BIODEPOSITS CONTRIBUTION TO NATURAL SEDIMENTATION IN A SUSPENDED Mytilus galloprovincialis Lmk MUSSEL FARM IN A GALICIAN RÍA (NW IBERIAN PENINSULA)

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Abstract

This work aimed to study how mussel culture of Mytilus galloprovincialis Lmk modulates the natural sedimentation rates through the water column as key controlling factor of the potential organic load reaching the bottom sediments. To achieve this objective, seston, faeces production and natural sedimentation dynamics were characterized during 4 seasonal campaigns at two mussel rafts and at a reference station not affected by the mussel culture in the Ría de Ares-Betanzos (NW Iberian Peninsula). Our results showed that seston content are below the pseudofaeces threshold (< 4 mg L^{-1}) with inorganic contents ranging between maximum values of 76 ± 4% in winter and minimum values of 6 ± 4% in spring. These seasonal variations controlled the faeces

(biodeposits) production by mussels (11-221 mg ind^{-1} d^{-1}) that increased with seston content, in particular under winter stormy conditions, when intense continental runoff and resuspension processes represented an important source of non-digestive inorganic particles for mussels.

Natural sedimentation fluxes recorded by means of sediment traps under the rafts (84-536 g m^{-2} d^{-1}) seasonally varied with mussel biodeposition being 6-7 fold the rates at the reference site. This fact corroborates that mussel feeding activity throughout the production of faeces increased natural sedimentation rates in the Ría de Ares-Betanzos. However, the relatively energetic water column conditions and the potentially high degradation rates of faeces in the culture area reduced up to 55-90% the biodeposits organic carbon potentially arriving to the seafloor. This indicates that in highly hydrodynamic environments the impact derived from shellfish culture may be reduced, attenuating a possible impact on the benthic communities.

1. INTRODUCTION

In coastal areas with high density of suspended bivalve cultures, the capacity of filter feeder organisms to clear large volumes of particles, repackage them and return them to the water column as faeces and pseudofaeces, hereafter referred as biodeposits, noticeably alters the transfer of matter and energy to the seafloor (see reviews Cranford et al., 2006; McKindsey et al., 2011). The study of this subject is of great importance not only to know the impact on the benthic ecosystem (Hatcher et al., 1994; Nizzoli et al., 2005; Danovaro et al., 2004; Cranford et al., 2003) but also to develop carrying capacity models for bivalve cultivations, which may aid to maximize sustainable bivalve production (Carver and Malet, 1990; Dame and Prins, 1998; McKindsey et al., 2006; Duarte et al., 2008; Weise et al., 2009; Duarte et al., 2010).

Many studies carried out under different laboratory and natural conditions have contributed to explain how mussel biodeposition increased the natural sedimentation underneath suspended cultures (e.g. Kautsky and Evans, 1987; Hatcher et al., 1994; Giles et al., 2006; Callier et al., 2006). Recent advances have used this information jointly with hydrodynamics to predict footprints of benthic loadings around mussel farms (Giles et al., 2009; McKindsey et al., 2009; Weise et al., 2009). From these models it was stated that dispersion/decay rate of biodeposits are modulated by the energy in the study environment, controlling in last term the biodeposits density below the culture areas.

The Galician Rías support the highest mussel production in Europe (250×10^6 kg yr^{-1}) with a total of 3335 mussel rafts enclosed in areas called polygons (Labarta et al., 2004). Given the large economic importance of this activity, the scientific community has focused on studying diverse aspects related with the biogeochemical impacts of mussel farm in the Rías (e.g. Maar et al., 2008; Otero et al., 2008; Petersen et al., 2008;

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Ysebaert et al., 2009; Alonso-Pérez et al., 2010; Froján et al., 2014). Since the 70s, it is known that the introduction of mussel aquaculture altered the trophic chain of the ecosystem and modified the natural sedimentation rates at the raft areas (Tenore and González, 1975; Cabanas et al, 1979). However, only one study (Alonso-Pérez et al., 2010) has subsequently quantified the natural sedimentation rates in the Ría de Vigo by considering different sites inside a raft polygon. These authors confirmed the important role that suspended mussel cultures play in the redistribution of organic material and nutrients. None of these previous studies have assessed the contribution of mussel faeces production to the natural sedimentation rates in the Rías.

In this regard, this study carried out in a Galician Ría (NW Iberian Peninsula) addresses, for the first time, the seasonal effect of both hydrodynamics and Mytilus galloprovicialis Lmk feeding activity (through faeces production) on the sedimentation rates and on the subsequent potential organic load reaching the bottom sediments in a natural environment. Results derived from this work are crucial as previous studies (see review McKindsey et al., 2011) have linked organic enrichment of the superficial sediments to alterations on sedimentary geochemical properties and changes in macrofaunal assemblage composition.

2. METHODS

2.1 Study site

The Lorbé raft polygon (local term to refer to a farm with tenths of mussel rafts) is situated in the southern side of the Ría de Ares-Betanzos (NW Iberian Peninsula) (43° 23' 24.74''N; 8° 17' 48.30''W) (Figure 1), being the main area of mussel Mytilus galloprovincialis culture in the Ría, with 107 rafts and a total production of 10,000 tons

year^{-1} (Labarta et al., 2004). The rafts have a size of 20 x 25 m, support 500 ropes 12 m long and are 100 m apart. They are anchored by an iron chain at the bow.

Mean total velocities at Lorbé raft polygon are around 5 cm s^{-1} and the variance of the current direction tidally dominated (Piedracoba et al., 2014). The seabed in Lorbé area is mainly composed by medium to fine sand with organic carbon contents < 2.8 % (Sánchez-Mata et al., 1999).

During 2010 and 2011, three stations located in the Ría de Ares-Betanzos (Figure 1) were monitored during 4 seasonal campaigns covering the most representative oceanographic scenarios for the Rías: i) summer upwelling (July 2010), ii) autumn bloom (October 2010), iii) winter mixing (February 2011) and iv) spring bloom (May 2011). Two stations were located in two commercial rafts from the outer (P46) and inner (P14) part of the Lorbé polygon moored at 16 and 14 m water depth, respectively (Figure 1). A third station, situated outside the polygon zone at 18 m water depth (Figure 1) was used as a reference (Ref) site, representing the hydrographic and biogeochemical conditions of the water column unaffected by mussel activity. During each sampling period, environmental data were obtained at the three stations. Physiological experiments with mussels were performed at the P46 and P14 raft stations to determine the egestion rates.

2.2 Hydrodynamic data and experimental design

Temporal series of wave data was supplied by Puertos del Estado and correspond to the propagation from the point WANA 3027034. The data were obtained from the WAM numerical model that works with a spatial resolution of 30 km in the Atlantic ocean. Ekman transport (-Q_{E-W}), an estimate of the volume of water upwelled per kilometre of coast, was calculated according to Bakun´s (1973) method:

$$-Q_{E-W} = - ((\rho_a \, C \, |V|) / (f \, \rho_{sw})) \, V_H$$

where ρ_a is the density of the air (1.22 kg m^{-3}) at 15 ºC, C is an empirical dimensionless drag coefficient (1.4 10^{-3}), f is the Coriolis parameter (9.946 10^{-5}) at 43ºN, ρ_{sw} is the seawater density (1025 kg m^{-3}) and |V| and V_H are the average daily module and northerly component of the geostrophic winds centred at 43ºN, 11ºW, respectively. Average daily winds were estimated from FNMOC model from NOAA institution at 44.5ºN and 7.5ºW. For calculations, sea level pressure was obtained from atmospheric model WXMAP. Positive values show the predominance of easterly winds that induces upwelling processes inside the Ría. On the contrary, negative values indicate downwelling processes.

Hydrographic data used in this study were obtained from a station (Intecmar station; Figure 1) maintained by Intecmar, an oceanographic institution in charge of monitoring the marine environment in the Galician Rías (http://www.intecmar.org/Ctd/Default.aspx).

Four FSI 2D-ACM current meters were moored at 1 m depth at the four sides (bow, port, starboard and stern) of both P46 and P14 rafts (Figure 1). Mean current flow statistical parameters for each sampling period were derived from 22-31 days starboard currentmenter data as this side of the raft was the most exposed to circulation (Aguiar et al. submitted).

Seston characterization, egestion (biodeposition) experiments with mussels and natural sedimentation rates at the raft stations were obtained during two consecutive days during each seasonal campaign. The egestion experiments (3-4 hours) were executed onboard of a boat docked to the corresponding raft to maintain ambient conditions of temperature, salinity, and food availability. Seawater from 3 m water depth was supplied by a peristaltic pump to a header tank and filtered through a 50 µm nylon mesh

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before being distributed at a constant flow rate to the experimental chambers (Filgueira et al., 2006). On each visit, mussels (n=15) between 50-60 mm were randomly sampled at 3 m water depth in the middle part of both P-46 and P-14 sampling rafts, cleaned of epibionts and placed in chambers with flowing seawater. Duplicate seawater samples from the outflow of an empty chamber were collected at the beginning, middle and end of the experiments to characterize the natural seston and Chlorophyll a (Chl a) content. At the end of the physiological experiments, the deposited mussel faeces of each tank were manually collected into 25 mL glass vials by means of a pipette.

Natural sedimentation rates were measured by using a multitrap collector system at the three stations. The multitrap collector is composed of 4 PVC trap baffled cylinders (6 cm diameter and a height/diameter ratio of 10.8) located at the edges of a PVC cross. Prior to deployment, the cylinders were filled with filtered seawater (membrane filter 0.4 µm pore size) and supplemented with NaCl (5 g L^{-1}) to avoid material exchange with surrounding waters. No preservatives were added. At the reference station a multitrap at 8 m depth was deployed during sampling intervals of 24 hours (twice each season). At each raft (P46 and P14) station two multitraps were deployed at 8 m depth (length of mussel ropes) at both the raft bow and stern during each egestion experiment interval. Data presented in this study are mean values of both traps.

Superficial sediments were collected with a Van Veen grab at the reference, P46 and P14 sampling sites. Samples were stored in sealed plastic bags at 4 °C until processing in the laboratory.

2.3 Analytical methods

Seston

Total (TPM), organic (POM) and inorganic (PIM) particulate matter concentrations were gravimetrically determined. Seston samples were filtered onto pre-ashed (450 °C for 4 h) and pre-weighed Whatman GF/F filters and rinsed with isotonic ammonium formate (0.5 M) to remove salts and prevent lysing of living algal cells. TPM was determined as the weight increment after drying the filters to constant weigh at 110 °C with precision of 0.001 mg. Filters were then ashed at 450 °C in a muffle furnace to determine the content of PIM. POM corresponded to the difference between the total dry mater weight and the ash weight. Chl a in seston was determined spectrophotometrically by filtering 1 L samples through 25 mm Whatman GF/F filters. The filters were then frozen at -20 °C until pigment extraction in 90% acetone over 24 hours in the dark at 4 °C. Quantification was performed following equation presented by Jeffrey and Welschmeyer (1997).

Biodeposits

After the collection into the glass vials, the faeces were settled onto formiate-pre-washed, pre-combusted (450 ºC, 4 h) Whatman GF/F filters (0.7 μm nominal pore size). The filters were then dried overnight (100 ºC) until constant weight and frozen at -20 ºC until analysis. Blank formiate-pre-washed and pre-combusted filters were also analyzed. For organic carbon (C_{org}) and nitrogen analysis in faeces the filters were de-carbonated using HCl vapours. Measurements of C_{org} were carried out with a Perkin Elmer 2400 CNH analyser, including daily acetanilide standards. The precision of the method was ± 0.3 μmol C L^{-1} and ± 0.1 μmol N L^{-1}.

To quantitatively study the contribution of faeces production to natural sedimentation rates units mg ind^{-1} d^{-1} were converted to g m^{-2} d^{-1} by considering the raft area (500 m^2) and the mussel biomass at each raft during each sampling period (Table 1). The mussel

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biomass was directly measured by counting the ropes of the raft and the number of mussels per meter of rope during each sampling experiment.

Sediment traps

From each multitrap collector, we determined total mass flux, particulate C_{org} and nitrogen, and Chl a contents. For total mass flux, 250 mL samples were filtered on pre-weight, pre-combusted 47 mm GF/F filters (0.7 μm nominal pore size). After being rinsed with Milli-Q water to remove the salts, filters were dried overnight at 40 °C and weighted. For C_{org} analysis, 100 mL samples were filtered on pre-weighted, pre-combusted (450 ºC, 4 h) Whatman GF/F filters (0.7 μm nominal size pore), dried overnight and frozen (-20 °C) before analysis. C_{org} and Chl a in the sediment trap samples were analyzed following steps previously described for faeces and seston, respectively.

Superficial sediments

At each station, three non-homogenized sub-samples of approximately 1 g each were used for grain size analysis with an LS100 Coulter Counter. The lithogenic material was described from a subsample treated with 10% H_2O_2 and 1 M HCl to remove organic matter and carbonates, respectively. Three main grain size categories were considered:

i) sand (>63 μm), ii) silt (4-63 μm) and iii) clay (<4 μm).

2.4. Statistical analyses

The mean regime applied to current speed allowed the extraction of average conditions for a given percentile of probability. In this way, it can be described the average conditions by the 50% percentile that represents the value exceeded 50% of the time.

Another relevant parameter is the 95% percentile as it gives information about more energetic conditions, which define the most intense events (Villacieros-Robineau et al., 2013).

Two-way analyses of variances (ANOVAs) were performed in order to evaluate significant effects of site (Reference, P46 and P14) and season (summer, autumn, winter and spring) over the environmental characteristics, faeces composition and natural sedimentation rates. Before performing statistical analysis, each variable was tested for normality conditions (Test de Shapiro-Wilk, $p > 0.05$) and homogeneity of variances (Test de Levene, $p > 0.05$). When the hypothesis of normality and homogeneity of variances were not assumed a two-way ANOVA by ranks (equivalent to the test of Kruskal-Wallis by ranks) was performed. Tukey´s HSD (Honestly Significant Difference) post-hoc test was selected for pairwise comparisons on main significant effects. Data analysis was performed with the statistical package R 2.12.2 (R Development Core Team 2011).

3. RESULTS

3.1 Hydrodynamic conditions

During summer 2010 cruise, the water column was characterized by a thermal stratification in the upper meters ($\Delta T = 18\text{-}14.5\,°C$) at the same time that upwelling favorable winds ($-Q_{E-W} > 0$) provoked the entrance of subsurface cold (14 °C) and salty waters into the Ría (Figure 2). During this sampling period the 50% percentile of the total current at P46 raft station was 6.1 cm s^{-1} with a tidal contribution of 6% (Table 1). In autumn 2010 campaign, under prevailing downwelling conditions we found a thermally mixed (15 °C) water column with relatively low sea surface salinity (<35 psu, not shown) due to freshwater input from Mondeo and Eume rivers. During this period

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total currents were less than 5 cm s^{-1} at both rafts for the 50% percentile, while significant wave height was relatively high favored by southwesterly winds (Figure 2; Table 1). A reduction in transmittance due to sediment resuspension seems to be associated to the wave action. The winter 2011 sampling period was carried out during a transition from upwelling to downwelling conditions under strong storm conditions reflected on relatively high wave height (> 3m). During this period, the 95% percentile of the total currents at P46 and P14 raft stations reached up to 9.6 cm s^{-1} and 9 cm s^{-1} respectively (Table 1). These highly dynamic conditions favored the resuspension of bottom sediments that introduced suspended particulate matter into the water column (low transmittance values). Finally, prevailing upwelling winds for the May cruise, forced the sea surface outcrop of cold and salty subsurface waters. During this period current speed showed differences between raft sites with the 50% percentile of the total current speed reaching values as high as 10 cm s^{-1} at the P46 raft station (Table 1).

3.2. Seston characteristics

Results of two-way ANOVA showed that the site, season and the interaction term (site × season) exerted a significant effect on TPM, PIM and POM (n = 36; Table 2). Values of TPM at the reference site varied over a range of 0.6-1.6 mg L^{-1} during the spring-winter period (Figure 3). With the exception of the high value (2.4 mg L^{-1}) recorded at P14 raft station during winter, TPM contents at both P46 and P14 raft stations were significantly lower than those founded at the reference site (Tukey HSD, p < 0.05) (Figure 3; Table 3), with maximum values (> 1.2 mg L^{-1}) recorded during winter and minimum values (< 0.5 mg L^{-1}) registered during spring. At the reference, P46 and P14 raft stations, PIM values ranged around mean annual values of 0.7 ± 0.5 mg L^{-1}, 0.3 ± 0.3 mg L^{-1} and 0.6 ± 0.9 mg L^{-1}, respectively, showing the same seasonal pattern than

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TPM (Tukey HSD, p<0.05) (Figure 3; Table 3). At the three stations PIM content represented more than 70 % of the total seston during winter and less than 12% during spring (Figure 3).

Chorophyll a

Results of two-way ANOVA showed that the sampling site and season exerted a significant effect on Chl a (n = 36; Table 2). Average Chl a content was 1.3 ± 0.5 µg L^{-1} at the reference site and 0.6 ± 0.3 µg L^{-1} and 0.7 ± 0.2 µg L^{-1} at both P46 (n = 15) and P14 (n = 15) raft stations, respectively (Figure 3). At the reference site, values of Chl a varied from a minimum value of 0.9 µg L^{-1} registered in winter and a maximum value of 2 µg L^{-1} in spring (Figure 3). Values of Chl a content at P46 and P14 raft stations were significantly lower than those founded at the reference site (Tukey HSD, p < 0.05) (Table 3), oscillating between 0.4 - 1 µg L^{-1} and 0.5 - 0.9 µg L^{-1}, respectively.

3.3 Biodeposits composition

Two ways ANOVA by ranks showed a significant effect of site, season and the interaction term (site x season) on the total (n = 96) and organic carbon (n = 24) biodeposition rates (Table 4). Post-hoc analyses showed that total faeces production at both rafts was higher in winter (> 100 mg ind^{-1} d^{-1}) relative to the other seasons (Tukey HSD, p < 0.01) (Table 3). For C_{org} biodeposition rates, post-hoc analyses did not show significant differences between seasons and stations, except at P14 raft site during winter, when maximum values were recorded (Tukey HSD, p < 0.05) (Table 3). C_{org} biodeposits ranged between values of 0.9 - 3.8 mg ind^{-1} d^{-1} and 2 - 13 mg ind^{-1} d^{-1} at P46 and P14 raft stations, respectively (Figure 4). At both raft sampling sites, the C_{org} content represented a minimum (< 6%) part of the total faeces production during winter,

achieving values higher than 14% during spring (Figure 4). C_{org}/N ratios ranged around mean annual values of 4.8 ± 0.1 and 5.3 ± 0.1 for P46 and P14 raft stations, respectively (Figure 4).

3.4 Natural sedimentation rates

ANOVA by ranks showed a significant effect of the site, season and the interaction term (site x season) on the total mass fluxes (n = 48; Table 4). Total mass flux at the reference site ranged between a minimum value of 12 g m^{-2} d^{-1} recorded during summer and spring and a maximum value of 71 g m^{-2} d^{-1} recorded during winter time (Figure 5). Total mass fluxes under the rafts were 6-7 fold the rates at the reference site, being significantly different (Tukey HSD, $p < 0.05$) (Table 3). At P46 raft station, total mass fluxes progressively increase from summer (145 g m^{-2} d^{-1}) to winter (536 g m^{-2} d^{-1}) and then, abruptly decrease to a minimum value of 111 g m^{-2} d^{-1} in spring. On the other hand, at P14 raft station, total mass flux achieved the maximum value (338 g m^{-2} d^{-1}) during autumn and gradually decreased until a value of 86 g m^{-2} d^{-1} in spring.

ANOVA by ranks also showed a significant effect of the site, season and the interaction term (site x season) on the organic carbon (C_{org}) fluxes (n = 48; Table 4). C_{org} fluxes at the reference site, that ranged between 0.5- 1 g m^{-2} d^{-1} were also significantly lower than those recorded at both P46 and P14 raft stations (Tukey HSD, $p < 0.05$) (Table 3), showing the same seasonal pattern than total mass fluxes (Figure 5). Underneath P46 and P14 rafts, C_{org} fluxes ranged between mean annual values of 4 ± 2 g m^{-2} d^{-1} and $5\pm$ 3 g m^{-2} d^{-1}, respectively; with values significantly higher during autumn (for P14 raft station) and winter (for both P46 and P14 raft stations) (Tukey HSD, $p < 0.05$) (Figure 5; Table 3). In terms of percentage, the C_{org} content at the reference site remained relatively lower during autumn and winter (around 1.6%) compared with summer (3.9 ±

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1.2 %) and spring (4.4 ± 0.3 %) sampling periods. The same pattern occurred at P46 raft station, with values ranging around 1.1 % for autumn and winter and around 2.6 % for summer and spring. On the contrary, C_{org} content at P14 raft sampling station showed no significant differences along the study year, ranging around a mean annual value of 2.4 ± 0.3 %. The C_{org}/N ratio remained relatively constant during the experimental year varying around mean annual values of 5.9 ± 0.4, 6.7 ± 0.6 and 6.2 ± 0.5 for reference, P46 and P14 stations, respectively (Figure 5).

3.5. Superficial sediments

The lithogenic fraction of the sediment was described as sandy-mud deposits at the three sampling stations with mean grain sizes ranging from a minimum value of 41 ± 8 µm at the reference station to a maximum value of 87 ± 23 µm at the shallower P14 raft site. Granulometric composition revealed that sands are significantly present in the lithogenic fraction at the three stations. Mean sand contents in the sediment samples were 23 ± 8%, 55 ± 2 %, 61 ± 1% for reference, P46 and P14 sampling stations, respectively.

4. DISCUSSION

The intense consumption of seston by filter organisms is partially routed to sustain metabolism and growth. The remaining fraction of filtered particles is packaged into biodeposits and is susceptible to settle down onto the seafloor, potentially enriching the underlying sediments with organic matter and affecting the benthic environment (Giles et al., 2006; Callier et al., 2006; Cranford et al., 2009). In this context, with our field experiments we proceed to evaluate the possible effect that the intense mussel raft culture in a Galician Ría may cause in the transfer of organic matter to the seafloor.

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In the Galician Rías, seston concentrations (<4 mg L^{-1}) are below the pseudofaeces thershold (Bayne et al., 1993) and thus, the removal of seston as a result of mussel feeding activity is only diverted to faeces production. Mytilus galloprovincialis biodeposition rates in the Ría de Ares - Betanzos (Figure 4) were of the order of previous observations (13 - 126 mg ind^{-1} d^{-1}) in other regions with suspended mussel cultures (Callier et al., 2006; Weise et al., 2009). However the high seston concentrations (8 - 27 mg L^{-1}) characterizing these systems made them uncomparable with the Galician Rías.

Based on lab experiments, Klerks et al. (1996) and Gergs et al. (2009) revealed that mussel filtering-biodeposition is positively correlated with seston concentration. Our results support these studies, demostrating that the intense mussel filtration-biodeposition processes in the Ría de Ares-Betanzos were related with suspended particle concentration (Figure 6a). We did not find any significative variation in faeces production under seston levels around 0.5 mg L^{-1}. However, during the stormy winter conditions that leaded to an abrupt increase in seston concentrations (> 1 mg L^{-1}), the faeces production was reinforced. This fact was more pronounced at the P14 raft station as the inner polygon zone was closer to the input of allocthonous material from continental runnoff. Besides, resuspension of sediment material is probably enhanced at P14 site during the stormy winter conditions, due to the shallowness of P14 site. In addition, our data also demonstrate that biodeposition rates are dependent of the food quality, as previously reported by Tenore and Dunstan (1973). In fact, we observed that the removal of non-digestive particles from mussels highly increases during winter time when continental runoff and resuspension processes supply inorganic particulate material to the polygon zone (Figure 6b). On the contrary, faeces production was reduced to minimum levels during spring (Figure 4) when phytoplanton cells with high

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Chl a content, dominated suspended particulate matter (Figure 3). Our results confirm the idea that when phytoplankton dominates the organic fraction of the seston, the digestive processes of the mussels are reinforced, and most of these high quality organic compounds are used by mussels to growth and to sustain their metabolism processes (Zúñiga et al., 2013; Froján et al., 2014). In this sense, Irisarri et al. (2013) found for the same experimental year that the seasonal pattern obtained for the absorption efficiency of mussel in Lorbé polygon highly correlated with the variations observed for the food quality (P46 (r^2 = 0.99 (n = 34; $p < 0.001$); P14 (r^2 = 0.94 (n = 39; $p < 0.001$)).

The link between Mytilus galloprovincialis biodeposition processes and the natural sedimentation to the seafloor constitutes an important aspect in the study of benthic-pelagic coupling. The sedimentation rates under the rafts were 6-7 fold the sedimentation at the reference site supporting the idea that mussel feeding activity increases natural sedimentation rates (Figure 5). Annual mean sedimentation rates (223 ± 46 g m^{-2} d^{-1}) underneath mussel rafts in the Ría de Ares-Betanzos were lower than values presented by Jaramillo et al. (1992) who presented a value of 553 g m^{-2} d^{-1} in a chilean estuary characterized by high seston content (> 2 g L^{-1}) and affected by the culture of Mytilus chilensis. For the Galician Rías, Cabanas et al. (1979) determined for the first time natural sedimentation rates under a mussel raft. They obtained an average value of 280 g m^{-2} d^{-1} during a 20 day experiment in the Ría de Arousa in winter. Later on, only Alonso-Pérez et al. (2010) presented natural sedimentation rates for a raft polygon in the Ría de Vigo based on a 4 day experiment, carried out under summer stratification conditions. They assessed an average value of 31.4 g m^{-2} d^{-1}, clearly lower than sedimentation rates recorded in our study area during summer 2010 (Figure 4). Unfortunately, the contribution of suspended mussel biodeposition to natural sedimentation rates is poorly understood since only one study carried out by Giles et al.

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(2006) has analyzed this important issue, indicating that faeces production make up between 6-14% to sedimentation fluxes.

In the Ría de Ares-Betanzos, natural sedimentation rates under the rafts followed a similar seasonal variation as mussel biodeposition, suggesting that mussel farming plays a key role in the transfer of matter and energy to the seafloor (Figure 4 and 5). Quantitatively, if we consider the polygon zone as a stationary system, it seems that the natural sedimentation rates under the rafts were mainly explained by mussel biodeposition (Figure 7a). However, the fact that the Ría de Ares-Betanzos is a dynamic system leads to the consideration that sedimentation rates under the rafts can be modulated by different hydrodynamic processes that add and/or disperse material into the system. In fact, only 45% to 10% of the potentially settling organic carbon due to mussel filtering activities was collected in the sediment traps and hence a large fraction (55-90%) did not reach the deep water column (Figure 7b). The final fate of this organic carbon attributed to biodeposits and not collected in the sediment traps, can be degradation and / or dispersion. Several studies (Fabiano et al., 1994; Giles and Pilditch, 2006; Carlsson et al., 2010; Jansen et al., 2012) have found that organic compounds of faeces are strongly affected by degradation processes as more labile components are rapidly release to the environment. In fact, the lower organic carbon content (1.1-2.7%) of the material collected by the sediment traps in comparison with biodeposits (3-19%) indicate that remineralization had occurred before material arrived to the deep water column (Figure 4 and 5). The increase in the C_{org} /N ratio in the trap material compared with the characteristic ratios for the faeces also points to remineralization processes during particles sinking (Figure 4 and 5). However, the presence of allocthonous refractory organic compounds (high C_{org} /N ratio) as part of the trapped material cannot be discarded.

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On the other hand, other studies have also found that in high energy environments mussel biodeposits may be dispersed by local currents, avoiding organic enrichments beneath sites of mussel culture sites. In the Lorbé raft polygon, the 95% percentile for total current speeds are between 7-9 cm s^{-1} (Table 1), similar to those recorded in other mussel farm areas characterized as high energy environments (Chamberlain et al. 2001, Hartstein and Rowden, 2004, Hartstein and Stevens 2005). In addition, significant wave action enhances the energetic conditions during the winter and autumn seasons, as previously described in the Ría de Vigo (Villacieros-Robineau et al., 2013). Such highly dynamic conditions may lead to intense dispersion processes that in last term avoid the settling of biodeposits under the rafts. In fact, bottom sediments in the Lorbé raft polygon were described as sandy mud sediments with mean grain size significantly higher under the rafts than at the reference site. This observation jointly with the low organic carbon contents (similar to organic percentages in the sediment traps) (<2.8%; Sanchez-Mata et al., 1999) also points to high energy conditions in the polygon zone that will avoid the settlement of the biodeposits under the rafts.

In summary, our results directly evidence that faeces production by Mytilus galloprovincialis mussel strongly alters the natural sedimentation rates, enhancing the transfer of particle fluxes towards the surface sediments. However, the intense degradation rates of mussel biodeposits and the high energy conditions in the Lorbé mussel farm area clearly reduce the amount of organic carbon arriving to the seafloor, attenuating the potential impact on the benthic communities.

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Figure captions

Figure 1. Bathymetric map of the Ría de Ares-Betanzos (NW Iberian Peninsula) showing the position of the Lorbé and Arnela polygons (squares) where mussel rafts are located. Positions of sampling stations (Intecmar; Ref: reference site; P46 and P14: raft sites) inside and outside the Lorbé polygon are also shown in the figure. Intecmar station is maintained by Intecmar, an oceanographic institution in charge of monitoring the marine environment in the Galician Rías (http://www.intecmar.org/Ctd/Default.aspx).

Figure 2. Temporal series of (a) upwelling index ($-Q_{E-W}$), (b) significant wave height, (c) water column temperature and (d) transmittance during the experimental year. The seasonal campaigns carried out in the Lorbé polygon and at the reference site are marked with vertical dashed lines.

Figure 3. Mean seasonal variations of (a) total particulate matter (TPM) (mg L^{-1}), (b) particulate inorganic matter (PIM) (mg L^{-1}), (c) PIM content (in %) and (d) Chl a content in seston (µg L^{-1}) at both the reference (black bars) and raft stations (gray and dark gray bars for P46 and P14 rafts, respectively). Error bars represent ± 1 SD (Standard Deviation). Sum10: summer 2010; Aut10: autumn 2010; Win11: winter 2011 and Spr11: spring 2011.

Figure 4. Mean seasonal (summer (Sum10), autumn (Aut10), winter (Win11) and spring (Spr11) variations of (a) total and (b) organic carbon (C_{org}) production of faeces (mg ind^{-1} d^{-1}), (c) C_{org} content (in %) and (d) C_{org}/N ratio of biodeposits at P46 (gray bars) and P14 (dark gray bars) raft stations. Error bars represent ± 1 SD (Standard Deviation).

Figure 5. Seasonal (summer (Sum10), autumn (Aut10), winter (Win11) and spring (Spr11) variations of total mass and organic carbon (C_{org}) fluxes at the reference (black

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bars) and raft (P46 and P14; gray and dark gray bars, respectively) stations. Seasonality of (c) C_{org} content (in %) and (d) C_{org}/N ratio are also shown. Error bars represent ± 1 SD (Standard Deviation).

Figure 6. Relationship between total faeces production and (a) total particulate matter (TPM) (mg L^{-1}) (r^2= 0.97) and (b) inorganic fraction of seston (PIM) (%) (r^2= 0.97) at both P46 and P14 raft sites. Values are mean ± 1 SD (Standard Deviation). Dots corresponding to winter period for both P46 (Win-P46) and P14 (Win-P14) raft stations are highlighted in the figure.

Figure 7. (a) Total mass fluxes as a function of total faeces production and (b) Organic carbon (C_{org}) fluxes plotted against C_{org} biodeposits production at both P46 and P14 raft sites. Values are mean ± 1 SD (Standard Deviation). Dots corresponding to winter period for both P46 (Win-P46) and P14 (Win-P14) raft stations are highlighted in the figure.
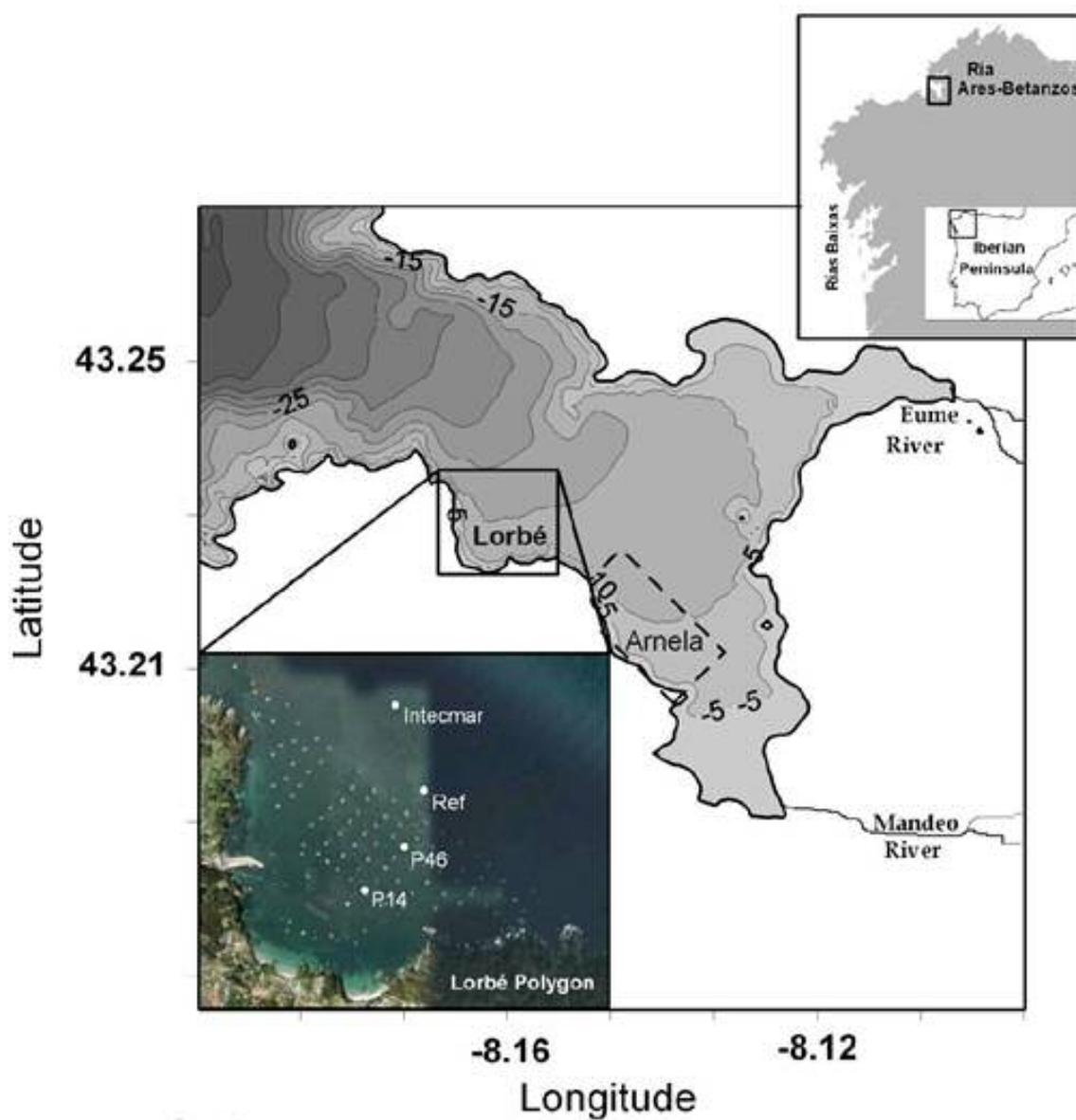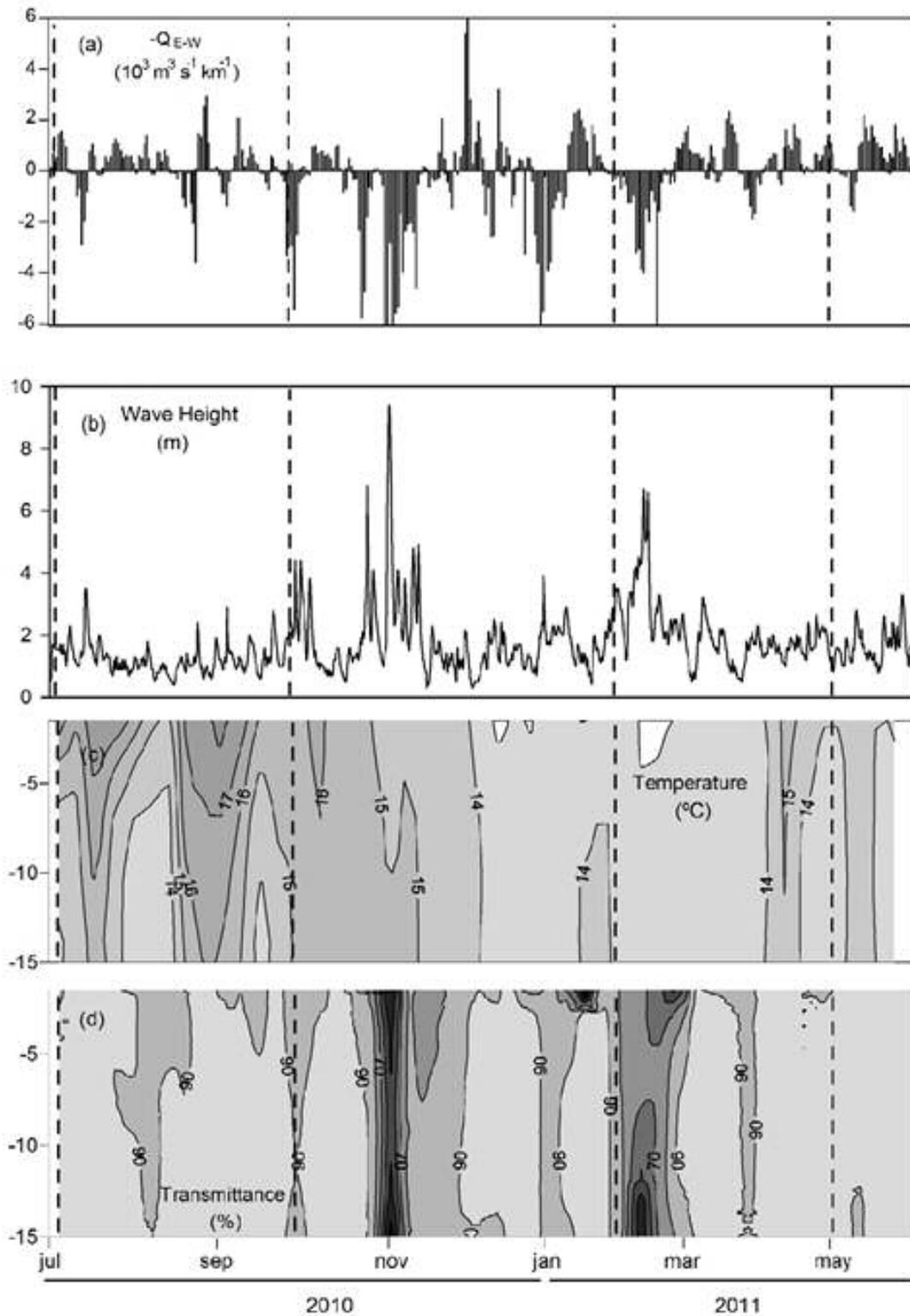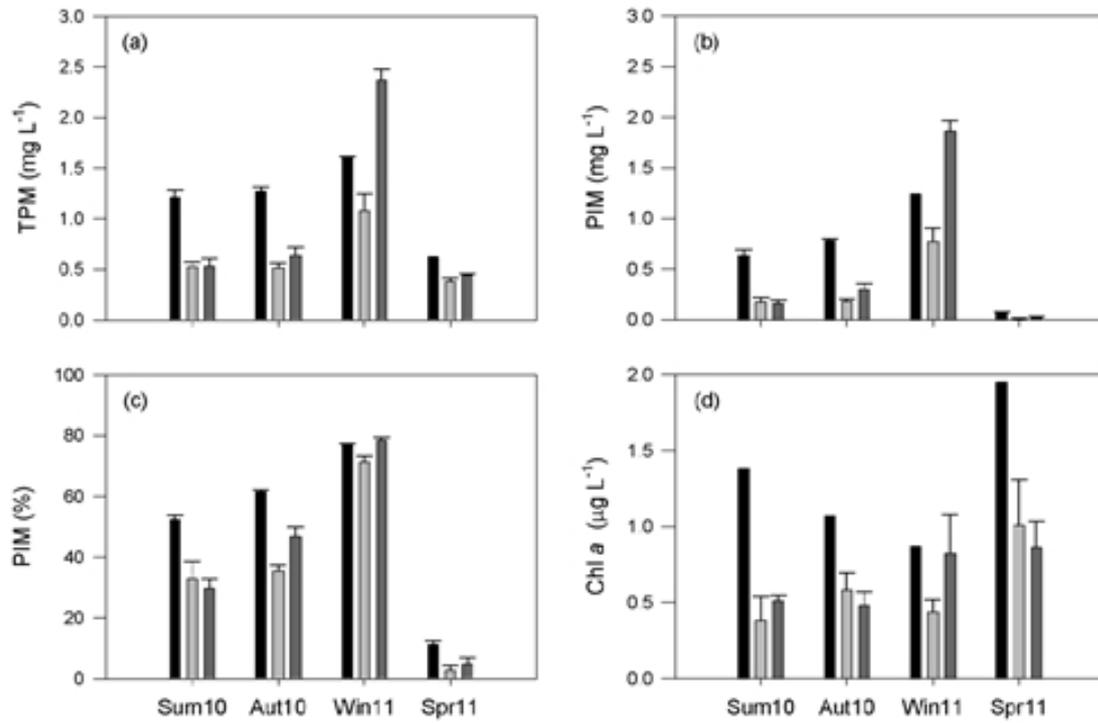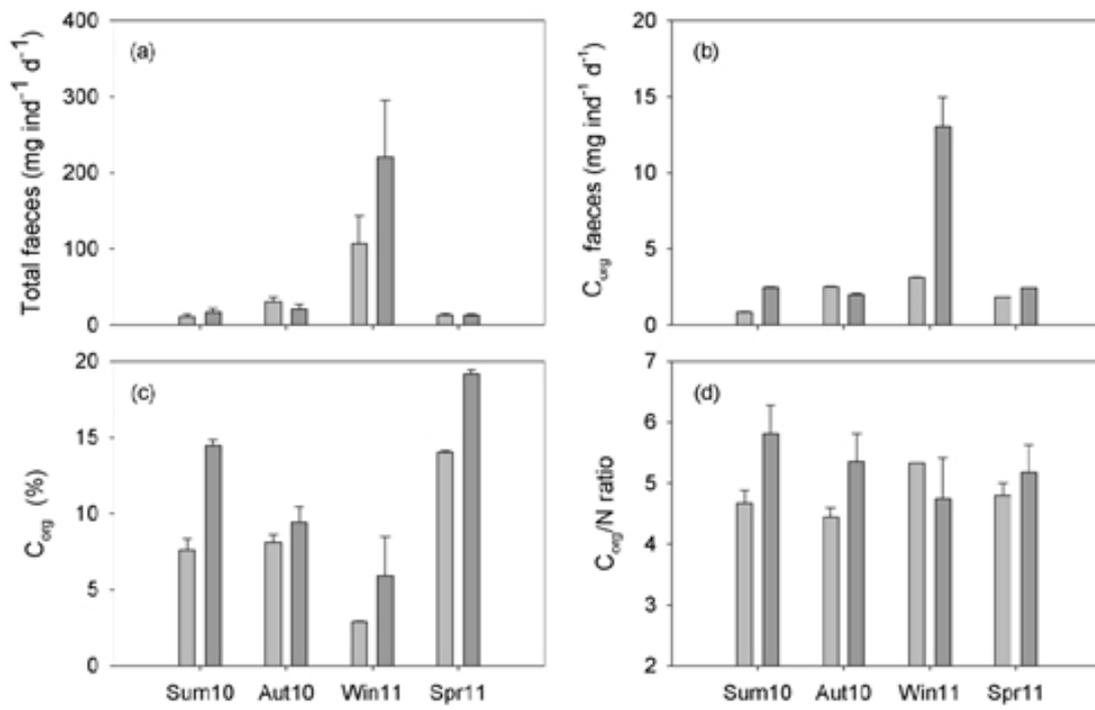
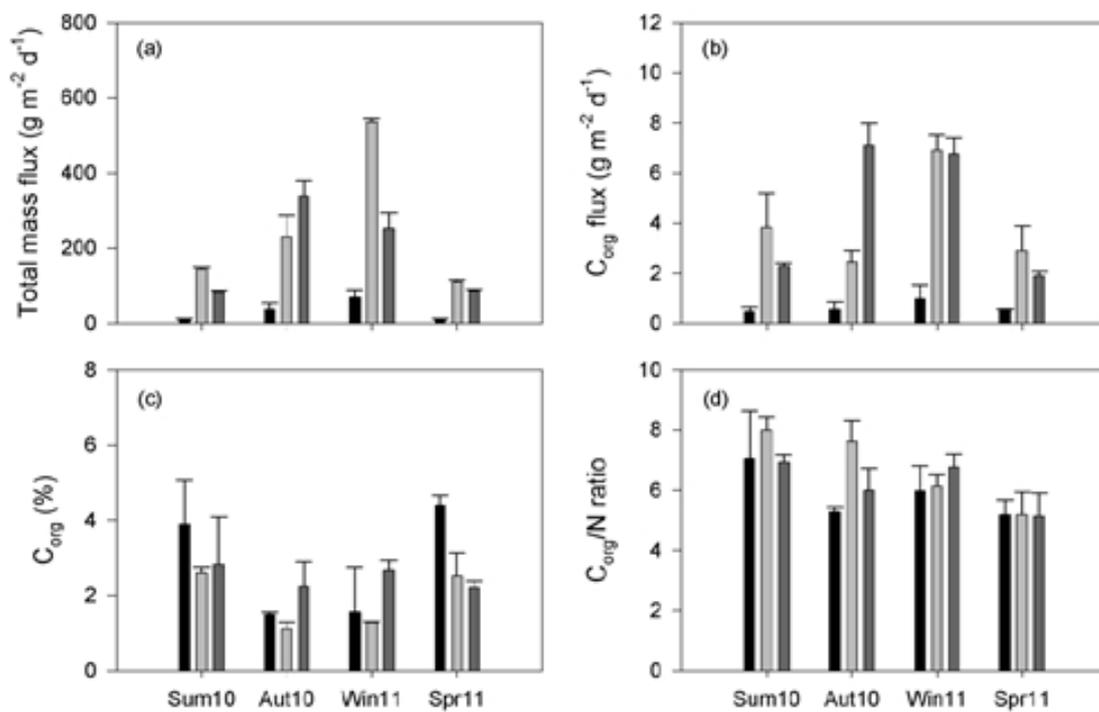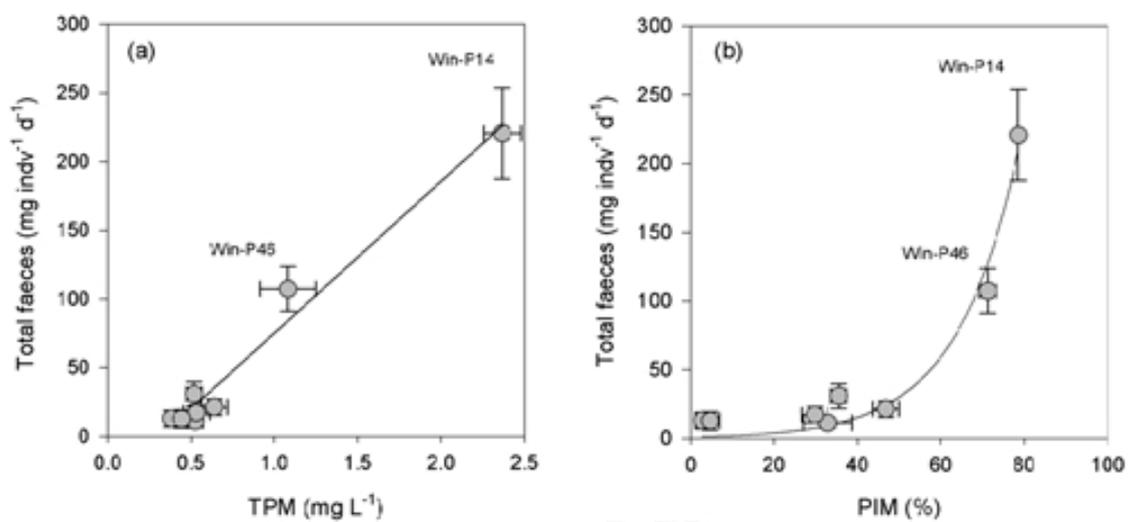Figure 1

Figure 2

Figure 3

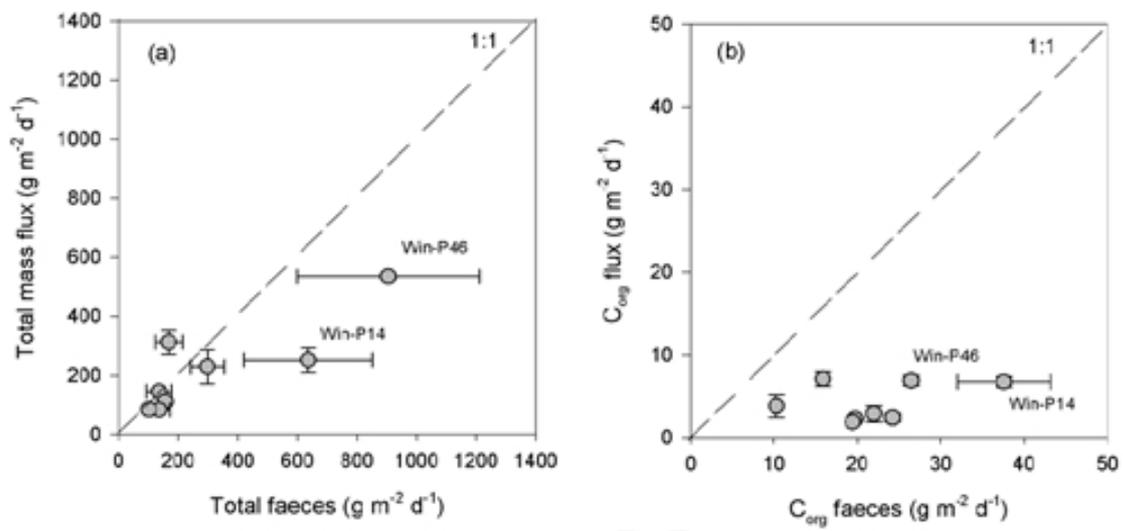Figure 4

Figure 5

Figure 6

Figure 7

Table 1. Mussel biomass and mean regime (percentiles 50% and 95%) for total, tidal and residual velocities at the raft (P46 and P14) stations during each sampling period. All velocities are expressed in cm s^{-1}. The variability (Var.) of the total current explained by the tide is also presented. Unfortunately, there is no current data for P14 raft station during summer 2010 sampling period due to technical problems.

Period/ Station	Mussel Biomass	Total		Tidal		Residual		Var.
Summer 2010	(nº mussels x 10^3 raft^{-1})	P50	P95	P50	P95	P50	P95	(%)
P46	6000	6.1	7.3	0.5	0.7	5.9	6.5	6
P14	3948							
Autumn 2010								
P46	4819	4.7	5.1	1.2	2.7	2.5	4.1	17
P14	3948	2.4	4.7	1.0	2.1	0.8	2.7	20
Winter 2011								
P46	4218	5.6	9.6	3.3	6.8	1.4	3.7	41
P14	1440	4.6	9.0	2.0	3.5	1.9	3.8	17
Spring 2011								
P46	2175	10	13.8	2.1	4.4	9.0	11.7	21
P14	3531	2.9	7.6	1.2	2.5	1.9	2.7	14

Table 2: Results of two-way analysis of variance (ANOVA) performed to evaluate significant effects of site (Reference, P46 and P14), season (summer, autumn, winter and spring) and interaction term (Site x Season) over the environmental characteristics (TPM: Total Particulate Matter, PIM: Particulate Inorganic Matter and Chl a: Chlorophyll a).

	TPM					PIM					Chl a				
	Df	SS	MS	F	P	Df	SS	MS	F	P	Df	SS	MS	F	P
Site	2	1.48	0.74	47.59	<0.001	2	1.02	0.51	58.43	<0.001	2	0.78	0.39	5.55	<0.05
Season	3	7.20	2.40	153.94	<0.001	3	8.32	2.71	319.46	<0.001	3	1.01	0.34	4.79	<0.01
Site x Season	6	2.39	0.40	25.56	<0.001	6	1.55	0.28	29.38	<0.001	6	0.74	0.12	1.75	0.15
Residuals	24	0.37	0.02			24	0.21	0.01			24	1.69	0.07		

Table 3. Homogeneous groups identified by Tukey´s HSD test ($\alpha = 0.05$) in terms of site (Reference, P46 and P14) and season (summer (Sum10), autumn (Aut10), winter (Win11) and spring (Spr11)) are given with letters (A, B, C, D, E, F, G, H). Analysis were performed over the environmental characteristics (TPM: Total Particulate Matter, PIM: Particulate Inorganic Matter), faeces (total and organic carbon (C_{org})) and fluxes (total and C_{org}) registered with the sediment traps.

	Seston		Faeces		Fluxes	
	TPM	PIM	Total	C_{org}	Total	C_{org}
Reference						
Sum10	B	C			A	A
Aut10	B	C			AB	AB
Win11	C	D			BC	ABC
Spr11	A	AB			A	A
P46						
Sum10	A	AB	A	A	DEF	EF
Aut10	A	AB	C	A	EFG	DE
Win11	B	C	D	A	H	F
Spr11	A	A	A	A	DE	DE
P14						
Sum10	A	AB	B	A	CD	CDE
Aut10	A	B	BC	A	GH	F
Win11	D	D	D	B	FGH	F
Spr11	A	A	A	A	BCD	BCD

Table 4: Results of two-ways analysis of variance (ANOVA) by ranks performed to analyze the influence of site (Reference, P46 and P14), season (summer, autumn, winter, spring) and interaction term (Site x Season) over both faeces (total and organic carbon (C_{org})) and mass fluxes (total and organic carbon (C_{org})) registered with the sediment traps.

	Total faeces					C_{org} faeces					Total mass flux					C_{org} flux				
	Df	SS	MS	F	P	Df	SS	MS	F	P	Df	SS	MS	F	P	Df	SS	MS	F	P
Site	1	1320.2	1320.2	11.0	<0.01	1	43.2	43.2	10.1	<0.01	2	5928.0	2964.0	190.9	<0.001	2	6001.6	3000.8	114.7	<0.001
Season	3	57109.0	19036.4	158.6	<0.001	3	190.7	63.6	14.8	<0.001	3	2364.5	788.2	50.8	<0.001	3	1246.5	415.5	15.9	<0.001
Site x Season	3	4686.0	1561.8	13.0	<0.001	3	88.4	29.5	6.9	<0.01	6	360.5	60.1	3.9	<0.01	6	1022.4	170.4	6.52	<0.001
Residuals	88	10564.0	120.0			16	65.6	4.3			36	559.0	15.5			36	941.5	26.2		

Highlights

Mussel culture seasonally alters natural sedimentation rates in the Ría de Ares-Betanzos (NW Iberian Peninsula)

Seasonal biodeposits production by mussels related with seston inorganic content

Potentially high degradation rates of faeces and hydrodynamics in the culture area controlled the biodeposit´s organic carbon arriving to the seafloor