FEEDBACK BETWEEN PHYSIOLOGICAL ACTIVITY OF *Mytilus galloprovincialis* Lmk AND BIOGEOCHEMISTRY OF THE WATER COLUMN: MICROBIAL PLANKTON CARBON AS A TRACER FOR FOOD QUALITY

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Abstract

The aim of this work was to study the filter feeding behaviour of individual mussels *Mytilus galloprovincialis* in the Ría de Vigo (Galicia, NW Iberian Peninsula) in order to assess the alteration of the water column biogeochemistry due to mussel culture and the effect of seston organic content on mussel physiological rates. The study was carried out during one experimental year under different oceanographic scenarios by comparing data from a station located in a raft area and from a reference sampling site with no direct effects of rafting culture activities. Results showed differences between stations in water column nutrient contents mainly on ammonium levels, with a mean annual excess of 40% at raft station due to mussel feeding activities. Otherwise, it was also observed a pronounced deficit of particulate matter at the raft respect to the reference sampling site

both in terms of chlorophyll *a* (- 33%) and microbial plankton carbon (-34%), reflecting preferential grazing of mussels on living plankton carbon.

The study of the seston organic compound during our experimental year also revealed that feeding activity and digestive behavior of mussel *Mytilus galloprovincialis* is highly dependent on the quality of the organic composition of the available food. Mussel organic ingestion rate and absorption efficiency were more strongly correlated with microbial plankton carbon contents than with particulate organic carbon and chlorophyll *a*, illustrating the importance of considering not only autotrophic but also heterotrophic microbial plankton carbon as high quality food for mussels in the Ría de Vigo.

Keywords: carbon; plankton; mussel; ammonium; ingestion rate; absorption efficiency; Galician Rías.

1. INTRODUCTION

The Galician Rías Baixas are four coastal embayments located (42° to 43°N) along the northern boundary of NW African upwelling system. From March-April to September-October, prevailing northerly winds cause the upwelling of nutrient-rich subsurface Eastern North Atlantic Central Water (ENACW) on the shelf and inside the Rías. The interaction between coastal upwelling and circulation patterns in the Rías promotes a massive response in the productivity of phytoplankton populations supporting a mean annual primary production of 912 g C m⁻² yr⁻¹ (Tilstone et al., 1999; Cermeño et al., 2006; Arbones et al., 2008). These primary production rates are in the upper range of highly productive systems where bivalve production constitutes an important economic activity, as for example Norwegian fjords (100 g C m⁻² yr⁻¹) (Erga, 1989; Aure et al.,

2007) , the Oosterschelde estuary in the Netherlands ($300 \text{ g C m}^{-2} \text{ yr}^{-1}$) (Smaal et al., 2001) or Thau lagoon in France ($400 \text{ g C m}^{-2} \text{ yr}^{-1}$) (Plus et al., 2006), and enable the Rías to support the highest mussel production in Europe, with a total estimated production of $250 \times 10^6 \text{ kg}^{-1} \text{ y}^{-1}$ of edible mussels.

Several studies revealed that feeding activity of suspended bivalve filter feeders may alter the trophic network and nutrient cycling existing in marine ecosystems (see reviews by Prins et al., 1998; Newell, 2004). The major pathways in which mussels interact with coastal nutrient cycling are principally due to i) their huge capacity to clear particles (including phytoplankton, zooplankton and detritus) from the surrounding waters to obtain nutrient requirements for growth (Navarro and Iglesias, 1993; Kreeger and Newell, 2001) and ii) the excretion of metabolic wastes at the same time that transfer particles to the bottom sea floor as faeces and pseudofaeces. (Dame 1993; Prins et al., 1998; Newell 2004; Cranford et al., 2007; Jansen et al., 2011). These processes show seasonal variability, reflecting environmental fluctuations in temperature, food supply and endogenous metabolic requirements of the mussels such as gametogenesis and somatic growth (Smaal and Vonck, 1997; Cranford and Hill, 1999).

For the Galician Rías Baixas, Tenore et al. (1982) stated that the introduction of this aquaculture system altered the trophic chain of the ecosystem, diverting primary production and energy flow from planktonic to benthic food web. In later years, *in situ* experiments have studied the culture of *Mytilus galloprovincialis* in relation to seston variables (quantity and quality) and chlorophyll in the Rías Baixas. Figueiras et al. (2002) suggested that the phytoplankton response to upwelling provides high-quality food enhancing absorption efficiency of *Mytilus galloprovincialis* ($AE \sim 0.6$). They estimated that mussel harvest in the Ría de Arousa extracts the equivalent to $\sim 10\%$ of primary production. Otherwise, Petersen et al. (2008) and Maar et al. (2008),

documenting food depletion in a mussel raft in the Ría de Vigo, emphasized the importance of physical forcing and phytoplankton composition for food availability, and stressed the relevance of including heterotrophic plankton in estimates potential bivalve production. Unfortunately, except for Alonso-Pérez et al. (2010), there has not been any study focused on the impact of suspended mussel culture on the biogeochemistry of the water column in the Rías Baixas by comparing different sampling sites and none of these studies were primarily concerned with the effect of different hydrographic and biogeochemical scenarios over mussel physiological response.

In this framework, the aim of our work was to assess “for the first time” the interaction between the effect of the seston organic content on mussel *Mytilus galloprovincialis* feeding activity, and the impact of mussel metabolism on water column biogeochemistry. Our study was based on a one year *in situ* experiment carried out in the Ría de Vigo by conducting seasonal campaigns at two stations, one inside a mussel raft and the other well outside of the mussel raft area

1.1 Study area

The Ría de Vigo is one of the four V-shaped embayments (Rías Baixas, NW Iberian Peninsula) that gradually widens seawards and it is partially enclosed by the Cíes Island (Figure 1). It reaches a maximum depth of 42 m along its central channel. Its main tributary is the river Oitabén-Verdugo which drains into the innermost part of the Ría with an average flow of $15 \text{ m}^3 \text{ s}^{-1}$ (Nogueira et al., 1997).

From March-April to September-October, the Rías Baixas (orientated in a NE-SW direction) are strongly influenced by prevailing northerly winds that cause the upwelling of enriched in nutrients Eastern North Atlantic Central Water (ENACW). These

upwelling pulses are responsible of the estuary-like circulation of the water inside the Ría, favouring the outflow of freshwater at sea surface and the inflow of water with high nutrient content through the bottom. On the contrary, during winter, the persistence of southerly winds primarily favours downwelling conditions that promote a negative estuarine-like circulation with sea surface entrance of nutrient-poor oceanic waters in the Rías and a subsurface outflow. A total of 478 mussel rafts organised in several polygons, differing in size, are located inside the Ría de Vigo, occupying ~5% of its surface (Figure 1). The average area of the rafts is 500 m² with an average of 500 hanging ropes 12 m long each (Labarta, 2000).

2. METHODS

During years 2007 and 2008 and in the framework of the RAFTING project, two stations located in the Ría de Vigo (Figure 1) were monitored during 4 campaigns in the most representative oceanographic scenarios: 1) autumnal bloom (17 September – 4 October), b) winter mixing (28 January – 14 February), c) spring bloom (14 April – 1 May) and d) summer upwelling (26 June – 14 July). One station was placed in a raft of a polygon located in the inner part of the Ría (RaS) and the other one was situated in the central channel of the Ría (ReS). This station has been used as a reference site representing the hydrographic and biogeochemical conditions of the water column with no effects of feeding mussel activities. During every sampling period hydrographic data (both sites) and physiological experiments with mussels (RaS station) were performed during six and two-three days, respectively.

2.1. Physical and biogeochemical data

Ekman transport ($-Q_x$), an estimate of the volume of water upwelled per kilometre of coast, was calculated according to Bakun's (1973) method:

$$-Q_x = -((\rho_a C |V|) / (f \rho_{sw})) V_H$$

where ρ_a is the density of the air (1.22 kg m^{-3}) at 15°C , C is an empirical dimensionless drag coefficient ($1.4 \cdot 10^{-3}$), f is the Coriolis parameter ($9.946 \cdot 10^{-5}$) at 43°N , 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 43°N , 11°W , respectively. Average daily winds were estimated from atmospheric pressure charts. Positive values show the predominance of northern and easterly winds that induces upwelling processes inside the Ría. On the contrary, negative values indicate the existence of downwelling processes.

The study of the oceanographic characteristics of the water column in the two sampling sites was carried out during six days every sampling period by conducting vertical profiles of temperature and salinity with a Seabird CTD-SBE911. Discrete water sampling at different depths (5, 10, 15, 20 m depth) for the determination of inorganic nutrients, dissolved oxygen (DO), dissolved organic carbon and nitrogen (DOC and DON, respectively), chlorophyll *a* (Chl *a*), particulate organic carbon and nitrogen (POC and PON, respectively) and microbial (pico, nano and micro) plankton counting were also collected by using a rosette sampler with 10 L PVC Niskin bottles, attached to the CTD-SBE911.

Inorganic nutrient samples were determined by segmented flow analysis with Alpkem autoanalysers following Hansen and Grassoff (1983) with some improvements proposed by Mouriño and Fraga (1985). The analytical errors were $\pm 0.02 \mu\text{M}$ for nitrite and ± 0.05

142 μM for nitrate and ammonium. Dissolved oxygen was determined by Winkler
143 potentiometric titration. The estimated analytical error was $\pm 0.35 \mu\text{M}$.

144 Aliquots of the filtrate were taken for dissolved organic carbon (DOC) and nitrogen
145 DON analyses. DOC and DON were measured simultaneously with a nitrogen-specific
146 Antek 7020 nitric oxide chemiluminescence detector, coupled in series with the carbon-
147 specific infra-red gas analyser of a Shimadzu TOC-5000 organic carbon analyser
148 (Álvarez- Salgado and Miller, 1998). The analytical errors were $\pm 1 \mu\text{M}$ for DOC and
149 $\pm 0.2 \mu\text{M}$ for DON.

150 Chl *a* concentration was determined by filtering seawater samples of 100 to 250 ml
151 through 25 mm Whatman GF/F filters. After filtration, samples were frozen (-20°C)
152 until pigment extraction in 90% acetone over 24 h in the dark at 4°C . Chl *a* contents
153 were determined by fluorescence of the pigment extracts using a Turner Designs
154 fluorometer calibrated with pure Chl *a* (Sigma). The estimated analytical error was 0.09
155 mg m^{-3} .

156 For POC and PON analysis, 250 ml samples were filtered on pre-weighted,
157 precombusted Whatman GF/F filters ($0.7 \mu\text{m}$ nominal size pore), dried overnight and
158 frozen (-20°C) before analysis. Measurements of POC and PON were carried out with a
159 Perkin Elmer 2400 CNH analyser, including daily acetanilide standards. The precision
160 of the method is $\pm 0.9 \mu\text{mol C l}^{-1}$ and $\pm 0.2 \mu\text{mol N l}^{-1}$.

161 For microplankton ($> 20\mu\text{m}$) counting a seawater volume of 100 ml was preserved with
162 Lugol's iodine until microscopic determination. Depending on the Chl *a* concentration,
163 a volume ranging from 10 to 50 ml was sedimented in composite sedimentation
164 chambers and observed through an inverted microscope. Organisms were counted and
165 identified to the species level and dimensions were taken to calculate cell biovolumes

after approximation to the nearest geometrical shape (Hillebrand et al., 1999). Cell carbon was calculated from these biovolumes following Strathmann (1967) for diatoms and dinoflagellates, Verity et al. (1992) for other flagellates ($> 20 \mu\text{m}$) and Putt and Stoecker (1989) for ciliates. A correction to estimate cell carbon for *Noctiluca scintillans* was applied following Tada et al. (2000). Differentiation between autotrophic and heterotrophic microplankton was done following Lessard and Swift (1986), Larsen and Sournia (1991) and our historical records of epifluorescence microscopy. Abundances of autotrophic and heterotrophic pico- ($< 2\mu\text{m}$) and nanoplankton ($2\text{-}20\mu\text{m}$) were determined from epifluorescence microscopy according to Figueiras et al. (2006). Autotrophic organisms were enumerated under blue light excitation while excitation with UV light was used to enumerate heterotrophic pico- and nanoflagellates. *Prochlorococcus* cannot be accurately counted with this technique, but their abundance is not important in this coastal system (Rodríguez et al., 2003). Biovolumes were converted to cell carbon following Verity et al. (1992) for pico- and nanoflagellates and Bratbak and Dundas (1984) for *Synechococcus*-type cyanobacteria (Syn). Hereafter, the carbon biomass representing pico-, nano- and microplankton cells will be so-called microbial plankton carbon (PC).

2.2 Mussel physiological experiments

The study of the metabolic activity and feeding and digestive behavior of mussels was performed *in situ* on the raft station (Figure 1) under ambient conditions of temperature, salinity and food availability. The experiments were conducted three times during autumn and twice for the other three periods. All these experiments were carried out simultaneously to the biogeochemical sampling of the water column.

Clearance, ingestion, absorption efficiency, respiration and excretion rates were determined according to Fernández-Reiriz et al. (2012). The experiments were performed on individual mussels, using 90 individuals of *Mytilus galloprovincialis* randomly collected from three different ropes (30 for each rope). After determination of physiological rates, the individuals were sacrificed for dry tissue (100°C, 24h) and organic weight (450°C, 24h) analyses. Mean values of size shell, dry tissue weight and condition index of the collected individuals for each sampling period are shown in Table 1.

2.2.1. Ammonium excretion rate ($\text{VNH}_4^+\text{-N}$) and oxygen uptake (VO_2)

Ammonium excretion rate ($\text{VNH}_4^+\text{-N}$) was determined after the mussels were placed in open cylindrical chambers with 250 ml of filtered seawater (0.2 μm Millipore membranes). Temperature was maintained during the determinations by immersing the chambers in an isothermal bath. Two chambers without animals were used as a control. After 90 min, water samples were collected from each chambers and frozen to -20°C until be analysed in the laboratory, according to the phenol-hypochlorite method described by Solorzano (1969). $\text{VNH}_4^+\text{-N}$'s were calculated from the difference in NH_4^+ concentration between the chambers with and without animals, respectively.

Oxygen uptake rates (VO_2) were determined by incubating the mussels in sealed 780 ml cylindrical chambers (height 85 mm, diameter 115 mm). Temperature was maintained during the determinations by immersing the chambers in an isothermal bath. Two chambers without animals were used as a control. The mussels were left undisturbed until most of their valves were opened, or at least for 45-60 minutes. Subsequently, oxygen measurements started using a manual probe (HACH HQ40). The depletion of oxygen in the chamber, due to respiration by the mussels was recorded for 30 to 60 min.

The measurements were stopped before the oxygen concentration dropped below 30% relative to control chamber without mussels. VO_2 's were calculated from the difference in oxygen concentration between the chambers with and without animals, respectively.

2.2.2. Organic ingestion rate (OIR) and absorption efficiency (AE).

The organic ingestion rate (OIR) was calculated as the product of clearance rate (CR) and food concentration. The CR was estimated by using mussels placed in a cylindrical chamber of 1200 ml with a water inflow in the lower part and a water outflow in the upper opposite side. The animals were placed in the chambers in such a way that the input flow was directed to the inhalant aperture and the exhalant aperture towards the water outflow, thus preventing re-filtration processes. Two chambers without mussels were used as blanks. The CR was estimated from the reduction in suspended particles concentration, measured as volume of particles ($mm^3 l^{-1}$) between the water surrounding the individuals and the outflow of the experimental chamber (Filgueira et al., 2006). Following the method of Conover (1966), absorption efficiency (AE) was estimated by determining the organic and inorganic content of the food and the faeces. Representative samples of the diet were collected during the experiments and the AE was calculated for a given pool of mussels by collecting the faeces in each experimental chamber. Samples of food and faeces were filtered through pre-combusted, pre-weighed Whatman GF/C membranes. Filters were rinsed with isotonic ammonium formate, dried to a constant weight at 80°C, and then weighed and combusted at 450°C for 3 hours. The filters were weighed again to estimate the organic and inorganic fraction contained in the food and faeces.

2.2.3. Standardisation of physiological rates

To preclude variability in physiological rates caused by size differences, these rates were corrected to a standard-size individual. To this end, once physiological measurements were completed, shell length of each individual was recorded to the nearest 0.1 mm with vernier callipers. Physiological rates were standardised to a mussel size of 60 mm length with the following formula:

$$Y_{\text{std}} = Y_{\text{exp}} (L_{\text{std}} / L_{\text{exp}})^b$$

where Y_{std} is the standardised physiological rate, Y_{exp} is the experimental physiological rate, L_{std} is the standardised size, L_{exp} the individual experimental size and b the exponent relating the physiological rate with size. In this study, a value of 1.85 was employed as a size standardisation exponent of the CR (Filgueira et al., 2006; Filgueira et al., 2008), 0.75 for the oxygen consumption (Bayne and Newel, 1983) and 0.72 for the ammonium excretion rate (Hawkins and Bayne, 1985).

2.3. Statistical analysis

The significance of the water column biogeochemical differences between ReS and RaS stations was analysed over the 12 m integrated values of a total of 24 paired observations (6 sampling days x 4 seasonal periods) by using a t-test ($p < 0.05$) (Table 2).

For the metabolic and physiological mussel rates analysis of variance was applied to analyse the seasonal pattern. A parametric ANOVA was used for variables that fulfil normality (Shapiro test) and homocedasticity (Levene test) assumptions, and a Kruskal-Wallis tests when this assumptions were rejected. The Tukey HSD (Honest Significant Differences) test was used as a *post hoc*. Data analysis was performed with the statistical package R 2.12.2 (R Development Core Team, 2011).

260 3. RESULTS

261 3.1. Hydrographic conditions and biogeochemistry of the water column

262 The thermohaline and biogeochemical conditions for the four sampling periods are
263 analysed based on temperature, nitrate (NO_3^-), ammonium (NH_4^+) and Chl *a* temporal
264 distributions at the reference (ReS) and raft (RaS) sampling sites (Figure 2 and 3,
265 respectively).

266 During autumn 2007 prevailing upwelling winds ($-\text{Q}_x$ positive values) favoured the
267 intrusion of nutrient-rich subsurface ENACW into the ría, breaking the thermal
268 stratification observed at the beginning of the study period at both ReS and RaS
269 stations. As expected, the entrance of this water parcel coming from the ocean was the
270 responsible of the maximum of Chl *a* during experimental day September 24th reaching
271 values as high as 6 and 3 mg m^{-3} at the reference and raft stations, respectively. During
272 winter 2008 the water column was characterised by relatively cold waters, only varying
273 0.5 °C, with high NO_3^- content (from 5 to 7 $\mu\text{mol kg}^{-1}$) and lower NH_4^+ levels than the
274 previous sampling period at the two sites. Chl *a* concentration was lower than 1 mg m^{-3}
275 at both stations. In spring 2008 we found that water column was thermally mixed with
276 very low NO_3^- (below 2 $\mu\text{mol kg}^{-1}$) and NH_4^+ (below 1 $\mu\text{mol kg}^{-1}$) levels during the first
277 half of the cruise. During this period, the maximum values of Chl *a* (5 mg m^{-3}) at sea
278 surface, decreased downwards to a marked minimum of 2 and 1 mg m^{-3} at the reference
279 and raft site, respectively. After April 21st, it was established a thermal stratification
280 which favoured the development of a subsurface Chl *a* maximum (6 mg m^{-3}) at ReS site
281 that disappeared at RaS station. Finally, during the first half of summer 2008 sampling
282 period the intrusion of ENACW, linked with very high positive values in upwelling

index, is clearly discernible by low temperature ($T < 14\text{ }^{\circ}\text{C}$), and high NO_3^- contents ($5\text{ }\mu\text{mol kg}^{-1}$) (Figure 2 and 3), always recorded close to the bottom. According to the upwelling conditions registered in the first half of this period, we observed a surface maximum of Chl *a* content at ReS site (5 mg m^{-3}) that was not registered at RaS station. After July 2nd, northerly winds relaxed and even shifted southward, developing a strong thermocline with relatively nutrient poor surface waters; NO_3^- and NH_4^+ levels lower than $1\text{ }\mu\text{mol kg}^{-1}$ and $2\text{ }\mu\text{mol kg}^{-1}$, respectively. During this sampling period the Chl *a* content maintained relatively constant along the whole water column at both ReS and RaS sampling sites (Figure 2 and 3).

In order to describe the effect of mussel rafts on the Ría ecosystem we present mean seasonal variations of nitrogen forms, dissolved oxygen (DO), particulate organic carbon (POC), Chl *a* and microbial plankton carbon (PC) at reference and raft stations (Figure 4). Despite average NO_3^- and nitrite (NO_2^-) concentrations showed no significant differences between sites, NH_4^+ contents were significantly different (Table 2) with higher values at RaS station. Mean integrated NH_4^+ levels ranged from maximum values of 27 mmol m^{-2} and 48 mmol m^{-2} registered in autumn and minimum values of 7 mmol m^{-2} and 15 mmol m^{-2} recorded during spring time for ReS and RaS stations, respectively (Figure 4a). Dissolved organic nitrogen (DON) presented no significant differences between sampling stations (Table 2) and seasonally varies between minimum integrated values of 75 mmol m^{-2} and 77 mmol m^{-2} in autumn for ReS and RaS sampling sites, respectively, and maximum integrated values of 89 mmol m^{-2} in spring at ReS station and 93 mmol m^{-2} in summer at RaS station (Figure 4a). Dissolved organic carbon (DOC) also presented no significant differences between sites ranging around mean annual integrated values of 1032 mmol m^{-2} and 1064 mmol m^{-2} at ReS and RaS stations, respectively (Table 2). We also found no-significant differences

in DO contents between sites being the mean annual deficit in the raft station respect to the reference site of 5% (Table 2). Maximum variations in mean seasonal DO contents between sampling sites were registered during autumn 2007 and summer 2008 (Figure 4b).

In terms of suspended particulate matter, although POC did not present significant differences between stations we registered clearly lower values of both Chl *a* and PC at the raft station by comparing with the reference site (Figure 2, 3 and Table 2). In terms of mean seasonal values of both Chl *a* and PC we found maximum values in spring and minimum values during winter time (Figure 4b).

3.2. Physiological rates during mussel experiments

In Figure 5 we present the box-plots with the seasonal evolution of both metabolic and physiological parameters. With our data, the test rejected the hypothesis of homogeneity between seasons in all physiological variables. In Table 3 it is presented a descriptive resume of the data showing the seasons that followed a homogeneous pattern (Tukey HSD) for all variables. In general, we observed that metabolic and physiological activity of *Mytilus galloprovincialis* reached minimum values in winter time and maximum values during spring and summer seasons (Figure 5).

The ammonium excretion rates ($\text{VNH}_4^+\text{-N}$) were significantly lower in autumn ($14.2 \mu\text{g h}^{-1}$) and winter ($13.7 \mu\text{g h}^{-1}$) than in spring and summer, when rates of $20.8 \mu\text{g h}^{-1}$ and $21.7 \mu\text{g h}^{-1}$ were registered, respectively (Table 3). The oxygen uptake presented a different pattern respect to the other variables, ranging between minimum values during winter (0.7 ml h^{-1}) and maximum values during spring (1.4 ml h^{-1}).

Seasonal variations of organic ingestion rates (OIR) presented minimum values in winter (0.8 mg h^{-1}) and maximum values in spring (2.7 mg h^{-1}) (Figure 5 and Table 3).

In terms of absorption efficiency (AE) mean values of 81.5% were registered during our experimental year, ranging from a minimum value of 72.5% in winter to a maximum value of 87.7% in summer (Table 3).

4. DISCUSSION

4.1. Alteration of water column biogeochemistry by feeding activity of mussels.

According to previous studies, the bivalve feeding activity can be important in the pathway of nutrient cycling on coastal ecosystems (Souchu et al., 2001; La Rosa et al., 2002; Nizzoli et al., 2006; Richard et al., 2007). Mussel cultures act as a source of recycled nutrients to the system by means of the respiration and excretion of inorganic metabolic waste products, while harvest of the mussels results in removal of nutrients from the system. In doing so, mussels act as a sink or source of nutrients and thereby interact with nutrient cycling in coastal ecosystems, particularly in areas with dense mussel communities (see reviews by Newell, 2004; Prins et al., 1998).

For the Ría de Vigo, it has been suggested that the intensive mussel culture has a great influence on the microbial food web structure and functioning (Figueiras et al., 2002; Petersen et al., 2008; Maar et al., 2008). However, only Alonso-Pérez et al. (2010) tried to elucidate the impact of suspended mussel culture on water column biogeochemistry by comparing conditions inside and outside of a raft area. Based on a short field campaign, they concluded that mussel farming did not significantly alter the pelagic nutrient cycling. On the contrary, the present seasonal study shows, for the first time, that mussel farming clearly modifies the water column biogeochemistry.

Data presented in this study reveal that thermohaline conditions were very similar at both the reference (ReS) and raft (RaS) stations, following the pattern presented in Figures 2 and 3. Nevertheless, the comparison of inorganic and organic (dissolved and particulate) nutrient forms at the the two stations showed contrasting results in terms of effects associated to mussel activity at the raft site. Focusing on nitrogen and beginning with the inorganic forms (NO_3^- , NO_2^- and NH_4^+), we did not observe any significant difference on NO_3^- content between stations (Figure 2, 3, 4 and Table 2). The relationship of this nutrient with the water column temperature pointed that is controlled by thermohaline properties observing the highest levels with the entrance of upwelled cold, nutrient-rich subsurface ENACW into the Ría (Fraga, 1981). Such correlation runs in parallel at both control and raft stations revealing that similar hydrographic conditions were affecting our two study sites (Figure 2 and 3). Likewise, NO_2^- distributions were also very similar between ReS and RaS sampling sites with no significant differences along the studied year (Figure 4 and Table 2) suggesting that this nitrogen form is not directly affected by mussel physiology either. These results clearly contrast with NH_4^+ distribution, that presented significant differences between the two sampling stations (Figure 2 and 3). Our ammonium excretion rates are of the same order than previously reported values (Hawkins and Bayne, 1985; Smaal and Vonck, 1997; Smaal et al., 1997; Jansen et al., 2011) and could explain the increase of NH_4^+ levels in the water column as the high correlation between these two parameters indicates (Figure 6). Our observations clearly evidence that mussel rope community plays an important role on nutrient recycling. NH_4^+ excess in the mussel farming zone respect to the reference site was highly significant in all sampling periods (Table 2) with values in the same range of data presented by Boucher et al. (1988) from a study of oyster farming, where the contribution of VNH_4^+ -N was 10-40%.

The role of dissolved organic matter as a potential food source has not been sufficiently studied and the few available manuscripts reached different conclusions (Roditi et al., 2000; Pang and Wang, 2004; Baines et al., 2007). Our results (Table 2), showing no significant differences in the quantity and quality of dissolved organic matter (expressed as DOC: DON ratio; p -value = 0.504) at the raft and reference stations, would support the idea proposed by Pan and Wang (2004). These authors stated that the contribution of DOC as nutrition source for marine bivalves is basically negligible due to the low carbon absorption efficiency (AE) from the dissolved phase, which is 3 orders of magnitude lower than the carbon AE from food particles.

In contrast, particulate organic matter as a food source for *Mytilus galloprovincialis* during our study presented a different contribution. While the lack of significant differences between PON and POC at the two sites would suggest that they were not suitable food sources, the reduction in microbial plankton carbon (PC) at the raft site points to preferential consumption by mussel activity. The PC content was depleted at the raft site (Figure 4b; Table 2) where PC represented, in annual terms, around $49 \pm 25\%$ of total POC in contrast to $73 \pm 37\%$ at the reference site. This fact reveals that there has to be an additional source of particulate organic matter at the raft site different from the non-planktonic carbon at the reference site. One possible additional source of particulate organic matter could be all raft epifauna community associated to the mussel ropes (Maar et al., 2008) but further studies should be focused on analyzing these other inputs of organic matter.

The annual deficits of both Chl *a* and PC at the raft station respect to the reference site (Figure 4b and Table 2) reflect the preferential grazing of mussels on plankton. The Chl *a* deficit at the mussel zone in Ría de Vigo was lower than values ($-44 \pm 4\%$) presented by Souchu et al. (2001), who reported how the filter feeders (*Crassostrea gigas*) were

able to alter water column nutrient cycling in a poorly flushed lagoon in the Mediterranean Sea. Otherwise, our Chl *a* (-26 - 53%) summer deficits in the raft station respect to the reference site were in good agreement with summer depletion rates (10-45%) found by Petersen et al. (2008) in a study carried out in the Ría de Vigo based on fluorescence data. They are also similar to previous levels of estimated phytoplankton reduction (30%) from mussel rafts in the Rías reported by Perez-Camacho et al. (1991). No previous studies have analysed the mussel consumption on plankton based on microbial plankton carbon.

4.2. Physiological responses of mussels to quantity and quality of the organic content in the available food.

The physiological responses of mussels to quantity and quality of the available food has been extensively studied mainly analysing the feeding and digestive behaviour under experimentally controlled levels of suspended particles load (SPM) and particulate organic matter (POM) content of the seston (e.g. Bayne et al., 1993; Cranford and Hill, 1999; Kreeger and Newell, 2001). In this framework, and taking into account that the Rías are characterised by low suspended particulate matter (Babarro et al., 2000) the goal of our study, carried out under natural conditions, is to understand how quantity and quality of the seston organic compounds can affect the feeding behaviour of suspended mussels and how these filter feeder organisms utilise these components to satisfy their carbon demands. To assess the nutritional value of the seston organic compounds we have used different proxies such as particulate organic carbon (POC), that represents the total organic carbon content of seston available for mussels, chlorophyll *a* (Chl *a*) that is used, as an indicator of phytoplankton biomass, and the microbial plankton carbon (PC), that considers the whole organic carbon derived from both autotrophic (phytoplankton) and heterotrophic microbial plankton.

From the relationship between organic ingestion rates (OIR) of mussels *Mytilus galloprovincialis* and the seston organic content in the Ría de Vigo during our experimental year, we can observe that 59% and 64% of the variance in OIR are explained based on quality of the ingested food derived from POC and PC estimates, respectively (Figure 7). These results corroborate that *Mytilus galloprovincialis* under natural conditions in the Rías Baixas are filter organisms that not only increase their feeding activity in terms of SPM contents (Figueiras et al., 2002) but also in terms of organic load of the available food. With this model we can assess that for maximum POC (247 mg m^{-3}) and PC (153 mg m^{-3}) contents, found in the Ría de Vigo during our experimental year, the predicted maximum OIR were 2.51 mg l^{-1} and 2.35 mg l^{-1} , respectively.

Otherwise the nutritional value of the seston carbon sources not only affects the feeding activity of mussels but also the digestive processes that optimize the consumed energy (Babarro et al., 2000; Perez-Camacho et al., 2000). Figueiras et al (2002) presented a first adjustment of mussel absorption efficiency (AE) to the nutritional value of seston, i.e. organic matter content of seston. Their approach assesses a maximum AE (~ 0.6) when the ratio between particulate organic matter and SPM is ~ 0.5 . However, during our experimental year in the Ría de Vigo, we found that AE is not highly related with POC contents ($r^2=0.34$). Taking into account this fact and considering primarily reported information (section 4.2) about an additional source of POC at RaS, we hypothesise that in our study this additional source of carbon is not accessible for mussel digestion, leading to an overestimation of the high quality available food. In fact, a significant relationship between AE and PC (not observed with Chl *a* content) was observed (Figure 7):

$$\text{AE (\%)} = 94.40 (\text{PC} / (9.06 + \text{PC})),$$

The model, that explains 85% of the variance in AE as dependent on the quality of ingested food due to microbial plankton carbon, predicts an asymptotic maximum AE of 89.4% for maximum PC contents registered in the Ría de Vigo during our experimental year. The adjustment of AE is maintained even if we consider the PC/POC ratio as the independent variable ($AE (\%) = 100 \text{ PC} / \text{POC} / (9.23 + \text{PC} / \text{POC})$; $r^2 = 0.83$). These models clearly reflect that when microbial plankton carbon dominates the organic content of the seston, the digestive processes of the mussels are reinforced and explain the high growth rates of mussel *Mytilus galloprovincialis* in the Rías. On the other hand, the fact that the good adjustments between both feeding (OIR) and digestive (AE) parameters with PC contents in the water column were lost when we used Chl *a* as a proxy of seston organic fraction (Figure 7) remarks the importance of consider the whole microbial plankton carbon, including not only autotrophic but also heterotrophic microbial plankton as high quality food for mussels in the Ría de Vigo. Otherwise, changes in PC/Chl *a* ratios can also help to explain differences in the adjustments with PC and Chl *a*.

In summary, the interaction between coastal upwelling and circulation patterns in the Rías triggers the high microbial plankton carbon content of seston (Margalef and Andreu, 1958; Tenore and González, 1975; Figueiras et al., 2002) which leads to maximum levels in the absorption of energy consumed by mussels, as presented in this study. In this way, the Rías Baixas have become into an ecosystem with the largest mussel production in Europe (Labarta, 2000). Future research increasing temporal and spatial resolution sampling, both in and out of the raft areas, will be necessary to corroborate our results.

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649

Table 1. Mean $\pm$ standard deviation of shell size, dry tissue weight and condition index (CI) for the individuals used in this study.

	Size shell (mm)	Dry weight (mg)	CI
Autumn07	1073 $\pm$ 199	56 $\pm$ 2	30 $\pm$ 5
Winter08	451 $\pm$ 77	57 $\pm$ 2	14 $\pm$ 2
Spring08	738 $\pm$ 107	58 $\pm$ 1	23 $\pm$ 3
Summer08	852 $\pm$ 175	61 $\pm$ 2	21 $\pm$ 3
Annual	811 $\pm$ 277	58 $\pm$ 3	23 $\pm$ 7

Table 2. Results derived from the t-test applied to the paired samples (n = 24; 6 samplings x 4 seasonal periods) between reference and raft stations. Values of p < 0.05 indicate a significant difference between sites. SD: standard deviation; NS. not significant.

	Significance level of t-test at $\alpha = 0.05$	Mean $\pm$ SD at reference site	Mean $\pm$ SD at raft site	Deficit or excess comparing raft and reference site (Mean $\pm$ SD in %)
	(n=24)	(n=24)	(n=24)	(n=24)
NO ₃ ⁻ , mmol m ⁻²	0.879	48 $\pm$ 39	46 $\pm$ 35	NS
NO ₂ ⁻ , mmol m ⁻²	0.562	3.4 $\pm$ 2.5	3.8 $\pm$ 2.6	NS
NH ₄ ⁺ , mmol m ⁻²	0.007	20 $\pm$ 14	33 $\pm$ 18	40 $\pm$ 31
DON, mmol m ⁻²	0.270	84 $\pm$ 20	87 $\pm$ 22	NS
DOC, mmol m ⁻²	0.239	1032 $\pm$ 80	1064 $\pm$ 81	NS
DO, mmol m ⁻²	0.049	3333 $\pm$ 309	3140 $\pm$ 350	-5 $\pm$ 7
POC, mg m ⁻²	0.064	2803 $\pm$ 1068	2229 $\pm$ 464	NS
Chl <i>a</i> , mg m ⁻²	0.023	37 $\pm$ 23	23 $\pm$ 16	-33 $\pm$ 26
PC, mg m ⁻²	0.015	2017 $\pm$ 750	1298 $\pm$ 561	-34 $\pm$ 28

Table 3. Mean $\pm$ standard deviation of ammonium excretion rate ($\text{VNH}_4^+\text{-N}$), oxygen uptake (VO_2), organic ingestion rate (OIR) and absorption efficiency (AE) for each sampling season. Homogeneous groups identified by Tukey's HSD test ($\alpha = 0.05$) are given in brackets (a,b).

	$\text{VNH}_4^+\text{-N}$ ($\mu\text{g h}^{-1}$)	VO_2 (ml h^{-1})	OIR (mg h^{-1})	AE (%)
Autumn07	14.2 ± 4.9 (a)	0.9 ± 0.2 (b)	2.0 ± 0.6	82.0 ± 5.3 (a)
Winter08	13.7 ± 4.5 (a)	0.7 ± 0.1 (a)	0.8 ± 0.2	72.5 ± 1.7
Spring08	20.8 ± 7.2 (b)	1.4 ± 0.3	2.7 ± 0.9 (a)	82.9 ± 4.6 (a,b)
Summer08	21.7 ± 5.7 (b)	0.8 ± 0.2 (a,b)	2.4 ± 0.6 (a)	87.7 ± 1.8 (b)
Annual	17.0 ± 6.6	1.0 ± 0.3	2.0 ± 0.9	81.5 ± 6.2

Figure 1. Map of the Ría de Vigo showing the location of the raft polygons (in black) and the positions of both reference (ReS) (black dot) and raft (RaS) (white dot) sampling sites. The position of RaS station inside the polygon is also shown in the figure.

Figure 2. Time-series of upwelling index, temperature, nitrate (NO_3^-), ammonium (NH_4^+) and chlorophyll *a* (Chl *a*) concentration registered at the reference station (ReS). The days when physiological experiments with mussels were carried out are shown as vertical dashed lines.

Figure 3. Time-series of temperature, nitrate (NO_3^-), ammonium (NH_4^+) and chlorophyll *a* (Chl *a*) concentration recorded at the raft station (RaS). The days when physiological experiments with mussels were carried out are shown as vertical dashed lines.

Figure 4. (a) Mean seasonal variations of 12 m water column integrated values of nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+) and dissolved organic nitrogen (DON) at both the reference (white bars) and raft (black bars) stations. (b) Mean seasonal variations of 12 m water column integrated values of dissolved oxygen (DO), chlorophyll *a* (Chl *a*), particulate organic carbon (POC) and microbial plankton carbon (PC) at both the reference (white bars) and raft (black bars) stations.

Figure 5. Box-plots showing the seasonal variation of metabolic (ammonium excretion rate ($\text{VNH}_4^+\text{-N}$) and oxygen uptake (VO_2)) and physiological (organic ingestion rate (OIR) and absorption efficiency (AE)) rates. The box itself goes from the lower quartile (Q1) to the upper quartile (Q3). The horizontal bar inside the box is the median. The whiskers above and below the box are drawn at the first value in the range $[\text{Q1} - (1.5 * \text{IQR}), \text{Q3} + (1.5 * \text{IQR})]$. Data points which exceed this range are drawn separately as outliers.

Figure 6. Dispersion diagram between ammonium content in the water column at the raft station ($\text{NH}_4^+_{\text{WC}}$) and the direct ammonium excretion rate ($\text{VNH}_4^+ \text{-N}$) obtained from the physiological experiments with mussels.

Figure 7. Dispersion diagrams showing the relationship between both organic ingestion rate (OIR) and absorption efficiency (AE) with the food quality variables (given as particulate organic carbon (POC), chlorophyll *a* (Chl *a*) and microbial plankton carbon (PC)) observed in the raft. The adjustment of the data was given by the hyperbolic equation $y = ax / (b + x)$. The coefficients *a* and *b* are shown in the figure for each adjustment.

Figure 1

Figure 2

Figure 3

Figure 4a

Figure 4b

Figure 5

Figure 6

Figure 7