Mussel farming impact on pelagic production and respiration rates in a coastal upwelling embayment (Ría de Vigo, NW Spain)

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Abstract: This paper provides the first diagnosis of the impact of mussel farming on the primary production (PP) and the metabolic balance in a coastal upwelling region (Ría de Vigo). Measurements of size-fractionated PP and microbial plankton metabolism were performed outside (reference station; ReS) and inside the farming area (raft station; RaS). At ReS, integrated PP was higher during upwelling ($1.05 \pm 0.45 \text{ g C m}^{-2} \text{ d}^{-1}$) with microphytoplankton dominating carbon fixation ($74 \pm 14\%$). The significance of nanophytoplankton and picophytoplankton increased during winter linked to lower PP ($0.24 \pm 0.03 \text{ g C m}^{-2} \text{ d}^{-1}$). Water column at ReS was always autotrophic with net community production (NCP) ranging from $186 \pm 67 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ during upwelling to $43 \pm 22 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ in winter. At RaS, there was a decrease in PP attributable not only to mussel consumption but also to the lower irradiance under mussel rafts. Concomitant decrease in NCP was also observed (by 56%), yet remained autotrophic, supporting the view that under current conditions food does not limit mussel growth in the Ría, thus securing the carrying capacity of the system in terms of production.

Keywords: Mussel impact, Chlorophyll *a*, Primary production, Microbial metabolism, NW Spain.

1. Introduction

World aquaculture is one of the most important food production sources, accounting for 44.1% of total fishery production in 2014 and it is expected to share around 52% in 2025 based on FAO projections (FAO 2016). Of the 73.8 million tonnes of total aquaculture production in 2014, around 21% corresponded to mollusc. The culture of this main species group has experienced a rapid development in the last two decades, becoming a crucial economic activity for the coastal communities where it is established. Although mollusc production has the benefit of being a non-fed aquaculture, there is a raising concern of reducing its environmental impact in order to achieve a sustainable development and management of this industry (Hishamunda *et al.*, 2014). The Rías Baixas of Galicia, 4 coastal inlets located on the NW Spain (Figure 1) with high density of mussel floating rafts, are an outstanding example of such coastal areas where mussel culture plays an important socioeconomic role (Figueiras *et al.*, 2002).

Fig. 1. Ría de Vigo with the two sampling sites (◆): Reference (ReS) and raft (RaS) stations. Locations of the raft polygons are shown as dark areas. Inset: The Rías Baixas on the NW Iberian Peninsula.

The culture of *Mytilus galloprovincialis* gives employment to 9.000 people directly and to 20.000 indirectly in the region, rendering a total mussel production of approximately 250×10^6 kg y⁻¹ (Labarta, 2000; Monfort, 2014). In fact, the Rías Baixas is the region with the highest mussel production in Europe, which is promoted by sheltering conditions and coastal upwelling that, through nutrient supply, stimulates phytoplankton growth and so provides food of high quality to mussels (Blanton *et al.*, 1987; Pérez-Camacho *et al.*, 1995; Figueiras *et al.*, 2002; Zúñiga *et al.*, 2013).

Microbial plankton abundance and composition in the Rías Baixas show strong dependency on seasonal upwelling dynamics. In this region, north winds along the coast force an Ekman transport towards the open sea, resulting in the upwelling of cold, nutrient-rich water into the euphotic zone which enhances primary production. From spring to autumn, upwelling events induce the dominance of large (> 20 µm) plankton (Figueiras *et al.*, 2002; Froján *et al.*, 2014). Large diatoms prevail during the first half of the upwelling season, while heterotrophic microplankton (dinoflagellates and ciliates) acquire higher importance during the second half, as a consequence of plankton succession in temperate seas (Figueiras and Ríos, 1993) that leads to the establishment of complex trophic relationships within the microbial community (Teixeira *et al.*, 2011). In contrast, small (< 20 µm) plankton dominates during the downwelling season (winter) as a result of the reduction in the abundance of large plankton rather than an increase in the abundance of small forms (Espinoza-González *et al.*, 2012; Froján *et al.*, 2014). These two contrasting situations are reflected on the degree of autotrophy-heterotrophy of the microbial plankton community (Cermeño *et al.*, 2006; Arbones *et al.*, 2008). Autotrophy predominates with increased abundance of large phytoplankton (mainly diatoms) during upwelling season; however, during the winter downwelling season, when large phytoplankton is scarce, the microbial plankton community is in metabolic balance or tends to be slightly heterotrophic.

The excess of photosynthesized organic matter produced during the upwelling season (spring to autumn) can support mussel growth in the Rías Baixas (Babarro *et al.*, 2000; Figueiras *et al.*, 2002). Studies conducted in the Ría de Vigo have shown strong dependence of mussel

physiology on microbial plankton. Organic ingestion rates and particularly absorption efficiencies were positively related to microbial plankton carbon, with mussel feeding rates achieving maximum values during the upwelling season (Zúñiga *et al.*, 2013). Also, there is evidence that mussel farming exerts a top-down control over the microbial plankton community of the Ría de Vigo, modifying its structure by consuming micro- and nanoplankton without affecting picoplankton (Froján *et al.*, 2014). In this regard, given the strong connection between the size distribution of primary producers and the trophic status of the pelagic ecosystem (Smith and Kemp, 2001; Cermeño *et al.*, 2006; Arbones *et al.*, 2008), mussel consumption could also have a strong impact on the metabolic balance of the system.

In this context, the objective of the present study is to investigate the impact of mussel farming on the ecosystem functioning by considering the ensuing effects on the metabolic balance of the water column, on size-fractioned carbon fixation as well as on size-fractioned chlorophyll *a*. Results from this study will provide relevant information for ecosystem models intended to assess the carrying capacity of densely cultured areas.

2. Materials and methods

2.1. Sampling strategy

A total of 24 oceanographic cruises were carried out between 2007 and 2008, covering the four seasonal periods characteristic of the region: autumn (September 17 to October 4), winter (January 28 to February 14), spring (April 14 to May 1) and summer (June 26 to July 14). During these sampling periods, water column observations were carried out on board R/V ‘Mytilus’ at two stations in the Ría de Vigo (Figure 1): a reference station (ReS) and a raft station (RaS). The ReS, located in the central channel of the Ría (42 m maximum depth) far from the mussel farming area, has been widely recognized as a reference site for studies regarding short-term and seasonal variability in the Ría de Vigo (e.g. Nogueira *et al.*, 2000; Nogueira and Figueiras, 2005; Crespo *et al.*, 2006). The RaS was situated inside the mussel farming area, within a raft polygon (group of rafts). Mussel rafts have an area of 500 m² which

are 100 m apart and from its wooden structure hang a maximum of 500 ropes 12 m long with mussels attached. Both sampling stations were visited on board the R/V 'Mytilus' every 2 to 3 days during each sampling period to collect water samples at 5 depths (surface, 5, 10, 15 and 20 m). Sampling took place with an oceanographic rosette equipped with 12 Niskin bottles to which a CTD SBE 911 (Sea-Bird) was attached. Subsamples were taken to determine nitrate and size-fractionated chlorophyll *a* (chl *a*) concentrations and to determine size-fractionated primary production and the metabolism of the microbial plankton community.

2.2. Nitrate and size-fractionated chl *a*

Nitrate concentrations ($\mu\text{mol kg}^{-1}$) were determined by segmented flow analysis with Alpkem autoanalysers (Hansen and Grasshoff, 1983). Chl *a* samples were size fractionated by sequential filtration of 250 ml of seawater through polycarbonate filters of 20 μm (microphytoplankton), 2 μm (nanophytoplankton) and 0.2 μm (picophytoplankton) pore size. The filters were frozen (-20 °C) until pigments were extracted in 90 % acetone over 24 h in the dark at 4 °C. Chl *a* concentrations (mg m^{-3}) were estimated by measuring fluorescence of the pigment extracts in a Turner Designs fluorometer calibrated with pure chl *a* (Sigma).

2.3. Size-fractionated primary production

Size-fractionated primary production was measured by ^{14}C uptake in 24 h *in situ* incubations at 4 depths in the photic layer (surface, 5, 10 and 15 m) with 3 light flasks and 2 dark flasks inoculated with 5.5×10^5 Bp (15 μCi) of $\text{NaH}^{14}\text{CO}_3$. Following incubation, samples were size fractionated by sequential filtration through polycarbonate filters of 20, 2, 0.2 μm pore size and the filters were immediately exposed to HCl fumes for 12 h to eliminate unincorporated inorganic ^{14}C . The incorporated radioactivity (dpm) was estimated in a liquid scintillator counter using the external standard and the channel ratio methods to correct for quenching.

2.4. Microbial plankton metabolism

Metabolism of the microbial plankton community was determined through oxygen production and consumption in 24 h *in situ* light-dark bottle incubations. At each of the 4 photic depths (surface, 5, 10 and 15 m) 4 light and 4 dark Winkler bottles were clamped in a specially constructed plexiglas holder. Only 4 dark samples were incubated at the aphotic depth of 20 m. For initial dissolved oxygen concentrations at each depth, 3 additional subsamples were taken and immediately fixed. Dissolved oxygen concentration in all bottles was determined with an automatic Winkler titration system. Net community production (NCP) is the increase of oxygen concentration in the light bottles in relation to the initial concentration; community respiration (CR) is obtained from the decrease in oxygen concentration in the dark bottles in relation to the initial concentration; gross primary production (GPP) is estimated by the addition of the oxygen consumed during respiration (CR) to NCP; $GPP = NCP + CR$.

2.5. Ekman transport

Ekman transport ($-Q_x$, $m^3 s^{-1} km^{-1}$), an estimate of the volume of water upwelled per kilometre of coast, was calculated following Bakun, (1973):

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

where ρ_a is the air density ($1.22 kg m^{-3}$) at $15^\circ C$, C is an empirical drag coefficient (1.3×10^{-3} , dimensionless), $|V|$ is the daily mean value of the module ($m s^{-1}$) of northerly winds (V_y) registered by the Silheiro buoy deployed on the shelf in front of the Ría de Vigo by 'Puertos del Estado', f is the Coriolis parameter ($9.95 \times 10^{-5} s^{-1}$) at $43^\circ N$ and ρ_{sw} is the density of seawater ($1025 kg m^{-3}$). Positive values of $-Q_x$ indicate the predominance of northerly winds responsible for upwelling within the Ría, while negative values are related to downwelling induced by southerly winds.

2.6. Presentation of the results and statistical analysis

Total and size-fractionated chl *a* concentration, total and size-fractionated primary production and rates of oxygen production and respiration are shown integrated over a 12 m water column, the length of the ropes holding mussels in the rafts. Although values integrated

over the entire water column were higher than the values integrated over the upper 12 m, the correlation between both values was always strong for all variables ($r^2 > 0.82$; $p < 0.001$), indicating that the pattern of variability for all variables did not change below 12 m depth.

A non-parametric analysis of variance (Kruskal-Wallis test) was utilised to test for significant effects of season (autumn, winter, spring and summer) and mussels (location: ReS vs. RaS) on chl *a*, primary production and metabolism of the microbial plankton community. Pairwise comparisons were made with the Dunn-Bonferroni post-hoc test. Statistical analysis was performed using SPSS software.

3. Results

3.1. Hydrographic scenario

Briefly, as baseline information, the hydrographic changes during this study reflect the seasonal variability usually found in the region (Figure 2). During the autumn sampling, the structure of the water column evolved from an upwelling of cold and nutrient rich subsurface water ($< 14\text{ }^{\circ}\text{C}$) to downwelling conditions. During this downwelling relatively high levels of nitrate ($> 6\text{ }\mu\text{mol kg}^{-1}$) were recorded in the water column due to river runoff (data not shown). During winter, winds were weak, especially for the second half of the sampling. At this time, nitrate levels were high ($> 5\text{ }\mu\text{mol kg}^{-1}$) in a homogenous water column. In spring, the water column progressed from well-mixed to stratified conditions and the nitrate levels were appreciably lower than in winter. Typical upwelling conditions occurred for the summer sampling. Upwelling favourable winds caused the advection of cold ($< 14\text{ }^{\circ}\text{C}$) and nutrient-rich subsurface water ($> 5\text{ }\mu\text{mol kg}^{-1}$) that turned into a thermally stratified water column with low nutrient levels at the sea surface as winds relaxed. Detailed environmental variability of this sampling can be found in Froján *et al.*, (2014), who showed that the thermohaline properties and nitrate concentrations measured at RaS were not significantly different to those recorded at ReS (Kruskal-Wallis test, $n = 240$, $p \geq 0.57$).

Fig. 2. (a) Upwelling index ($\times 10^3 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-1}$), (b) temperature ($^{\circ}\text{C}$), and (c) nitrate concentration ($\mu\text{mol kg}^{-1}$) at the reference station (ReS); (d) temperature ($^{\circ}\text{C}$), and (e) nitrate concentration ($\mu\text{mol kg}^{-1}$) at the raft station (RaS) during the 4 sampling periods.

3.2. Total and size-fractionated chl *a*

Total integrated chl *a* at ReS showed considerable temporal variability (Figure 3a). Mean values were high in autumn and spring (Table 1, S1) but were significantly lower ($p \leq 0.05$) in winter. Average concentration was also high in summer though differences with winter values were not significant. The $> 20 \mu\text{m}$ size-fraction was responsible for the variability observed in total chl *a*. Thus, this size-class showed significant differences ($p \leq 0.05$) between the values recorded in winter and those measured in spring and autumn (Table S1). The mean chl *a*

concentration in the $> 20 \mu\text{m}$ fraction was high in autumn, summer and namely in spring (Table 1), when this fraction accounted for $77 \pm 10 \%$ of the total chl *a*. The contribution of the $> 20 \mu\text{m}$ size-class to total chl *a* was appreciably lower in winter, albeit this was also the size-fraction with the highest mean concentration in this period (Table 1). The picophytoplankton size-class ($< 2 \mu\text{m}$) exhibited the lowest chl *a* concentration ($5 \pm 2 \text{ mg chl } a \text{ m}^{-2}$) during the 4 periods (Figure 3a), but accounted for a substantial portion ($26 \pm 3\%$) of the total chl *a* in winter (Table 1). Chl *a* values in the fraction corresponding to nanophytoplankton ($2\text{-}20 \mu\text{m}$) were between these two extremes, with an annual average concentration of $10 \pm 5 \text{ mg m}^{-2}$, representing $26 \pm 12 \%$ of total chl *a*. However, differences among periods for the integrated chl *a* concentration in both pico- and nanophytoplankton fractions were not significant.

Fig. 3. Size-fractionated chlorophyll *a* (chl *a*) concentration integrated in the upper 12 m of the water column during each sampling period at (a) reference (ReS) and (b) raft (RaS) stations.

Integrated values of total chl *a* were lower at RaS than at ReS (Figure 3) and the decrease was particularly important in autumn ($p < 0.05$) with a reduction of $50 \pm 25 \%$ in total chl *a* (Table 1). This reduction primarily occurred in the $> 20 \mu\text{m}$ fraction ($p < 0.05$), which showed

250 **Table 1.** Average ($\pm$ SD) (mg m^{-2}) values of total chlorophyll *a* (chl *a*), picophytoplankton ($< 2\mu\text{m}$), nanophytoplankton ($2\text{-}20\mu\text{m}$) and microphytoplankton ($> 20\mu\text{m}$)
251 integrated in the upper 12 m of the water column during each sampling period ($n = 6$) and for all periods ($n = 24$) at reference (ReS) and raft (RaS) stations. Shaded values
252 show the average ($\pm$ SD) relative contributions (%) of picophytoplankton ($< 2\mu\text{m}$), nanophytoplankton ($2\text{-}20\mu\text{m}$) and microphytoplankton ($> 20\mu\text{m}$) to total integrated chl *a*.
253 Bold *p*-values denote significant differences between ReS and RaS (Kruskal-Wallis test), and finally the percentage of reduction between ReS and RaS. Average values
254 sharing the same superscript are not significantly different from each other. n.s denotes non-significant.
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		mg m^{-2}		% (total chl <i>a</i>)		p-value	% reduction
		ReS	RaS	ReS	RaS		
Total chl <i>a</i>	Autumn	61 ± 27^a	28 ± 19			0.025	50 ± 25
	Winter	17 ± 5^b	11 ± 3			0.05	31 ± 13
	Spring	66 ± 30^a	54 ± 19			0.873	n.s
	Summer	43 ± 26^{ab}	31 ± 15			0.749	n.s
$> 20 \mu\text{m}$	Autumn	43 ± 25^a	19 ± 16	69 ± 14	61 ± 15	0.037	47 ± 40
	Winter	7 ± 3^b	3 ± 1	39 ± 7	29 ± 6	0.016	46 ± 16
	Spring	52 ± 27^a	44 ± 19	77 ± 10	80 ± 11	0.873	n.s
	Summer	28 ± 21^{ab}	15 ± 12	59 ± 20	46 ± 23	0.262	n.s
2 - 20 μm	Autumn	12 ± 4^a	6 ± 2	21 ± 10	26 ± 8	0.016	39 ± 30
	Winter	6 ± 1^a	4 ± 1	36 ± 4	36 ± 2	0.037	27 ± 17
	Spring	10 ± 6^a	7 ± 5	16 ± 8	14 ± 8	0.337	n.s
	Summer	11 ± 5^a	11 ± 9	31 ± 14	38 ± 18	0.522	n.s
$< 2 \mu\text{m}$	Autumn	6 ± 3^a	3 ± 2	10 ± 6	13 ± 9	0.150	n.s
	Winter	4 ± 1^a	4 ± 1	26 ± 3	35 ± 4	0.575	n.s
	Spring	5 ± 2^a	3 ± 1	8 ± 4	6 ± 4	0.055	n.s
	Summer	3 ± 1^a	4 ± 2	10 ± 7	16 ± 10	0.522	n.s

in autumn a concentration at RaS less than a half of the concentration recorded at ReS (Table 1); Even so, the $> 20 \mu\text{m}$ size-class still accounted for the largest amount of total chl *a* at RaS during autumn, spring and summer (Figure 3b, Table 1). However, the microphytoplankton fraction ($> 20 \mu\text{m}$) showed the lowest chl *a* concentration in winter (Figure 3b, Table 1) representing a mean decrease of $46 \pm 16 \%$ of chl *a* concentration in this fraction at ReS. Conversely, the $< 2 \mu\text{m}$ size-class ($35 \pm 4 \%$ of the total chl *a*) and the $2 - 20 \mu\text{m}$ size-class ($36 \pm 2 \%$ of the total chl *a*) attained more importance during this period (Table 1), leading to a change in the size-structure of chl *a* at RaS (Figure 3). Also noted that chl *a* concentrations in the picophytoplankton fraction ($< 2 \mu\text{m}$) at RaS and ReS were not significantly different for the 4 sampling periods ($0.06 \geq p \geq 0.58$).

3.3. Total and size-fractioned primary production

Total primary production at ReS (Figure 4a) displayed continuous fluctuations during autumn, spring and summer, in close relation to the variability observed in hydrographic conditions (Figure 2). The best example of this extreme variability was found in summer, when the maximum value of primary production recorded on July 14 ($2.0 \text{ g C m}^{-2} \text{ d}^{-1}$) occurred only 7 days after measuring the minimum value ($0.17 \text{ g C m}^{-2} \text{ d}^{-1}$). Despite this variability, average integrated primary production during these 3 periods (autumn, spring and summer) was relatively high ($1.05 \pm 0.45 \text{ g C m}^{-2} \text{ d}^{-1}$). In contrast, primary production in winter ($0.24 \pm 0.04 \text{ g C m}^{-2} \text{ d}^{-1}$) showed less variability (Figure 4a, Table 2) and was significantly lower ($p \leq 0.05$) than in the other three periods (Table S2).

Size-fractionated primary production at ReS (Figure 4a) followed a similar trend to that previously described for chl *a* (Figure 3a). Primary production in the $> 20 \mu\text{m}$ fraction, which was significantly lower ($p \leq 0.05$) in winter than in the other 3 sampling periods (Table S2), was responsible for the variability exhibited by total primary production (Figure 4a). This was also the size-class with the highest contributions to total primary production during the 4 periods, mainly in spring when accounted for $85 \pm 4 \%$ of total primary production (Table 2). Primary

production of pico- ($< 2 \mu\text{m}$) and nanophytoplankton ($2\text{-}20 \mu\text{m}$) and their contribution to total carbon fixation was noticeably lower in all sampling periods (Figure 4a, Table 2).

Fig. 4. Size-fractionated primary production (PP) integrated in the upper 12 m of the water column during each sampling period at (a) reference (ReS) and (b) raft (RaS) stations.

Total primary production at RaS was $63 \pm 14 \%$ lower ($p < 0.001$) than at ReS (Figure 4) and this decrease occurred in all three size fractions (Table 2). Nevertheless, phytoplankton $> 20 \mu\text{m}$ remained as the size-class with highest contributions in autumn, spring and summer (Figure 4b, Table 2). In contrast, the 3 size-classes showed similar contributions ($\sim 33\%$) to total primary production during winter (Figure 4b, Table 2).

3.4. Metabolism of the microbial plankton community

Variability in gross primary production (GPP) determined at ReS was large (Figure 5a), ranging from $18 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ measured in summer to $292 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ recorded in autumn when the highest average GPP was registered (Table 3). Mean GPP significantly

305 **Table 2.** Average ($\pm$ SD) ($\text{mg C m}^{-2} \text{d}^{-1}$) values of total primary production (PP), picophytoplankton ($< 2\mu\text{m}$), nanophytoplankton ($2\text{-}20\mu\text{m}$) and microphytoplankton ($>$
 306 $20\mu\text{m}$) integrated in the upper 12 m of the water column during each sampling period ($n = 6$) and for all periods ($n = 24$) at reference (ReS) and raft (RaS) stations. Shaded
 307 values show the average ($\pm$ SD) relative contributions (%) of picophytoplankton ($< 2\mu\text{m}$), nanophytoplankton ($2\text{-}20\mu\text{m}$) and microphytoplankton ($> 20\mu\text{m}$) to total integrated
 308 PP. Bold p -values denote significant differences between ReS and RaS (Kruskal-Wallis test), and finally the percentage of reduction between ReS and RaS. Average values
 309 sharing the same superscript are not significantly different from each other. n.s denotes non-significant.
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		$\text{g Cm}^{-2} \text{d}^{-1}$		% (total PP)		p-value	% reduction
		ReS	RaS	ReS	RaS		
Total PP	Autumn	1.13 ± 0.37^a	0.33 ± 0.18			0.004	70 ± 15
	Winter	0.24 ± 0.04^b	0.08 ± 0.02			0.004	67 ± 5
	Spring	1.04 ± 0.23^a	0.47 ± 0.28			0.010	56 ± 22
	Summer	0.98 ± 0.70^a	0.42 ± 0.30			0.109	n.s
$>20 \mu\text{m}$	Autumn	0.81 ± 0.42^a	0.24 ± 0.16	70 ± 21	68 ± 16	0.010	57 ± 51
	Winter	0.12 ± 0.02^b	0.03 ± 0.01	49 ± 9	35 ± 8	0.004	76 ± 5
	Spring	0.89 ± 0.21^a	0.42 ± 0.25	85 ± 4	89 ± 4	0.010	54 ± 22
	Summer	0.73 ± 0.59^a	0.33 ± 0.28	66 ± 16	64 ± 26	0.200	n.s
2 - 20 μm	Autumn	0.20 ± 0.16^a	0.04 ± 0.01	19 ± 16	16 ± 8	0.004	72 ± 9
	Winter	0.07 ± 0.02^a	0.02 ± 0.01	29 ± 6	29 ± 3	0.004	67 ± 3
	Spring	0.08 ± 0.04^a	0.02 ± 0.01	8 ± 3	6 ± 3	0.006	69 ± 23
	Summer	0.17 ± 0.08^a	0.06 ± 0.02	24 ± 12	23 ± 14	0.010	62 ± 5
$< 2 \mu\text{m}$	Autumn	0.12 ± 0.06^a	0.04 ± 0.02	11 ± 5	15 ± 9	0.006	62 ± 10
	Winter	0.05 ± 0.01^a	0.03 ± 0.01	22 ± 4	35 ± 6	0.016	47 ± 13
	Spring	0.07 ± 0.03^a	0.03 ± 0.02	7 ± 2	5 ± 2	0.010	61 ± 32
	Summer	0.08 ± 0.05^a	0.03 ± 0.01	10 ± 5	13 ± 12	0.006	56 ± 24

decreased ($p < 0.01$) in winter and increased again in spring (Table S3). Mean GPP was also relatively high during the first half of the summer sampling ($138 \pm 45 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$) but markedly decreased during the second half ($29 \pm 12 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$), when particularly low values were measured (18 and 27 $\text{mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ on July 7th and 10th, respectively) (Figure 5a).

Fig. 5. (a) gross primary production (GPP), (b) net community production (NCP) and (c) community respiration (CR) integrated in the upper 12 m of the water column during each sampling period at reference (ReS) station.

Community respiration (CR) also showed significant ($p < 0.05$) seasonal variability at ReS (Figure 5c) with minimum values in winter that progressively increased throughout spring and summer to reach maximum values in autumn (Table 3, S3). Likewise, net community production (NCP) exhibited significant variability ($p < 0.01$) at ReS (Figure 5b). Average NCP in autumn doubled the mean NCP in spring and was 3-fold and 4-fold that determined in

336 **Table. 3.** Average ($\pm$ SD) ($\text{mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$) values of gross primary production (GPP), net community production (NCP), community respiration (CR) and f -ratios
337 (NCP/GPP) for the microbial plankton community, integrated in the upper 12 m of the water column during each sampling period ($n = 6$) and for all periods ($n = 24$) at
338 reference (ReS) and raft (RaS) stations. Bold p -values denote significant differences between ReS and RaS (Kruskal-Wallis test), and finally the percentage of reduction
339 between ReS and RaS. Average values sharing the same superscript are not significantly different from each other. n.s denotes non-significant.
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		ReS	RaS	p-value	% reduction
GPP	Autumn	226 ± 68^a	75 ± 55	0.006	68 ± 17
	Winter	47 ± 19^b	27 ± 11	0.055	n.s
	Spring	105 ± 35^b	65 ± 37	0.100	n.s
	Summer	84 ± 66^b	61 ± 67	0.631	n.s
NCP	Autumn	186 ± 67^a	49 ± 54	0.016	62 ± 19
	Winter	43 ± 22^b	25 ± 11	0.200	n.s
	Spring	92 ± 35^b	44 ± 40	0.068	n.s
	Summer	56 ± 76^b	48 ± 74	0.873	n.s
CR	Autumn	40 ± 27^a	26 ± 14	0.423	n.s
	Winter	4 ± 4^b	3 ± 4	0.568	n.s
	Spring	14 ± 14^{ab}	21 ± 16	0.465	n.s
	Summer	28 ± 23^{ab}	13 ± 10	0.128	n.s
NCP/GPP (f-ratios)	Autumn	0.82 ± 0.14^a	0.55 ± 0.30	0.078	n.s
	Winter	0.89 ± 0.12^a	0.90 ± 0.12	0.683	n.s
	Spring	0.87 ± 0.11^a	0.67 ± 0.35	0.234	n.s
	Summer	0.46 ± 0.43^a	0.38 ± 0.47	0.852	n.s
	All periods	0.76 ± 0.29	0.65 ± 0.35	0.257	n.s

summer and winter, respectively (Table 3). Microbial plankton community was clearly autotrophic during the 4 sampling periods (Figure 5b), though slight heterotrophy was observed during the second half of the summer sampling period (on 7th and 10th July) when a sharp decrease in GPP occurred (Figure 5a).

Fig. 6. (a) gross primary production (GPP), (b) net community production (NCP) and (c) community respiration (CR) integrated in the upper 12 m of the water column during each sampling period at raft (RaS) station.

There were no significant differences ($p \geq 0.13$) for CR values recorded at RaS and at ReS (Figures 5c, 6c; Table 3). In contrast, both GPP and NCP measured in autumn at RaS showed a significant ($p < 0.05$) decrease of $\sim 65\%$ in relation to the values determined at ReS (Figures 5, 6; Table 3). Although GPP and NCP did not show significant differences in winter, spring and summer both rates were generally lower at RaS than at ReS (Figures 5, 6; Table 3). Negative

NCP ($-17 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$) was recorded at RaS on April 17 when CR was relatively high (Figure 6b-c).

As NCP informs on the amount of primary production that is not utilized by the microbial community and GPP represents the total primary production, the ratio NCP:GPP provides an estimate of the fraction of primary production available to be exported from the microbial plankton community without causing its collapse. Values of this ratio (Table 3), which is analogous to the f -ratio (Eppley and Peterson, 1979), reveal that the microbial community in summer at ReS had a variable export capacity (f -ratio = 0.46 ± 0.43) that was about a half of the export capacity ($p < 0.01$) during the other 3 periods (f -ratio ≥ 0.82). Table 3 also shows that, although differences between the f -ratios recorded at ReS and at RaS were not significant, the average f -ratio at RaS (0.65 ± 0.35) was slightly lower than the mean f -ratio at ReS (0.76 ± 0.29).

4. Discussion

Changes in the hydrographic conditions resulted in a considerable short-term variability in the chl a concentration and carbon fixation in the Ría de Vigo, outside the farming area. Superimposed on this short-term variability, there was also a seasonality characterised by low values in winter and high values during the upwelling season. This study shows that the variability in chl a concentration and carbon fixation in the Ría de Vigo, outside the mussel farming area, was characterised by low values in winter and high values during the upwelling season. Similar seasonal patterns have been previously described for the Galician Rías (Bode and Varela, 1998; Tilstone *et al.*, 1999; Cermeño *et al.*, 2006; Arbones *et al.*, 2008; Froján *et al.*, 2014). These variations in chl a and PP levels were mainly due to variations in the phytoplankton $> 20 \mu\text{m}$, in which diatoms prevailed (Froján *et al.*, 2014). The dominance of $> 20 \mu\text{m}$ size-fraction from spring to autumn is explained by the intensification of upwelling in the Ría interior, resulting in an excess of photosynthesized organic matter ready to be exported to higher trophic levels (Teixeira *et al.*, 2011) and so converting the Ría into a suitable ecosystem

for mussel farming (Figueiras *et al.*, 2002). However, this Ría situation differs from the adjacent shelf (Lorenzo *et al.*, 2005; Espinoza-González *et al.*, 2012) where pico- and nanophytoplankton prevail all year around. Consequently, in addition to potential technical limitations, biological constraints could also hinder the expansion of mussel farming to offshore waters in Galicia due to the dominance of the small fractions of phytoplankton.

On the other hand, GPP and NCP outside the mussel farm exhibited similar seasonal variability to PP with winter minimum and high values for the upwelling season. Recurrent positive values of NCP highlight the predominance of autotrophy in the microbial plankton community for the entire study year. Only a slight heterotrophy was detected on 7 and 10 July (Figure 5b) when the large heterotrophic dinoflagellate *Noctiluca scintillans* was present (Froján *et al.*, 2014). The two variables, NCP and GPP, showed a positive and significant linear relationship ($\text{NCP} = [-7.9 \pm 7.57] + [0.88 \pm 0.05] \times \text{GPP}$; $r^2 = 0.93$, $p < 0.001$), with the intercept being not significant. The non-significance of the intercept indicates that autotrophy was virtually permanent in the microbial plankton community, while the slope of the regression points to the high export capacity (88% of GPP) of this microbial community. Autotrophy and covariation of GPP and NCP were possible because CR was relatively low ($21 \pm 16 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$) compared with previous reported data (Arbones *et al.*, 2008).

The results of this study also evidence that the mussel farming area presents an alteration in phytoplankton community and microbial metabolism in comparison with the reference levels for the Ría de Vigo discussed in the above paragraphs. Although the observed differences will henceforth be attributed to mussel farming, other factors, both physical and biological, are likely to induce a certain degree of variability in the results, a fact that should be taken into account when interpreting the data. Previous studies conducted in the Ría de Vigo (Petersen *et al.*, 2008; Froján *et al.*, 2014) and in other mussel culture areas (Ogilvie *et al.*, 2000; Strohmeier *et al.*, 2008; Cranford *et al.*, 2014) have shown that mussel activity causes significant decreases in chl *a* concentration. Here we show that this decrease does not equally affect the 3 size-classes, with large size-classes being much more affected. The review by Ward and Shumway, (2004) reveals

that small particles ($< 3 \mu\text{m}$) are not effectively retained by bivalves and certainly our results show that chl a $< 2 \mu\text{m}$ (picophytoplankton) fraction was not affected by mussels, while the decreases observed in the other two fractions ($2 - 20 \mu\text{m}$ and $> 20 \mu\text{m}$) were probably caused by mussel consumption at RaS. The lack of significance of these decreases in spring and summer must be due to the high variability recorded in chl a concentrations during these two sampling periods (Figure 3). However, our results contrast with those obtained by Jabobs *et al.*, (2015), (2016) in the Dutch Wadden Sea (The Netherlands) and Sonier *et al.*, (2016) in St. Peters Bay (Canada), since they found evidence that picophytoplankton constitutes a trophic resource for mussels. Contrast is even higher with the results reported by Jiang *et al.*, (2016) who showed that picophytoplankton can be stimulated by oyster culture. This discrepancy may be related with the low availability of larger sizes of phytoplankton in those places.

Although the percentage of chl a $> 20 \mu\text{m}$ was lower at RaS than at ReS, the impact of mussel culture was not strong enough to cause a dominance shift in the size-structure of chl a at RaS for all seasons except winter. The selective consumption by mussels on the fraction $> 20 \mu\text{m}$ was able to induce a change in the size structure of the phytoplankton community during winter, leaving microphytoplankton as a minor component (Figure 3, Table 1). This winter situation is similar to that reported for other regions (Courties *et al.*, 1994; Vaquer *et al.*, 1996; Dupuy *et al.*, 2000; Souchu *et al.*, 2001; Cranford *et al.*, 2008), where microphytoplankton is not so abundant and the selective consumption by bivalves can lead to dominance of picophytoplankton.

In addition to analyse the impact of mussel farming in terms of phytoplankton biomass, as broadly reported in the literature, we have done one step forward and study the impact in terms of carbon fixed in the ecosystem. Contrary to expectations based on chl a decreasing, there is an acute and significant decrease recorded in the carbon fixed not only on the large size classes but also for the smallest one (Figure 4, Table 2). Carbon fixation in the $< 2 \mu\text{m}$ fraction was also lower at RaS than at ReS, while differences in chl a were not significant for this size-fraction due to the low retention efficiency of picophytoplankton by mussels (Norén *et al.*, 1999;

Newell, 2004; Petersen *et al.*, 2008). Therefore, we suppose that decreases in carbon fixation should also be attributed to stronger light attenuation that surely occurs in a raft zone where wooden beams and many hanging ropes exist. This contrasts with the increases in primary production reported for other culture zones based on ‘long-lines’ (Trottet *et al.*, 2008), where probably such light attenuation does not occur.

Mussel culture also alters the metabolic balance of the microbial community in the Ría de Vigo. This influence basically occurs through the decrease of GPP due to phytoplankton consumption by mussels and through the light attenuation caused by raft structures. Microbial respiration appears to be unaffected, probably because respiration mainly takes place in heterotrophic picoplankton (heterotrophic bacteria), a plankton component that is not effectively removed by mussels. The most evident consequence of these two contrasting effects on GPP and CR was the decrease in NCP at RaS (Figure 6b), a reduction that was more pronounced when diatoms (microphytoplankton) prompted high GPP in the Ría (autumn and spring). This reduction (56%) is similar to that observed in carbon fixation (63%) resulting in an appreciably lower export capacity (lower *f*-ratio) of the microbial plankton community at RaS than in waters far from mussel influence at ReS (Table 3). However, metabolism of the microbial plankton community at RaS continued being autotrophic, supporting the view that food does not limit mussel growth under the current conditions.

Overall, our results are relevant to assess the carrying capacity and thus the sustainability of mussel aquaculture (Filgueira *et al.*, 2013, 2015; Bricker *et al.*, 2016). In the Ría de Vigo, the dominance of the > 20 µm size-class in fixed carbon (Figure 4b), as well as in chl *a* at RaS (Figure 3b), during the 3 productive periods (spring, summer and autumn) indicates that mussel pressure on phytoplankton community was not sufficiently strong to cause food limitation, thus securing the carrying capacity of the system in terms of production (Inglis *et al.*, 2000). However, this does not necessarily indicates that the Ría can support higher mussel production, because an increase in production could induce changes in environmental conditions (Otero *et*

471 *al.*, 2005; Alonso-Pérez *et al.*, 2010) that could affect the ecological carrying capacity of the
472 system (Inglis *et al.*, 2000).

473 Concerning potential food limitation of mussel growth, it is also important to consider that
474 the size-structure of the phytoplankton community responds to changes in environmental
475 conditions. An increasing trend in water column stratification and a decrease in the intensity and
476 frequency of coastal upwelling, as reported by recent investigations (Levitus *et al.*, 2012; Barton
477 *et al.*, 2013), would be translated in a diminishing of the input of nutrient-rich upwelled water in
478 the rías that could result in a shift in the size-structure of the phytoplankton community with
479 picophytoplankton becoming the more relevant fraction during the productive season, from
480 spring to autumn (Crespo *et al.*, 2012). Under this new situation, primary production would
481 possibly decrease, dropping matter and energy transfer to higher trophic levels and thus turning
482 the Ría into a system unable to maintain the current mussel production. Nevertheless, other
483 factors, such as spatial heterogeneity and zooplankton grazing, could influence food availability
484 for mussels in growing areas, inducing inshore-offshore gradients in phytoplankton biomass
485 (Varela *et al.*, 2008) and mussel biomass changes in rafts over time (Duarte *et al.*, 2008), which
486 add some uncertainty in the results. More studies increasing temporal and spatial resolution of
487 sampling, both inside and outside the farming areas, are necessary to corroborate our
488 conclusions.

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