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

Zúñiga, D.[1*], Froján, M.[1], Castro, C.G.[1], Alonso-Pérez, F.[1], Labarta, U.[1], Figueiras, F.G.[1], Fuentes-Santos, I.[1], Fernández-Reiriz, M.J.[1]

[1] Instituto de Investigaciones Marinas (IIM), CSIC, Vigo, 36208, Spain.

* Corresponding author: Instituto de Investigaciones Marinas (IIM), CSIC, Vigo, 36208, Spain. Telf.: +34 986 231930, Fax: +34 986 292762, e-mail: dianazuniga@iim.csic.es

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.,

47 2007) , the Oosterschelde estuary in the Netherlands (300 g C m^{-2} yr^{-1}) (Smaal et al.,
48 2001) or Thau lagoon in France (400 g C m^{-2} yr^{-1}) (Plus et al., 2006), and enable the
49 Rías to support the highest mussel production in Europe, with a total estimated
50 production of 250 x 10^6 $kg^{-1}y^{-1}$ of edible mussels.

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

63 For the Galician Rías Baixas, Tenore et al. (1982) stated that the introduction of this
64 aquaculture system altered the trophic chain of the ecosystem, diverting primary
65 production and energy flow from planktonic to benthic food web. In later years, *in situ*
66 experiments have studied the culture of *Mytilus galloprovincialis* in relation to seston
67 variables (quantity and quality) and chlorophyll in the Rías Baixas. Figueiras et al.
68 (2002) suggested that the phytoplankton response to upwelling provides high-quality
69 food enhancing absorption efficiency of *Mytilus galloprovincialis* (AE ~ 0.6). They
70 estimated that mussel harvest in the Ría de Arousa extracts the equivalent to ~10% of
71 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 $m^3 s^{-1}$ (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 m^2 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 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 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 ±0.02 μM 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 ±0.35 μM.

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 ±1 μM for DOC and
149 ±0.2 μM 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 μm 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 ±0.9 μmol C l^{-1} and ±0.2 μmol N l^{-1}.

161 For microplankton (> 20μm) 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 μm) 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- (< 2μm) and nanoplankton (2-20μm) 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 heterototrophic 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.

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

197 **2.2.1. Ammonium excretion rate (VNH_4^+-N) and oxygen uptake (VO_2)**

198 Ammonium excretion rate (VNH_4^+-N) was determined after the mussels were placed in
199 open cylindrical chambers with 250 ml of filtered seawater (0.2 μm Millipore
200 membranes). Temperature was maintained during the determinations by immersing the
201 chambers in an isothermal bath. Two chambers without animals were used as a control.
202 After 90 min, water samples were collected from each chambers and frozen to -20ºC
203 until be analysed in the laboratory, according to the phenol-hypochlorite method
204 described by Solorzano (1969). VNH_4^+-N´s were calculated from the difference in NH_4^+
205 concentration between the chambers with and without animals, respectively.

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

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

216 **2.2.2. Organic ingestion rate (OIR) and absorption efficiency (AE)**.

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

235 **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:

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

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).

3. RESULTS

3.1. Hydrographic conditions and biogeochemistry of the water column

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

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

283 index, is clearly discernible by low temperature (T < 14 ºC), and high NO_3^- contents (5
284 µmol kg^{-1}) (Figure 2 and 3), always recorded close to the bottom. According to the
285 upwelling conditions registered in the first half of this period, we observed a surface
286 maximum of Chl *a* content at ReS site (5 mg m^{-3}) that was no registered at RaS station.
287 After July 2nd, northerly winds relaxed and even shifted southward, developing a strong
288 thermocline with relatively nutrient poor surface waters; NO_3^- and NH_4^+ levels lower
289 than 1 µmol $kg^{-1}$and 2 µmol kg^{-1}, respectively. During this sampling period the Chl *a*
290 content maintained relatively constant along the whole water column at both ReS and
291 RaS sampling sites (Figure 2 and 3).

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

308 in DO contents between sites being the mean annual deficit in the raft station respect to
309 the reference site of 5% (Table 2). Maximum variations in mean seasonal DO contents
310 between sampling sites were registered during autumn 2007 and summer 2008 (Figure
311 4b).

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

317 **3.2. Physiological rates during mussel experiments**

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

325 The ammonium excretion rates (VNH_4^+-N) were significantly lower in autumn (14.2 µg
326 h^{-1}) and winter (13.7 µg h^{-1}) than in spring and summer, when rates of 20.8 µg h^{-1} and
327 21.7 µg h^{-1} were registered, respectively (Table 3). The oxygen uptake presented a
328 different pattern respect to the other variables, ranging between minimum values during
329 winter (0.7 ml h^{-1}) and maximum values during spring (1.4 ml h^{-1}).

330 Seasonal variations of organic ingestion rates (OIR) presented minimum values in
331 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.

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

379 The role of dissolved organic matter as a potential food source has not been sufficiently
380 studied and the few available manuscripts reached different conclusions (Roditi et al.,
381 2000; Pang and Wang, 2004; Baines et al., 2007). Our results (Table 2), showing no
382 significant differences in the quantity and quality of dissolved organic matter (expressed
383 as DOC: DON ratio; p-value = 0.504) at the raft and reference stations, would support
384 the idea proposed by Pan and Wang (2004). These authors stated that the contribution of
385 DOC as nutrition source for marine bivalves is basically negligible due to the low
386 carbon absorption efficiency (AE) from the dissolved phase, which is 3 orders of
387 magnitude lower than the carbon AE from food particles.

388 In contrast, particulate organic matter as a food source for *Mytilus galloprovinciallis*
389 during our study presented a different contribution. While the lack of significant
390 differences between PON and POC at the two sites would suggest that they were not
391 suitable food sources, the reduction in microbial plankton carbon (PC) at the raft site
392 points to preferential consumption by mussel activity. The PC content was depleted at
393 the raft site (Figure 4b; Table 2) where PC represented, in annual terms, around 49 ±
394 25% of total POC in contrast to 73 ± 37% at the reference site. This fact reveals that
395 there has to be an additional source of particulate organic matter at the raft site different
396 from the non-planktonic carbon at the reference site. One possible additional source of
397 particulate organic matter could be all raft epifauna community associated to the mussel
398 ropes (Maar et al., 2008) but further studies should be focused on analyzing these other
399 inputs of organic matter.

400 The annual deficits of both Chl *a* and PC at the raft station respect to the reference site
401 (Figure 4b and Table 2) reflect the preferential grazing of mussels on plankton. The Chl
402 *a* deficit at the mussel zone in Ría de Vigo was lower than values (-44 ± 4%) presented
403 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 galloprovinciallis* 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 galloprovinciallis* 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 asses 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):

AE (%) = 94.40 (PC / (9.06+PC)),

454 The model, that explains 85% of the variance in AE as dependent on the quality of
455 ingested food due to microbial plankton carbon, predicts an asymptotic maximum AE of
456 89.4% for maximum PC contents registered in the Ría de Vigo during our experimental
457 year. The adjustment of AE is maintained even if we consider the PC/POC ratio as the
458 independent variable (AE (%) = 100 PC / POC/(9.23+PC/POC); r^2 = 0.83). These
459 models clearly reflect that when microbial plankton carbon dominates the organic
460 content of the seston, the digestive processes of the mussels are reinforced and explain
461 the high growth rates of mussel *Mytilus galloprovincialis* in the Rías. On the other hand,
462 the fact that the good adjustments between both feeding (OIR) and digestive (AE)
463 parameters with PC contents in the water column were lost when we used Chl *a* as a
464 proxy of seston organic fraction (Figure 7) remarks the importance of consider the
465 whole microbial plankton carbon, including not only autotrophic but also heterotrophic
466 microbial plankton as high quality food for mussels in the Ría de Vigo. Otherwise,
467 changes in PC/Chl *a* ratios can also help to explain differences in the adjustments with
468 PC and Chl *a*.

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

477 **Acknoledgements**

The authors thank the crew of the 'R/V Mytilus' and the members of the Oceanography and Physiology and Culture of Bivalves Molluscs from the Instituto de Investigacións Mariñas de Vigo (CSIC) for their valuable help. Financial support came from MICINN RAFTING project (CTM2007–61983/MAR, CTM2007-30624-E/MAR). D.Z. was funded by a postdoctoral fellowship (Jae-Doc) from the CSIC. M.F. was financed by a research grant (BI/PTDC/MAR/102045/2008/2010-022) from Fundação para a Ciência e a Tecnologia (FCT) in the framework of CALIBERIA project (FCOMP-01-0124-FEDER-010599).

References

Alonso-Pérez F, Ysebaert T, Castro CG (2010) Effects of suspended mussel culture on benthic–pelagic coupling in a coastal upwelling system (Ría de Vigo, NW Iberian Peninsula). J Exp Mar Biol Ecol 382 (2): 96–107.

Álvarez-Salgado XA, Miller AEJ (1998) Simultaneous determination of dissolved organic carbon and total dissolved nitrogen in seawater by high temperature catalytic oxidation: conditions for precise shipboard measurements. Mar Chem 62 (3, 4): 325–333.

Arbones B, Castro CG, Alonso-Pérez F, Figueiras FG (2008) Phytoplankton size structure and water column metabolic balance in a coastal upwelling system: the Ría de Vigo, NW Iberia. Aquat Microb Ecol 50: 169-179.

Aure J, Strand Ø, Erga SR, Strohmeier T (2007) Primary production enhancement by artificial upwelling in a western Norwegian fjord. Mar Ecol Prog Ser 352: 39-52.

500 Babarro JMF, Fernández-Reiriz MJ, Labarta U (2000) Metabolism of the mussel
501 *Mytilus galloprovinciallis* from two origins in the Ría de Arousa (north-west
502 Spain). J Mar Biol Ass UK 80: 865-872.
503 Baines SB, Fisher NS, Cole JJ (2007) Dissolved organic matter and persistence of the
504 invasive zebra mussel (*Dreissena polymorpha*) under low food conditions. Limnol
505 Oceanogr 52 (1): 70-78.
506 Bakun A (1973) Coastal upwelling indices, West Coast of North America , US Dept. Of
507 Commerce, NOAA Tech. Rep., NMFS SSRF-671, 103 pp. 1946–71.
508 Bayne BL, Newell RIE (1983) Physiological energetics of marine mollusca. In:
509 Saleuddin ASM, Wilbur KM (eds). The Mollusca vol 4. New York, Academic
510 Press: 407-515.
511 Bayne BL, Iglesias JIP, Hawkins AJS, Navarro E, Héral M, Deslous-Paoli JM (1993)
512 Feeding behaviour of the mussel *Mytilus edulis*: Responses to variations in quantity
513 and organic content of the seston. J Mar Biol Ass U K 73: 813-829.
514 Boucher G, Boucher-Rodoni R (1988) *In situ* measurement of respiratory metabolism
515 and nitrogen fluxes at the interface of oyster beds. Mar Ecol Prog Ser 44: 229-238.
516 Bratbak G, Dundas I (1984) Bacterial dry matter content and biomass estimations. Appl
517 Environ Microbiol 48: 755-757.
518 Cermeño P, Marañón E, Pérez V, Serret P, Fernández E, Castro CG (2006)
519 Phytoplankton size structure and primary production in a highly dynamic coastal
520 ecosystem (Ría de Vigo, NW-Spain): Seasonal and short-time scale variability. Est
521 Coast Shelf Sci 67: 251-266.
522 Conover RJ (1966) Assimilation of organic matter by zooplankton. Limnol Oceanogr
523 11: 338-345.

524 Cranford PJ, Hill PS (1999) Seasonal variation in food utilization by the suspensión-
525 feeding bivalve molluscs *Mytilus edulis* and *Placopecten magellanicus*. Mar Ecol
526 Prog Ser 190: 223-239.
527 Cranford PJ, Strain PM, Dowd M, Hargrave BT, Grant J, Archambault MC (2007)
528 Influence of mussel aquaculture on nitrogen dynamics in a nutrient enriched coastal
529 embayment. Mar Ecol Prog Ser 347: 61-78.
530 Dame RF (1993). Bivalve filter feeders in estuarine and coastal ecosystem processes.
531 NATO ASI Series, Springer-Verlag, New York.
532 Erga SR (1989) Ecological studies on the phytoplankton of Boknafjorden, westerm
533 Norway: Environmental control of photosynthesis. J Plankton Res 11: 785-812.
534 Fernández-Reiriz MJ, Range P, Álvarez-Salgado XA, Espinosa J, Labarta U (2012)
535 Tolerance of juvenile Mytilus galloprovincialis to experimental seawater
536 acidification. Mar Ecol Prog Ser 454: 65-74.
537 Figueiras FG, Labarta U, Fernández-Reiriz MJ (2002) Coastal upwelling, primary
538 production and mussel growth in the Rías Baixas of Galicia. Hidrobiol 484: 121-
539 131.
540 Figueiras FG, Zdanowski MK, Crespo BG (2006) Spatial variability in bacterial
541 abundance and other microbial components in the NW Iberian margin during
542 relaxation of a spring upwelling event. Aquat Microb Ecol 43: 255–266.
543 Filgueira R, Labarta U, Fernández-Reiriz MJ (2006) Flow-through chamber method for
544 clearance rate measurements in bivalves: design and validation of individual
545 chambers and mesocosm. Limnol Oceanogr Methods 4: 284-292.
546 Filgueira R, Labarta U, Fernández-Reiriz MJ (2008) Effect of Condition index on
547 Clearance Rate allometric relationships in Mytilus galloprovincialis Lamarck. Rev
548 Biol Mar Ocean 43:391-398

549 Fraga F (1981) Upwelling off the Galician coast, Northwest Spain. In Richards, F.A.
550 (ed), coastal upwelling series, vol. 1 AGU, Washington DC: 176-182.
551 Hansen HP, Grassoff K (1983) Automated chemical analysis. In: Grassoff, K., Ehrardt,
552 M., Kermling, K. (Eds.), Methods of Seawater Analysis. Verlag Chemie,
553 Weinheim: 347–379.
554 Hawkins AJS, Bayne BL (1985) Seasonal-variation in the relative utilization of carbon
555 and nitrogen by the mussel *Mytilus edulis*: budgets, conversion efficiencies and
556 maintenance requirements. Mar Ecol Prog Ser 25: 181-188.
557 Hillebrand H, Dürselen C, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume
558 calculation for pelagic and benthic microalgae. J Phycol 35: 403–424.
559 Jansen HM, Strand Ø, Verdegem M, Smaal A (2011) Accumulation, release and
560 turnover of nutrients (C-N-P-Si) by the blue mussel *Mytilus edulis* under
561 oligotrophic conditions. J Exp Mar Biol Ecol 416-417: 185-195.
562 Kreeger DA, Newell RIE (2001) Seasonal utilization of different seston carbon sources
563 by the ribbed mussel, *Geukensia demissa* (Dillwyn) in a mid-Atlantic salt marsh. J
564 Exp Mar Biol Ecol 260: 71-91.
565 La Rosa T, Mirto S, Favaloro E, Savona B, Sarà G, Danovaro R, Mazzolam A (2002)
566 Impact on the water column biogeochemistry of a Mediterranean mussel and fish
567 farm. Water Res 36: 713-721.
568 Labarta U (2000) La Acuicultura en Galicia. A Economía Galega. Serie Informes anuais
569 n◦ 14, Informe 1998/1999. Fundación Caixa Galicia. CIEF Centro de Investigación
570 Económica y Financiera.
571 Larsen J, Sournia A (1991) The diversity of heterotrophic dinoflagellates. In. Patterson
572 DJ, Larsen j, (Eds.) The Biology of Free-Living Heterotrophic Flagellates. Oxford
573 University Press, New York, 313-332.

574 Lessard EF, Swift E (1986) Dinoflagellates from the North Atlantic classified as
575 phototrophic or heterotrophic by epifluorescence microscopy. J Plankton Res 8.
576 1209-1215.
577 Maar M, Nielsen TG, Petersen JK (2008) Depletion of plankton in a raft culture of
578 *Mytilus galloprovincialis* in Ría de Vigo, NW Spain. II. Zooplankton. Aquat Biol 4:
579 127-141.
580 Margalef R, Andreu B (1958). Componente vertical de los movimientos del agua en la
581 ría de Vigo y su posible relación con la entrada de sardina. Inv.Pesq. XI: 105-126.
582 Mouriño C, Fraga F (1985) Determinación de nitratos en agua de mar. Investigaciones
583 Pesqueras 49: 81–96.
584 Navarro E, Iglesias JIP (1993). Infaunal filter-feeding bivalves and the physiological
585 response to short-term fluctuations in food availability and composition. In: Dame
586 RF (ed) Bivalve filter feeders in Estuarine and Coastal Ecosystem Processes. Ecol
587 Sci 33, NATO ASI series, pp: 25-56.
588 Newell RIE (2004) Ecosystem influences of natural and cultivated populations of
589 suspension-feeding bivalve molluscs: a review. J Shellfish Res 23: 51-61.
590 Nizzoli D, Welsh DT, Fano EA, Viaroli P (2006) Impact of clam and mussel farming on
591 benthic metabolism and nitrogen cycling, with emphasis on nitrate reduction
592 pathways. Mar Ecol Prog Ser 315: 151–165.
593 Nogueira E, Pérez FF, Ríos AF (1997) Seasonal patterns and long-term trends in an
594 estuarine upwelling ecosystem (Ría de Vigo, NW Spain). Est Coast Mar Sci 44:
595 285–300.
596 Pan JF, Wang WX (2004) Differential uptake of dissolved and particulate organic
597 carbon by the marine mussel *Perna viridis*. Limnol Oceanogr 49 (6): 1980-1991.

598 Perez-Camacho A, Labarta U, Navarro E (2000) Energy balance of mussels *Mytilus*
599 *galloprovincialis*: the effect of length and age. Mar Ecol Prog Ser 199: 149-158.
600 Petersen JK, Nielsen TG, Van Duren L, Maar M (2008) Depletion of plankton in a raft
601 culture of *Mytilus galloprovincialis* in Ría de Vigo, NW Spain. I. Phytoplankton.
602 Aquat Biol 4: 113-125.
603 Plus M, La Jeunesse I, Bouraoui F, Zaldiver JM, Chapelle A, Lazure P (2006)
604 Modelling water discharges and nitrogen inputs into a Mediterranean lagoon.
605 Impact on the primary production. Ecol Model 193: 69-89.
606 Prins T, Smaal A, Dame R (1998) A review of feedbacks between bivalve grazing and
607 ecosystem processes. Aquat Ecol 31: 349-359.
608 Putt M, Stoecker DK (1989) An experimentally determined carbon: volume ratio for
609 marine ‘‘oligotrichous’’ ciliates from estuarine and coastal waters. Limnol
610 Oceanogr 34 (6): 1097–1103.
611 R Development Core Team (2011). R: A language and environment for statistical
612 computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-
613 900051-07-0, URL http://www.R-project.org/.
614 Richard M, Archambault P, Thouzeau G, Desrosiers G (2007) Summer influence of 1
615 and 2 yr old mussel cultures on benthic fluxes in Grande-Entrée lagoon, Îles-de-la-
616 Madeleine (Québec, Canada). Mar Ecol Prog Ser 338: 131–143.
617 Roditi HA, Fisher N, Sanudo-Wilhelmy S (2000) Uptake of dissolved organic carbon
618 and trace elements by zebra mussels. Nature 407: 78–80.
619 Rodríguez F, Pazos Y, Maneiro J, Zapata M (2003) Temporal variation in
620 phytoplankton assemblages and pigment composition at a fixed station of the Ría of
621 Pontevedra. Est Coast Shelf Sci 58: 499–515.

622 Smaal AC, Vonck A (1997) Seasonal variation in C, N and P budgets and tissue
623 composition of the mussel *Mytilus edulis*. Mar Ecol Prog Ser 153: 167-179.
624 Smaal AC, Vonck A, Bakker M (1997) Seasonal variation in physiological energetics of
625 *Mytilus edulis* and *Cerastoderma edule* of different size classes. J Mar Biol Ass UK
626 77: 817-838.
627 Smaal AC, Van Stralen M, Schuiling E (2001) The interaction between shellfish culture
628 and ecosystem processes. Can J Fish Aquat Sci 58: 991-1001.
629 Solorzano L (1969) Determination of ammonia in natural seawaters by the phenol-
630 hypochlorite method. Limnol Oceanogr 14: 799-801.
631 Souchu P, Vaquer A, Collos Y, Landrein S, Deslous-Paoli JM, Bibent B (2001)
632 Influence of shellfish farming activities on the biogeochemical composition of the
633 water column in Thau lagoon. Mar Ecol Prog Ser 218: 141-152.
634 Strathmann R (1967) Estimating the organic carbon content of phytoplankton from cell
635 volume or plasma volume. Limnol Oceanogr 12: 411–418.
636 Tada K, Pithakpol S, Yano R, Montani S (2000) Carbon and nitrogen content of
637 *Noctiluca scintillans* in the Seto Inland Sea, Japan. J Plankton Res 22 (6): 1203-
638 1211.
639 Tenore KR (1982) Coastal upwelling in the Rías Bajas, NW Spain: Contrasting the
640 benthic regimes of the Rias de Arosa and de Muros. J Mar Res 40: 701-772.
641 Tenore KR, González N (1975) Food chain patterns in the Ría de Arosa, Spain: an area
642 of intense mussel aquaculture. 10th Euro Symp Mar Biol 2: 601-619.
643 Tilstone GH, Figueiras FG, Fermín EG, Arbones B (1999) Significance of
644 nanophytoplankton photosynthesis and primary production in a coastal upwelling
645 system (Ría de Vigo, NW Spain). Mar Ecol Prog Ser 183: 13-27.

646 Verity PG, Robertson CY, Tronzo CR, Andrews MG, Nelson JR, Sieracki ME (1992)
647 Relationships between cell volume and the carbon and nitrogen content of marine
648 photosynthetic nanoplankton. Limnol Oceanogr 37 (7): 1434–1446.

649

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

652

653

654

655

656

657

658

659

660

661

662

663

664

665

666

667

668

669

670

671

672

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

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

677

	Significance level of t-test at $\alpha = 0.05$	Mean ± SD at reference site	Mean ± SD at raft site	Deficit or excess comparing raft and reference site (Mean ± SD in %)
	(n=24)	(n=24)	(n=24)	(n=24)
NO_3^-, mmol m^{-2}	0.879	48 ± 39	46 ± 35	NS
NO_2^-, mmol m^{-2}	0.562	3.4 ± 2.5	3.8 ± 2.6	NS
NH_4^+, mmol m^{-2}	0.007	20 ± 14	33 ± 18	40 ± 31
DON, mmol m^{-2}	0.270	84 ± 20	87 ± 22	NS
DOC, mmol m^{-2}	0.239	1032 ± 80	1064 ± 81	NS
DO, mmol m^{-2}	0.049	3333 ± 309	3140 ± 350	-5 ± 7
POC, mg m^{-2}	0.064	2803 ± 1068	2229 ± 464	NS
Chl *a*, mg m^{-2}	0.023	37 ± 23	23 ± 16	-33 ± 26
PC, mg m^{-2}	0.015	2017 ± 750	1298 ± 561	-34 ±28

678

679

680

681

682

683

684

685

686

687

688 Table 3. Mean ± standard deviation of ammonium excretion rate (VNH_4^+-N), oxygen
689 uptake (VO_2), organic ingestion rate (OIR) and absorption efficiency (AE) for each
690 sampling season. Homogeneous groups identified by Tukey´s HSD test ($\alpha = 0.05$) are
691 given in brackets (a,b).

692

693

	VNH_4^+-N ($\mu g\ h^{-1}$)	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

694

695

696

697

698

699

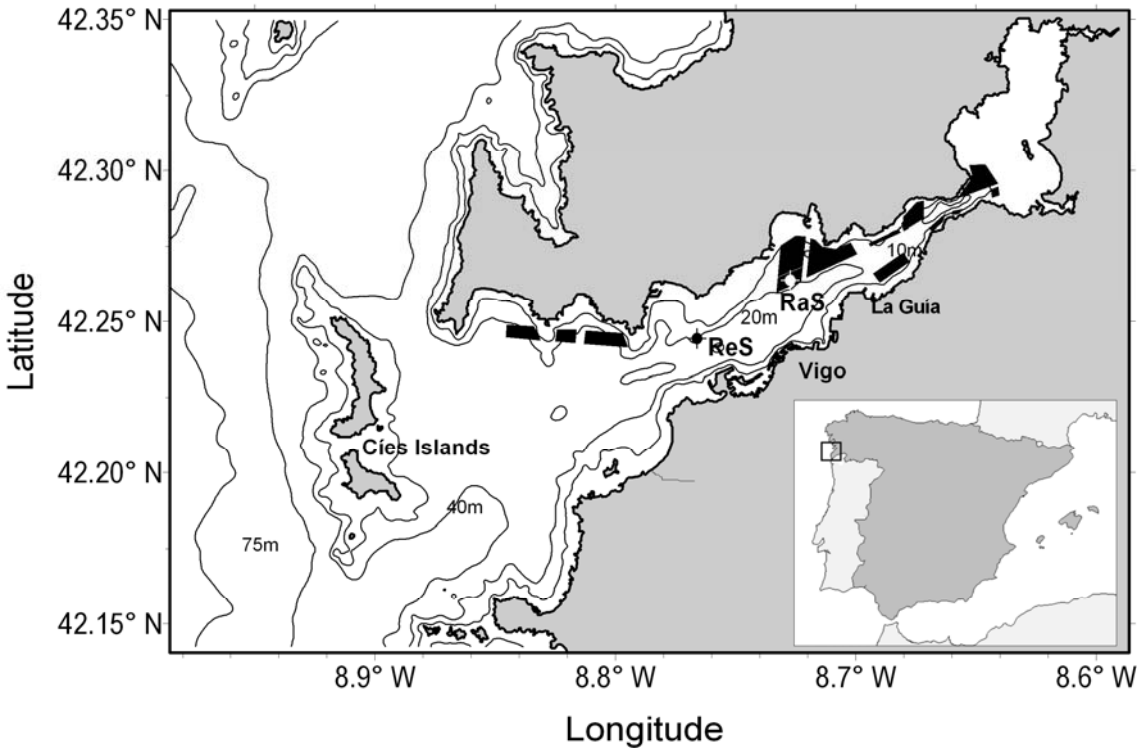
700

701 Figure 1. Map of the Ría de Vigo showing the location of the raft polygons (in black)
702 and the positions of both reference (ReS) (black dot) and raft (RaS) (white dot)
703 sampling sites. The position of RaS station inside the polygon is also shown in the
704 figure.
705 Figure 2. Time-series of upwelling index, temperature, nitrate (NO_3^-), ammonium
706 (NH_4^+) and chlorophyll *a* (Chl *a*) concentration registered at the reference station (ReS).
707 The days when physiological experiments with mussels were carried out are shown as
708 vertical dashed lines.
709 Figure 3. Time-series of temperature, nitrate (NO_3^-), ammonium (NH_4^+) and chlorophyll
710 *a* (Chl *a*) concentration recorded at the raft station (RaS). The days were physiological
711 experiments with mussels were carried out are shown as vertical dashed lines.
712 Figure 4. (a) Mean seasonal variations of 12 m water column integrated values of nitrate
713 (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+) and dissolved organic nitrogen (DON) at both
714 the reference (white bars) and raft (black bars) stations. (b) Mean seasonal variations of
715 12 m water column integrated values of dissolved oxygen (DO), chlorophyll *a* (Chl *a*),
716 particulate organic carbon (POC) and microbial plankton carbon (PC) at both the
717 reference (white bars) and raft (black bars) stations.
718 Figure 5. Box-plots showing the seasonal variation of metabolic (ammonium excretion
719 rate (VNH_4^+-N) and oxygen uptake (VO_2)) and physiological (organic ingestion rate
720 (OIR) and absorption efficiency (AE)) rates. The box itself goes from the lower quartile
721 (Q1) to the upper quartile (Q3). The horizontal bar inside the box is the median. The
722 whiskers above and below the box are drawn at the first value in the range [Q1 - (1.5 *
723 IQR), Q3 + (1.5 * IQR)]. Data points which exceed this range are drawn separately as
724 outliers.

725 Figure 6. Dispersion diagram between ammonium content in the water column at the
726 raft station ($NH_4^+{}_{WC}$) and the direct ammonium excretion rate (VNH_4^+-N) obtained from
727 the physiological experiments with mussels.
728 Figure 7. Dispersion diagrams showing the relationship between both organic ingestion
729 rate (OIR) and absorption efficiency (AE) with the food quality variables (given as
730 particulate organic carbon (POC), chlorophyll *a* (Chl *a*) and microbial plankton carbon
731 (PC)) observed in the raft. The adjustment of the data was given by the hyperbolic
732 equation y = ax / (b + x). The coeficients a and b are shown in the figure for each
733 adjustment.
734
735

736 Figure 1

737

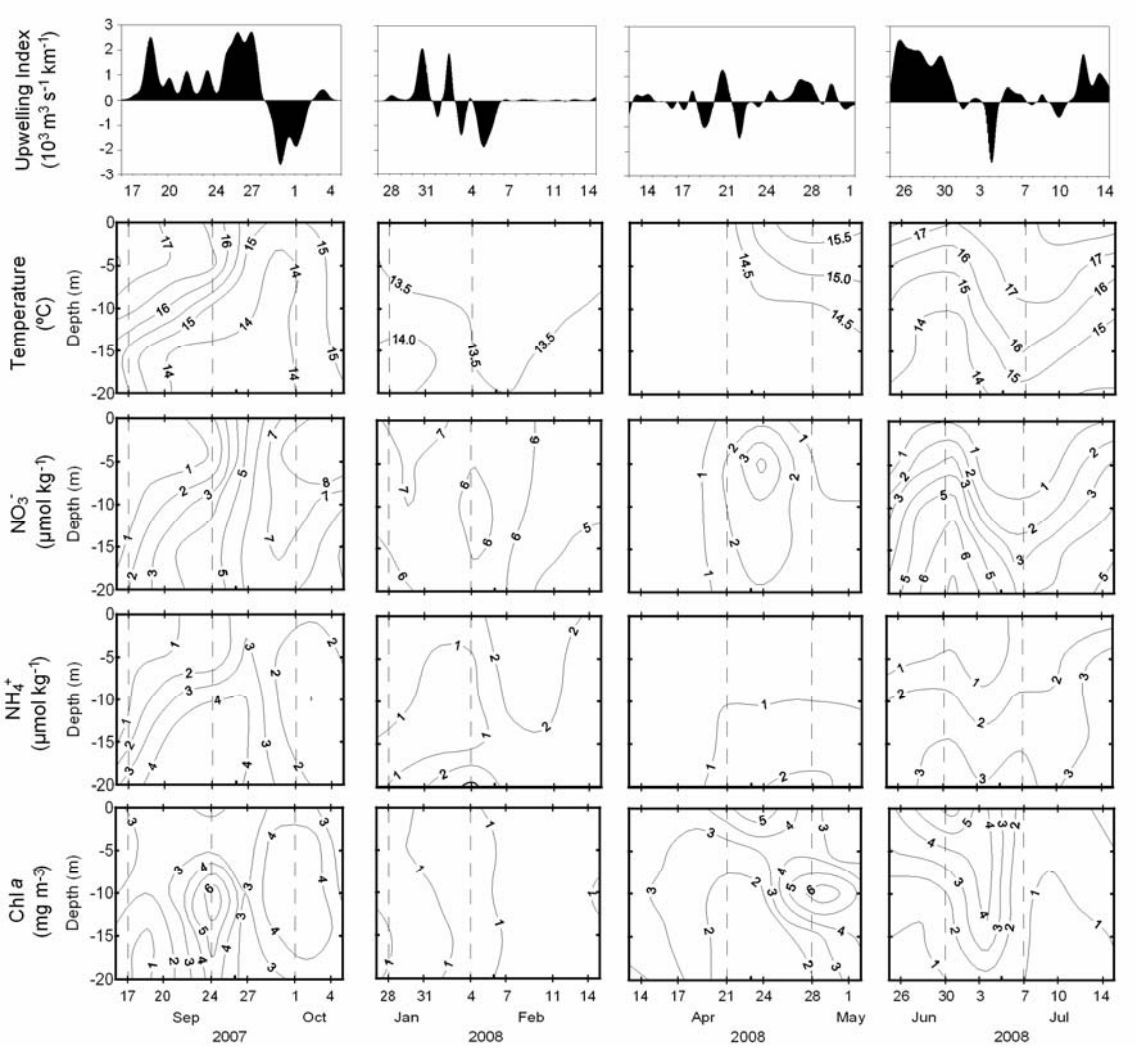


738

739

740 Figure 2

741

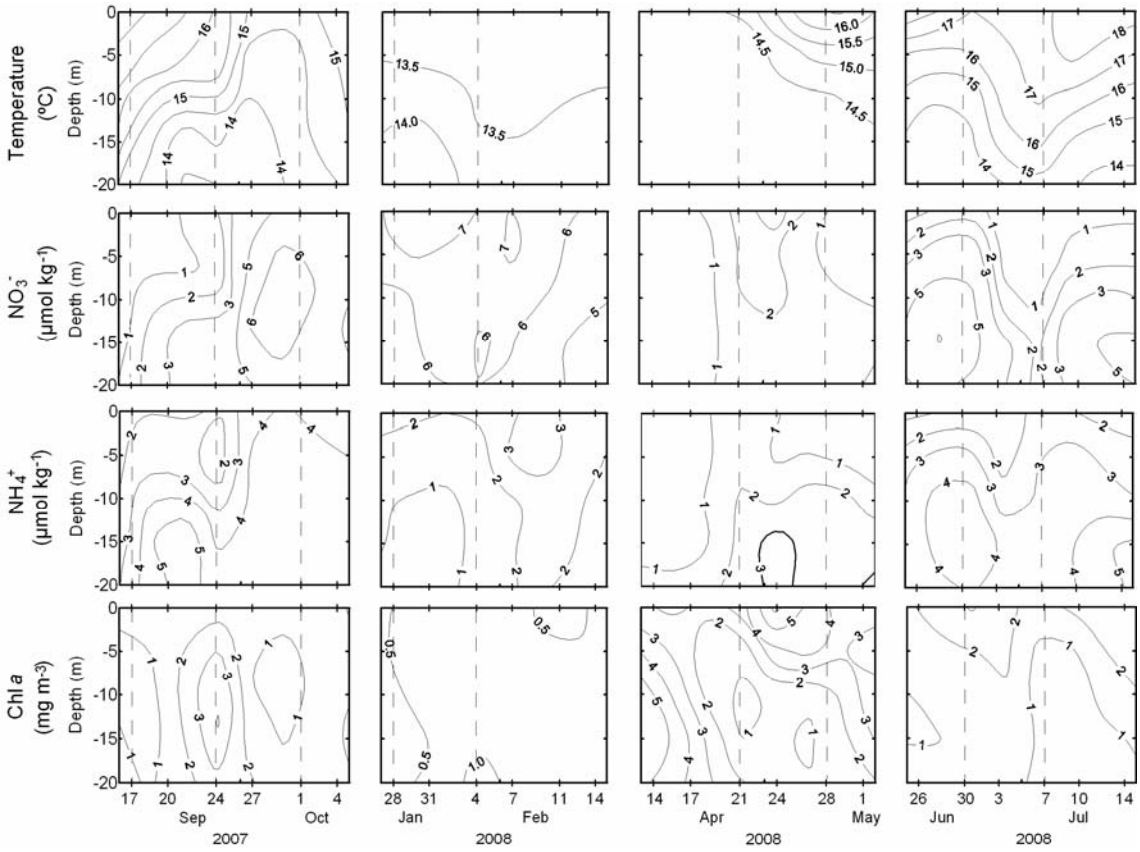


742

743

744 Figure 3

745

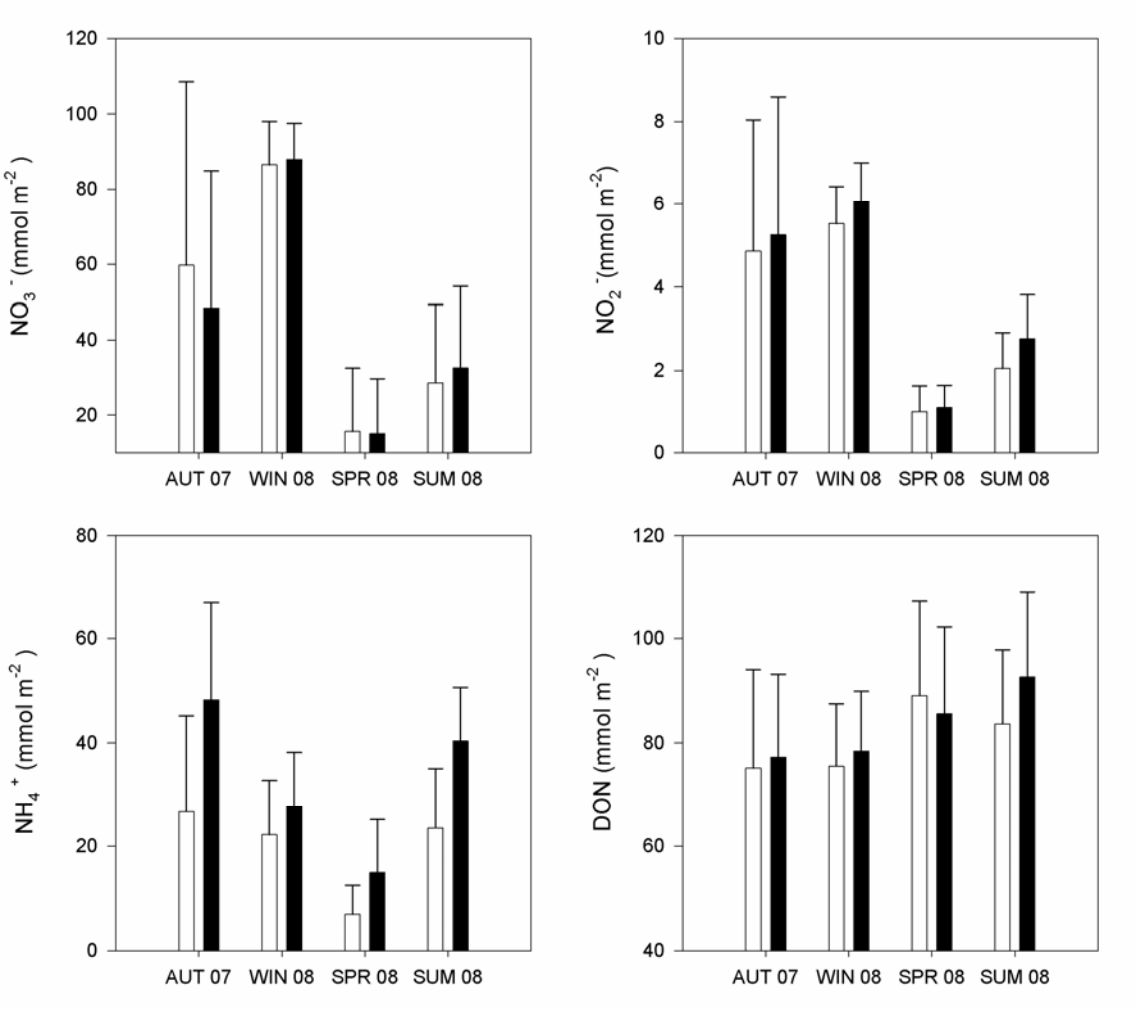


746

747

748 Figure 4a

749

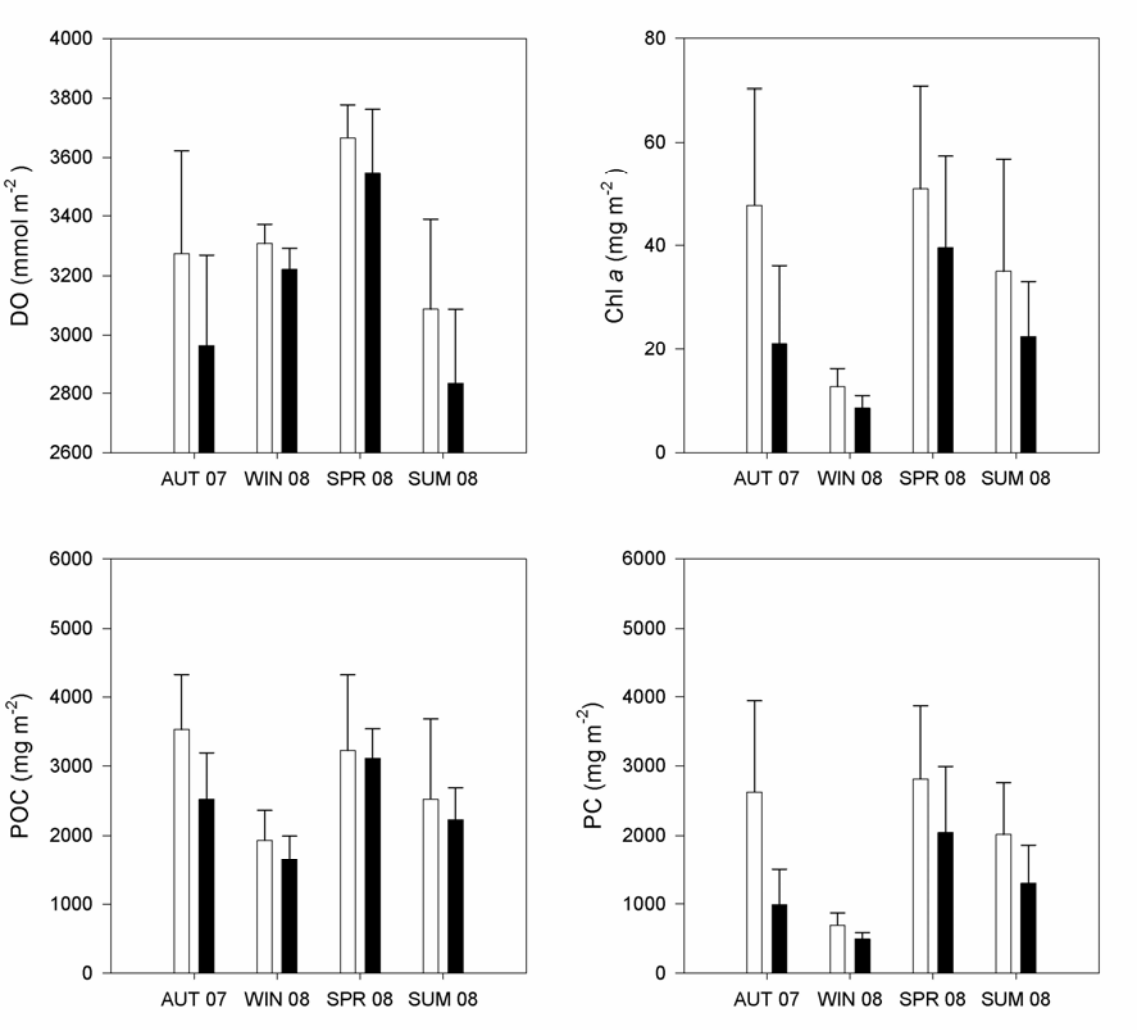


750

751

752 Figure 4b

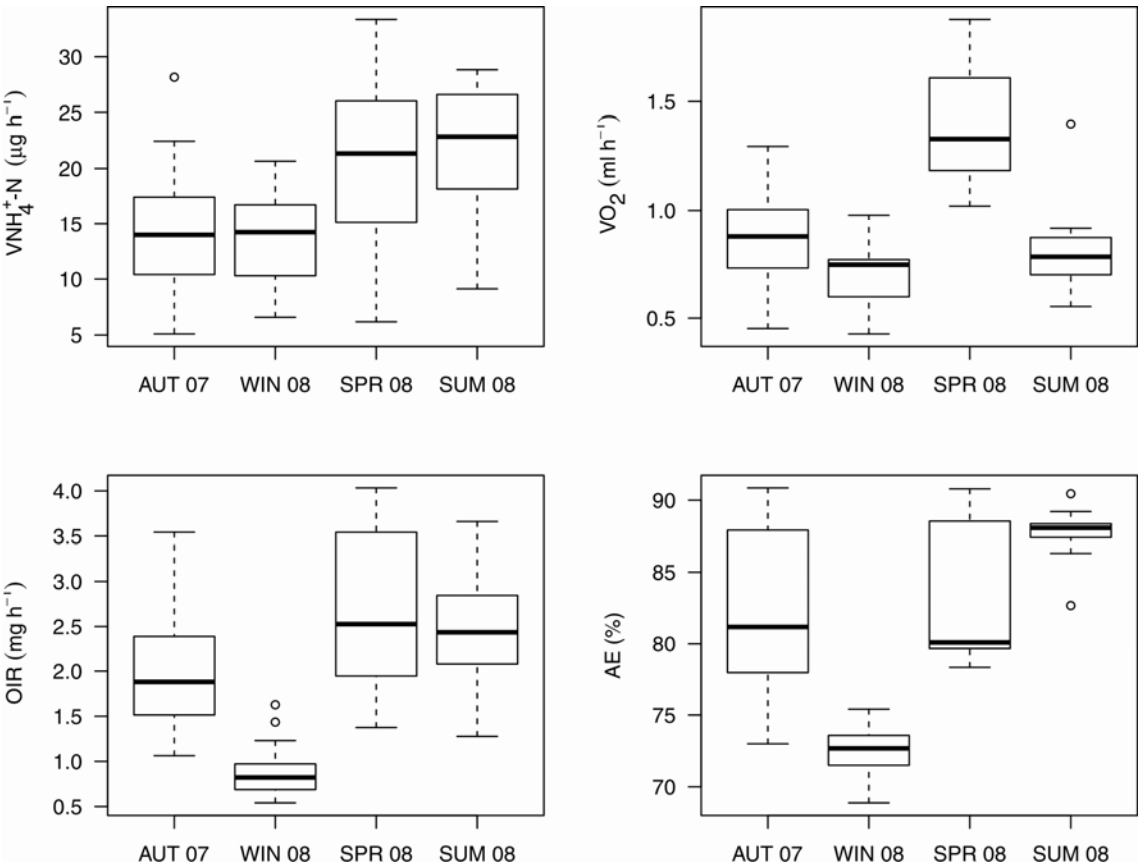
753



754

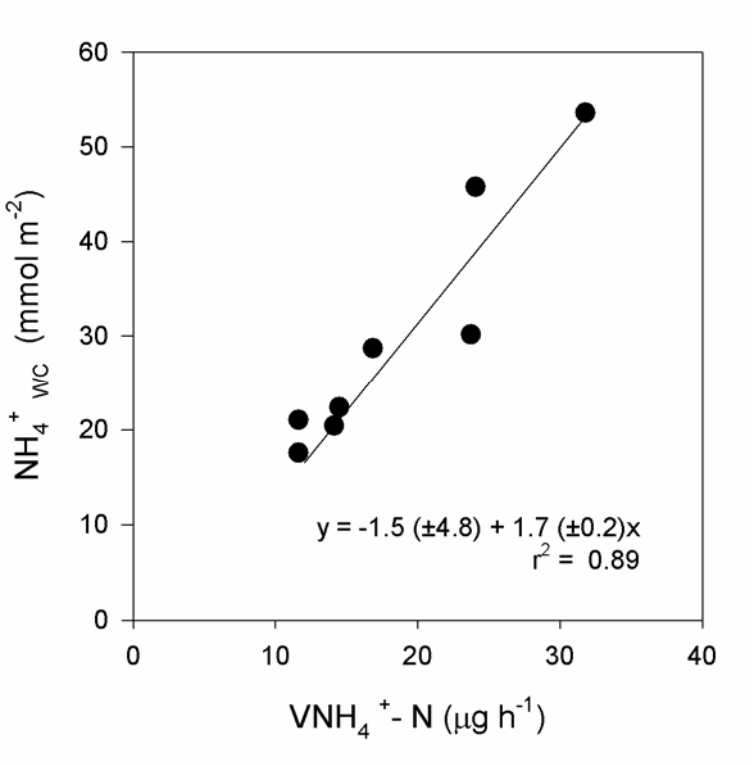
755

Figure 5


VNH_4^+-N (µg h^{-1})
30
25
20
15
10
5
AUT 07
WIN 08
SPR 08
SUM 08
VO_2 (ml h^{-1})
1.5
1.0
0.5
AUT 07
WIN 08
SPR 08
SUM 08
OIR (mg h^{-1})
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
AUT 07
WIN 08
SPR 08
SUM 08
AE (%)
90
85
80
75
70
AUT 07
WIN 08
SPR 08
SUM 08

760 Figure 6

761

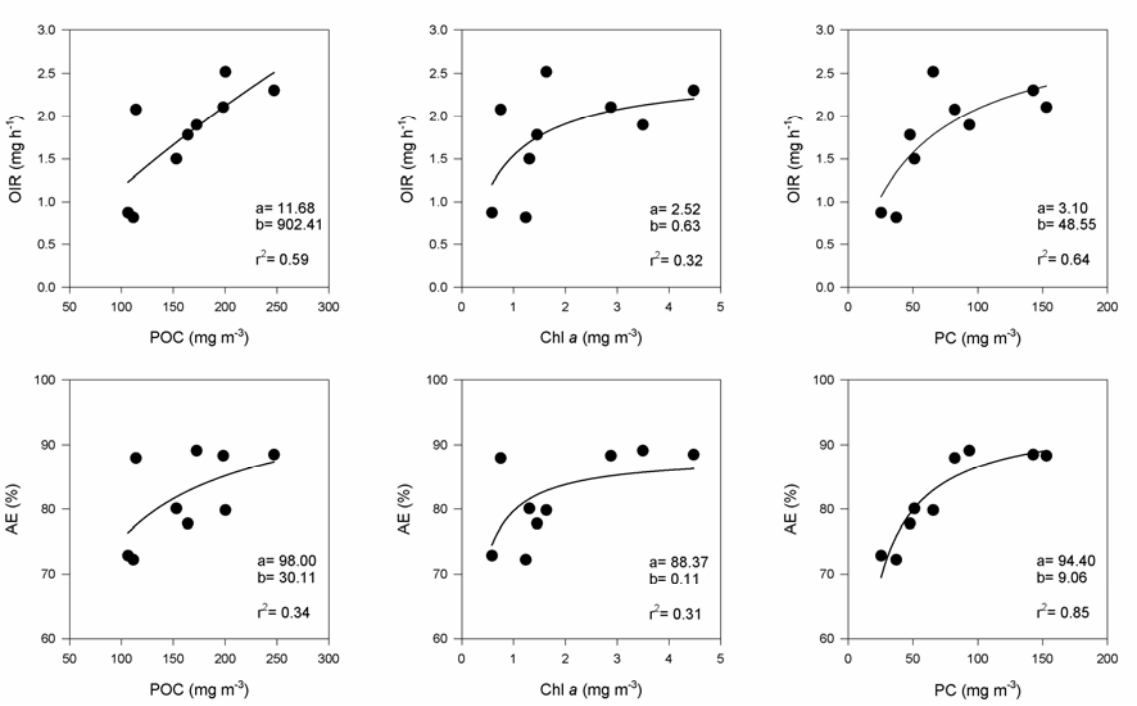


762

763

764 Figure 7

765



766

767