 cm of the sediment, as initial concentration. From the four remaining cores (cores M–P in Fig. 1), the final concentration of AVS-S was obtained. In non-inoculated incubations, the sediment oxygen uptake (SOU), TAN flux (TAN_f) and AVS-S production ($AVS\text{-}S_p$) were calculated from the following equation:

$$(\mathrm{SOU}),\ (\mathrm{TAN_f}),\ (\mathrm{AVS\text{-}S_p}) = \frac{(C_8 - C_0) \times V}{(S \times t)}$$

In the corresponding inoculated incubations, the fluxes were calculated taking into account the additional organic load and using the following equation:

$$(\mathrm{SOU}),\ (\mathrm{TAN_f}),\ (\mathrm{AVS\text{-}}S_p) = \frac{(C_8 - C_0) \times V}{(S \times t \times \mathrm{OM_{add}})}$$

where C_0 and C_8 are the concentrations of DO, TAN or AVS-S at the beginning or the end of the incubation (mmol L^{-1}), V is the volume of the water column (L), S is the sediment surface (m^2), t is the incubation time (days) and OM_{add} is the added OM (mmol C added for SOU and $AVS\text{-}S_P$; mmol N added for TAN_f).

Statistical treatment

Physical characteristics, FF and p, in impacted and non-impacted sediments were compared using one-way ANOVA. With regard to the non-inoculated incubations, the differences in SOU, TAN_f and $AVS\text{-}S_P$ between non-impacted and impacted zones were also compared using one-way ANOVA. For the inoculation assays with feed or faeces, a correlation matrix was first made between all variables to determine any association or agreement between them. Then, those that might act as co-variables of the dependent variables SOU, TAN_f and $AVS\text{-}S_P$ were selected and their effect on statistical contrast of the factors SS and IT were examined. Following the criteria proposed by Hair, Anderson, Tatham and Black (1999), the differences in SOU, TAN_f and $AVS\text{-}S_P$ between SS and IT were contrasted without (two-way ANOVA) and with (two-way ANCOVA) the co-variables, to see whether the inclusion of the co-variables improves the statistical power of the comparisons. If no considerable improvement is observed, the co-variables are not taken into consideration, as they would also reduce the degrees of freedom available (residuals) for the factor comparison. Finally, a principal components analysis (PCA) was made as an exploratory technique for determining the structure of the data as a whole and to establish which variable or group of variables best explains most of the variance (Hair *et al.* 1999). For this analysis, data of SOU, TAN_f and $AVS\text{-}S_p$ of inoculated incubations were expressed without relativizing to the additional organic load; in this way, data of inoculated and non-inoculated incubations are expressed with the same units. Normalized VARIMAX factorial rotation was applied. Thus, the communality of each variable was calculated as a representative measure of the proportion of variance contributed by each variable to the final solution or, in other words, the proportion of variance explained by the extracted factors. Factorial loads above 0.7 and communalities above 0.5 were considered to be significant (Hair *et al.* 1999).

Results

Physical characteristics of impacted and non-impacted sediments were very similar (Table 1). No significant differences ($P > 0.05$) between zones were detected with regard to particle size distribution, and although significant ($P < 0.05$), the difference in porosity was not very large. Regardless whether or not corers had been inoculated, benthic metabolism was always higher in the impacted sediments (Fig. 2a–d). In the non-inoculated assays (Fig. 2a), SOU was significantly higher (37% on average) in the impacted sediment samples ($P < 0.05$). $AVS\text{-}S_p$ was also significantly higher in the impacted sediment samples ($P < 0.05$) but to a greater extent (87% on average). On the other hand, TAN_f was also greater in the impacted sediments (80% on average) but not to a statistically significant extent ($P > 0.05$) because of the great variability observed in impacted sediments for this variable.

Benthic metabolism was also always higher in impacted than non-impacted sediments regardless of IT (Fig. 2a–d). The variables that were significantly

Table 1 Particle size distribution and porosity (*p*) of sediments from the impacted and non-impacted areas (mean ± standard error)

Area	% Gravel	% Sand	% Silt and clay (FF)	*p*
Impacted	11.61 ± 0.86[a]	84.75 ± 0.71[a]	3.64 ± 0.60[a]	0.78 ± 0.05[a]
Non-impacted	11.40 ± 1.93[a]	85.06 ± 1.43[a]	3.58 ± 0.48[a]	1.11 ± 0.08[b]

Comparisons using one-way ANOVA. Different superscripts in the same column indicate statistically significant between the areas ($P < 0.05$).
FF, finest fraction.

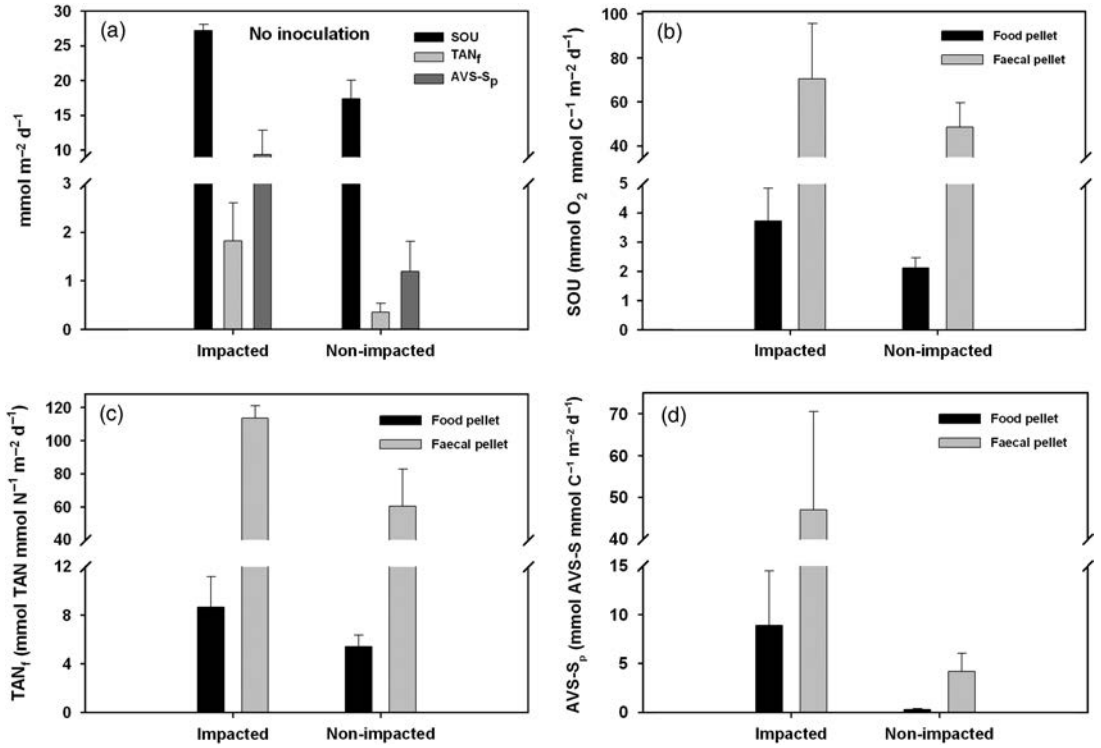

Figure 2 Benthic fluxes in impacted and non-impacted sediments. (a) SOU, TAN_f and AVS-S_p (mmol m^{-2} day^{-1}) in non-inoculated sediments. (b) SOU (mmol O_2 mmol C^{-1} m^{-2} day^{-1}) after inoculating feed or faeces. (c) TAN_f (mmol TAN mmol N^{-1} m^{-2} day^{-1}) after inoculating feed or faeces. (d) AVS-S_p (mmol AVS-S mmol C^{-1} m^{-2} day^{-1}) after inoculating feed or faeces.

correlated (Table 2) were considered to be potential co-variables: In this case, *T* was significantly and positively correlated with SOU, SOU was significantly and positively correlated with TAN_f and SOU was significantly and positively correlated with AVS-S_p, so the higher the *T*, the higher the SOU, and the higher the SOU, the higher the TAN_f and the AVS-S_p. Other interesting, but not significant, correlations were the positive correlation between FF and *p*, the negative correlation between AVS-S_p and both FF and *p* and the positive correlation between AVS-S_p and TAN_f.

In assays involving inoculations, contrasts were first made using two-way ANOVA with SOU, TAN_f and AVS-Sp as dependent variables and SS and IT as factors, subsequently incorporating the corresponding potential co-variables to see whether the statistical significance improved. The only significant co-variable was *T* with SOU ($P < 0.001$). Table 3 shows the results obtained by ANOVA and ANCOVA. In the case of SOU, once the influence of *T* had been removed, significant differences were observed for the IT ($P < 0.001$). Bearing in mind the proportion of the load added, SOU was higher when faeces were inoculated. With regard to the two different collection zones, no significant differences were detected ($P > 0.05$), although SOU was always higher in the

Table 2 Correlation matrix between the water, sediment and benthic metabolism variables: water temperature (*T*), sediment porosity (*p*), sediment finest fraction (FF), sediment oxygen uptake (SOU), total ammonium nitrogen flux (TAN_f) and acid volatile sulphide production ($AVS\text{-}S_p$)

	T	FF	*p*	TAN_f	SOU
FF	− 0.11				
p	− 0.36	0.49			
TAN_f	0.28	0.13	0.15		
SOU	**0.61**	0.06	0.06	**0.73**	
$AVS\text{-}S_p$	0.49	− 0.41	− 0.25	0.46	**0.61**

Significant correlations ($P < 0.05$) are given in bold.

Table 3 Summary of the statistical results for the two-way ANCOVA for SOU and two-way ANOVA for TAN_f and $AVS\text{-}S_p$ with the sampling site (SS, impacted and non-impacted) for both inoculum type (IT, feed and faeces)

Dependent variable	Sources of variation	d.f.	MS	F
SOU	*T* (co-variable)	1	9638.28	26.55^{***}
	SS	1	520.06	1.43^{NS}
	IT	1	9955.12	27.42^{***}
	SS × IT	1	1470.19	4.05^{NS}
	Residual	11	363.03	
TAN_f	SS	1	3386.51	5.53^{**}
	IT	1	27664.36	45.19^{**}
	SS × IT	1	2673.44	4.37^{NS}
	Residual	12	612.20	
$AVS\text{-}S_p$	SS	1	3533.95	5.34^{**}
	IT	1	1279.50	1.93^{NS}
	SS × IT	1	1781.32	2.69^{NS}
	Residual	12	662.04	

$^{*}P < 0.05$.
$^{**}P < 0.01$.
$^{***}P < 0.001$.
$AVS\text{-}S_p$, production of acid volatile sulphides; FF, finest fraction; NS, not significant; SOU, sediment oxygen uptake; TAN, total ammonia nitrogen.

impacted zone (Fig. 2b). Similar results were obtained for TAN_f. When faeces were inoculated, TAN_f was significantly higher ($P < 0.001$) than when the feed was inoculated, and in the impacted zone TAN_f was greater (Fig. 2c). AVS-Sp was significantly higher in the impacted zone ($P < 0.01$) regardless of the inoculum used. However, although not to a statistically significant extent, faeces caused a proportionally higher AVS-Sp (Fig. 2d).

Table 4 shows the results of the PCA. The first two factors extracted explained 59.72% of the variance. The first factor explained 38.41% of the total variance, with *T* (0.91) and SOU (0.86) showing a significant factorial load. The second factor explained 21.31%, although AVS-Sp was the only variable with a significant factorial load in this case (0.79). Communality was significant for *T* (0.53) and SOU (0.59) but not for AVS-Sp.

Table 4 PCA results: factor loadings, communality and explained variance

	Factor 1	Factor 2	Communality (R^2 multiple)
T	**0.91**	− 0.15	**0.53**
FF	− 0.11	− 0.66	0.15
p	− 0.41	− 0.60	0.24
TAN_f	0.44	0.34	0.15
SOU	**0.86**	0.24	**0.59**
$AVS\text{-}S_p$	− 0.07	**0.79**	0.20
% explained variance	38.41	21.31	Total: 59.72

Significant factor loading and communality ($P < 0.05$) are given in bold.
$AVS\text{-}S_p$, production of acid volatile sulphides; FF, finest fraction; PCA, principal components analysis; SOU, sediment oxygen uptake; TAN, total ammonia nitrogen.

Discussion

This work confirms that benthic metabolism in sediments impacted by fish farming activity is significantly more accentuated, as it had been observed previously by other authors (Hargrave, Duplisea, Pfeiffer & Wildish 1993; Christensen *et al.* 2000; Holmer *et al.* 2002; Ferrón, Ortega & Forja 2009; Papageorgiou *et al.* 2010). The benthic processes occurring seem to be interrelated because biogeochemical variables are usually correlated, as have been described in various studies concerning sediment organic enrichment (Papageorgiou *et al.* 2010). Hargrave *et al.* (1993) in a study on salmon farms showed that sediment fluxes increased during periods of higher water temperature, and also how oxygen uptake and ammonium flux were related. Similar results have been obtained in our work; oxygen consumption was highly correlated with the water temperature, and the former was significantly and positively correlated with ammonium flux and sulphide production. The dependence of temperature on multiple biochemical processes, particularly on the oxygen consumption is well known (Hargrave *et al.* 1993). This influence may be critical at the end of the warmest season when warm waters sink, coinciding with the period of maximum growth rate and maximum waste output of many fish species

(Piedecausa, Aguado-Giménez, Cerezo-Valverde, Hernández-Llorente & García-García 2010).

Modification of the oxic conditions of the sediment surface leads to changes in the way in which the OM decomposes. The addition of organic material, such as the wastes from aquaculture, increases oxygen demand as a result of the mineralization carried out by aerobic microbial activity, but also as a result of chemical oxidative processes such as the re-oxidation of the sulphides produced previously by anaerobic sulphate-reducing bacteria (Holmer *et al.* 2005; Preisler, De Beer, Lichtschlag, Lavik, Boetius & Jorgensen 2007). Fish farm sediments are characterized by the production and accumulation of sulphides (Samuelsen, Ervik & Solheim 1988; Hall *et al.* 1990; Holmer & Kristensen 1992; Holmer *et al.* 2005; Aguado-Giménez *et al.* 2007). This increase in sulphides enhances oxygen consumption in the upper layers of sediment (Hargrave *et al.* 1993; Wildish *et al.* 1999; Rickard & Morse 2005; Shin, Cheung & Cheung 2006), and hence the observed correlation between SOU and AVS-Sp. Hargrave, Holmer and Newcombre (2008) observed that sulphides are accumulated to a greater extent in aquaculture-impacted sediments, which is to be expected because such sediments favour sulphate-reducing activity. The physical characteristics of sediment and oxygen availability play an important role in benthic processes and how benthic communities respond to the addition of OM (Gray, Wu & Or 2002; Hargrave *et al.* 2008). Whether or not the sediment has or has not suffered, some aquaculture waste-derived impact has some influence previously on the benthic rates, due to the participation of organic compounds already existing in the sediments (Kristensen & Blackburn 1987; Faganeli & Ogrinc 2009). Thus, the lower porosity of the sediments of the impacted zone suggests that water-sediment exchanges are more limited, which would have favoured anoxification and the accumulation of sulphides.

Analysis of the PCA reveals which variables had greater specific weight when explaining the observed phenomena. The SOU was the most influential variable and its correlation with both TAN_f and AVS-Sp can be understood as the reduction in oxygen availability favours the proliferation of anaerobic processes such as the reduction of sulphate to sulphide and the release of ammonia. The strong influence of temperature on oxygen consumption and the latter on sediment biogeochemistry imply that these variables determined, to a greater extent, the magnitude and the predominance of one or another biogeochemical pathway. The accumulation of sulphides is just a consequence of the limited oxygen availability, which would explain its minor contribution to the explained variance. Therefore, the oxygen depletion caused by the organic enrichment appeared as the trigger for subsequent biogeochemical alterations. The scarce importance of TAN_f within the dataset could be due to the fact that TAN_f mainly comes from leaching, because of the short term of the incubations (Piedecausa *et al.* 2009). The physical variables measured in the sediment (FF and *p*) did not show a significant relevance on the processes measured, although it is known that less porous sediments, such as those from the impacted zone, which hinder the diffusion of gases and solutes, favour anaerobic conditions (Avnimelech, Ritvo, Meijer & Kochba 2001)

Holmer and Kristensen (1994) indicated that both the quantity and quality of sediment OM affect microbial reactions and processes. Ding and Sun (2005) also suggested that the degradation rate of OM in different redox conditions depended more on its chemical composition than on the quantity. Inoculation with faeces resulted in a more marked (relative to organic material addition) benthic metabolism than when feed was inoculated. The most likely explanation for this behaviour is that, whereas the feed is relatively sterile and does not create an oxygen demand until colonized by bacteria, the faeces are already richly colonized with fish gut bacteria and could start to consume oxygen without the lag phase experienced in the incubations with feed, in spite of the fact that faeces composition could seem hardly attackable by bacteria. Almost certainly, in a longer incubation the reverse results would have been expected. Also, the fact that faeces are easier-to-leach than feed (Fernández-Jover *et al.* 2007; Piedecausa *et al.* 2009) would explain the enhanced benthic processes when faeces were inoculated.

The results of this work corroborate that sediments impacted by fish farming activity display a stressed metabolism. Different fish farming-derived wastes, feed and faecal pellets, stimulate the benthic processes in a different way as a function of their quality and bioavailability, but the short-term duration of the incubations does not allow describing the complete dynamic of wastes degradation, although the initial phase of mineralization was shown.

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