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Microbial plankton community in the Ría de Vigo (NW Iberian upwelling system): impact of the culture of *Mytilus galloprovincialis*

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ABSTRACT: Variability in size structure and composition of the microbial plankton community in the Ría de Vigo (NW Iberian coastal upwelling system) was studied as a function of the prevailing oceanographic conditions during 4 seasonal sampling periods (autumn, winter, spring and summer). The impact of mussel culture on this microbial plankton community was also evaluated by comparing the results obtained at a reference station (ReS) located outside the farming area with those found inside the farming area (raft station, RaS). Integrated microbial plankton biomass remained relatively constant (2.5 ± 0.4 g C m^{-2}) at ReS during autumn, spring and summer, when microplankton clearly dominated, accounting for 64 ± 13% of the total microbial plankton biomass. Pico- and nanoplankton were present in the microbial community all year round, with mean biomass values of 0.32 ± 0.09 and 0.42 ± 0.23 g C m^{-2}, respectively. These 2 fractions became more relevant during winter, when the contribution of microplankton to total microbial plankton biomass decreased (to 23 ± 9%), and a balanced trophic structure between autotrophs and heterotrophs was established. At RaS, a significantly lower biomass of microplankton (by 46 ± 32%) and nanoplankton (by 35 ± 22%) was observed compared to ReS, regardless of their trophic nature. Picoplankton biomass did not differ between sites. These results suggest that mussel farming exerts a top-down control over the microbial plankton community by consuming micro- and nanoplankton without affecting picoplankton. An excess of ammonium, probably excreted by mussels, and a lower autotrophic carbon:chlorophyll ratio at RaS suggest that mussel culture could also exert a bottom-up-like control on the phytoplankton that escape mussel consumption in farming zones.

KEY WORDS: Microbial plankton · Size structure · *Mytilus galloprovincialis* · Mussel impact · Coastal upwelling · NW Iberia

INTRODUCTION

The size structure of the microbial plankton community is one of the main factors controlling the transfer of matter and energy through marine food webs. In the open ocean, where nutrient levels are low, small phytoplankton dominates and recycling of the photosynthesised organic matter prevails (Pomeroy 1974, Azam et al. 1983). In contrast, where nutrients are abundant, as is usual in coastal areas, the dominance of large diatoms in the microbial community implies the existence of a short food web (Cushing 1989), which means that a significant fraction of the biogenic carbon is available to be exported outside the microbial community and fuel higher trophic levels (Eppley & Peterson 1979, Wassmann 1990).

Food webs in coastal upwelling systems have been traditionally considered to be short and efficient (Ryther 1969). Indeed, these ecosystems are highly productive areas that support the most important fisheries of the world's oceans (Pauly & Christensen

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1995). However, coastal upwelling systems are particularly dynamic, and the structure of the microbial community is affected by this environmental variability. Small organisms (<20 µm) dominate during periods of downwelling and during upwelling relaxation, while microplankton thrives during during upwelling events (Varela et al. 1991, Iriarte & González 2004, Espinoza-González et al. 2012). Moreover, upwelling can be intensified or weakened in response to the interaction of the wind field with coastal singularities, such as bays or capes, generating multiple environments in which different microbial communities develop (Castro et al. 1997, Pitcher et al. 2010). The NW Iberian Peninsula is one of these highly dynamic coastal upwelling zones, where northerly winds prevail from March to October, inducing upwelling, and during the rest of the year, the dominant southerly winds cause downwelling (Fraga 1981). It is known that changes in the structure and composition of the microbial plankton community in shelf waters occur in response to this environmental variability on seasonal and short-term scales (Varela et al. 1991, Crespo et al. 2011, Espinoza-González et al. 2012).

The Rías Baixas (see Fig. 1), 4 V-shaped bays with a NE–SW orientation that exchange water with the adjacent shelf, are located on the NW Iberian coast. The singular topography of the Rías, shallower and narrower inward, and their estuary-like circulation enhance upwelling and downwelling processes occurring at the continental shelf (Figueiras et al. 2002). However, despite their uniqueness, the size and trophic structure of the microbial plankton community as a whole has not received much attention in the Rías Baixas. Studies conducted to date were either focused on the composition and dynamics of microplankton (Figueiras et al. 1994, Tilstone et al. 1994, Fermín et al. 1996) or indirectly addressed the size structure of phytoplankton through chlorophyll fractionation (Cermeño et al. 2006, Arbones et al. 2008). Therefore, no comprehensive characterisation of the microbial plankton community has been done in the Rías Baixas in relation to the environmental conditions recorded at annual scales.

While such a characterisation is needed for its own sake, it is even more important considering the intensive culture of the edible mussel *Mytilus galloprovincialis* Lamarck that exists in the Rías. The Galician Rías support the highest mussel production in Europe (250×10^6 kg yr^{-1}), with a total of 3335 mussel rafts enclosed in areas called polygons (Labarta et al. 2004). Although previous research suggests that mussel culture significantly alters the food web in the Rías (Tenore et al. 1982), very few studies have been conducted to assess this issue (Cabanas et al. 1979, Maar et al. 2008, Petersen et al. 2008, Zúñiga et al. 2013). In terms of chlorophyll, Cabanas et al. (1979) and Petersen et al. (2008) showed a reduction inside the mussel area between 20 and 60% of the outside concentration, mainly affecting chlorophyll *a* (chl *a*) in the fraction >2 µm. More recently, Zúñiga et al. (2013) showed strong correlations between microbial plankton carbon biomass and ingestion rate or absorption efficiency of *M. galloprovincialis.* These results point to the important role that the microbial plankton community plays in mussel growth and production in the Rías Baixas. Nevertheless, more research is required to establish to what extent mussel culture causes modifications in the structure and composition of the microbial plankton community originally dependent on the prevailing oceanographic conditions. The aim of the present work was to characterise the structure and composition of the microbial plankton community in the Ría de Vigo (one of the Rías Baixas) in relation to the hydrographic conditions recorded during 4 seasonal campaigns, and to analyse the impact of mussel culture on this microbial plankton community for each study period. This knowledge should contribute to improving the management capacity of the mussel culture in this upwelling region.

MATERIALS AND METHODS

Sampling strategy

The study was carried out at 2 sampling stations, a reference station (ReS) and a raft station (RaS), in the Ría de Vigo (Fig. 1) in 2007 and 2008. The ReS was positioned in the central channel of the Ría, well outside of the mussel farming area. The RaS was located slightly inwards in the Ría within a polygon (group of rafts). Four seasonal periods, autumn (September 17 to October 4), winter (January 28 to February 14), spring (April 14 to May 01) and summer (June 26 to July 14), were sampled. The 2 stations were visited aboard the RV 'Mytilus' every 2 to 3 d during each period, providing 6 sampling days per period. Water samples at both stations were collected at 5 depths (surface, and 5, 10, 15 and 20 m) using a CTD SBE 9/11 (Sea-Bird) fitted to an oceanographic rosette equipped with 12 Niskin bottles. Subsamples were taken to determine nitrate, ammonium and chl *a* concentrations, and to evaluate picoplankton (<2 µm), nanoplankton (2–20 µm) and microplankton (>20 µm) biomass. Daily values of the Ekman transport ($-Q_x$,

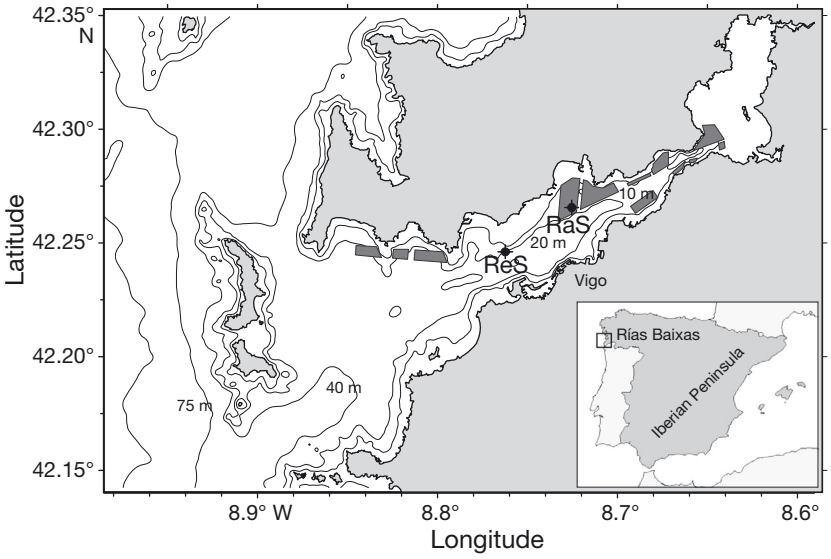

Fig. 1. Ría de Vigo, with the 2 sampling sites (✦): reference (ReS) and raft (RaS) stations. Locations of the raft polygons are also shown (dark grey areas). Inset: the Rías Baixas on the NW Iberian Peninsula

m^3 s^{-1} km^{-1}) perpendicular to the coast were calculated according to Bakun (1973) using the daily average of shelf winds available from the seawatch buoy off Cape Silleiro, belonging to Puertos del Estado (Spanish government port authorities; www.puertos.es).

Nutrients and chl *a*

Nitrate and ammonium concentrations (µmol kg^{-1}) were determined by segmented flow analysis using an Alpkem autoanalyser following Hansen & Grasshoff (1983). For chl *a*, 250 ml of seawater was filtered through 25 mm Whatman GF/F filters. The filters were then frozen (–20°C) until pigments were extracted in 90% acetone for 24 h in the dark at 4°C. Chl *a* concentrations (mg m^{-3}) were determined by measuring the fluorescence of the extracted pigments in a Turner Designs fluorometer calibrated with pure chl *a* (Sigma).

Picoplankton biomass

Subsamples of 1.8 ml were collected in sterile cryovials to determine the biomass of heterotrophic bacteria (HB) and autotrophic picoplankton (APP). After fixation with a P+G solution (1% paraformaldehyde + 0.05% glutaraldehyde) at 10% final concentration, samples were frozen and kept in liquid nitrogen at –80°C until analysis in the laboratory. The analysis was performed in a FACScalibur flow cytometer using aliquots of 0.6 ml for APP and 0.4 ml for HB, and 10 µl of yellow-green 1 µm Polyscience latex beads as an internal standard (Calvo-Díaz & Morán 2006). HB were previously stained with 4 µl of SYBR Green dye. Abundances of HB, *Synechococcus* and autotrophic eukaryotic picoflagellates (APF) were obtained. *Prochlorococcus* did not appear in any sample, revealing that their presence in the Ría is insignificant, as previously pointed out by Rodríguez et al. (2003). HB biomass was calculated using a conversion factor of 20 fg C $cell^{-1}$ (Lee & Fuhrman 1987), and the biomass of *Synechococcus* was estimated following Bratbak & Dundas (1984). The biomass of APF was determined according to Verity et al. (1992).

Heterotrophic eukaryotic picoflagellates (HPF) were determined in subsamples of 10 ml fixed with buffered 0.2 µm filtered solution of formaldehyde (2% final concentration) and stained with DAPI (0.1 µg ml^{-1} final concentration) for 10 min in the dark (Porter & Feig 1980). The samples were then filtered through 0.2 µm black Millipore-Isopore filters, and HPF abundance was obtained using an epifluorescence microscope, illuminating the filters with UV light. The biomass was estimated according to Verity et al. (1992).

Nanoplankton biomass

Subsamples of 30 ml were used to determine abundance and biomass of autotrophic nanoflagellates (ANF) and heterotrophic nanoflagellates (HNF). The subsamples were fixed and stained as described above for HPF. Subsequently, these subsamples were filtered through 0.8 µm black Millipore-Isopore filters to enumerate ANF and HNF by epifluorescence microscopy. Autotrophic organisms were distinguished by their reddish colour when the sample was illuminated with blue light, whereas heterotrophic organisms were distinguished by their blue colour under UV light illumination. At least 300 cells were counted in each sample. The biovolumes of ANF and HNF were calculated by measuring the diameter of several individuals (at least 25 in each group and sample) and assuming a spherical shape. Biomass in carbon units was calculated according to Verity et al. (1992).

Microplankton biomass

Subamples of 100 ml preserved in Lugol's iodine were sedimented in composite sedimentation chambers and observed with an inverted microscope to identify and count microplankton cells. The organisms were counted and identified at species level when possible. The smaller species (<20 µm) were enumerated from 2 perpendicular transects scanned at 400×, medium-size individuals (20–50 µm) were counted in 1 or 2 transects at 200×, and larger organisms (>50 µm) were counted by scanning the whole slide at 100×. At least 500 cells were counted in each sample. Differentiation between autotrophic microplankton (AMP) and heterotrophic microplankton (HMP) was made following Lessard & Swift (1986) and Larsen & Sournia (1991) and also using our historical records of epifluorescence microscopy. Cell volumes were estimated according to Hillebrand et al. (1999), and the biovolumes of diatoms and dinoflagellates were converted to carbon biomass following Strathmann (1967). However, the cellular carbon of *Noctiluca scintillans* was estimated applying the correction suggested by Tada et al. (2000). Carbon biomass was estimated following Verity et al. (1992) for flagellates other than dinoflagellates and Putt & Stoecker (1989) for ciliates. Diatoms, dinoflagellates, flagellates and ciliates <20 µm were included in the nanoplankton fraction, whereas chain-forming diatoms <20 µm were ascribed to microplankton.

The biomass values of all microbial plankton components are presented integrated over a 12 m water column because this is the length of the ropes containing mussels in the rafts. In this way, biomass comparisons between ReS and RaS stations are made for the environment (upper part of the water column) potentially affected by mussels. Although biomass values integrated over the entire water column were higher than the biomass values integrated over the upper 12 m depth of the water column, both values showed in all cases (size classes and trophic structure) strong correlation ($r^2 > 0.9$; $p < 0.001$), indicating that the structure of the microbial community did not change in the water column.

Statistical analysis

A non-parametric analysis of variance (Kruskal-Wallis test) was applied to test significant effects of location (ReS vs. RaS) and season (autumn, winter, spring and summer) over the environmental variables and microbial plankton components using the statistical software SPSS.

RESULTS

Wind forcing and water column response

The thermohaline properties and nitrate, ammonium and chl *a* levels at ReS for the 4 study periods (Fig. 2) showed the seasonal and short-time variability typically recorded in the Ría de Vigo in response to dominant winds.

Upwelling favourable winds ($-Q_x$ positive values) prevailed during the first half of autumn, shifting to southerly winds in the second half. These changes in wind regime modified the water column structure, from an initial stratification (>16°C and nutrients <1 µmol kg^{-1} at sea surface), to an upwelling of subsurface cold (<14°C), saline (>35.6) and nutrient-rich water, and finally to the occurrence of an intense downwelling. The subsurface chl *a* maximum on September 24 (6 mg m^{-3}) was probably associated with phytoplankton accumulation at the pycnocline during the upwelling pulse, whereas the subsequent downwelling caused a homogeneous chl *a* distribution (~4 mg m^{-3}). Weak winds prevailed throughout the winter sampling, especially in the second half, when the water column displayed thermal homogeneity and weak saline stratification. Nitrate levels were high (5 to 6 µmol kg^{-1}) and chl *a* concentration remained low (<1 mg m^{-3}). In spring, winds were relatively weak. The hydrographic conditions evolved from a well-mixed water column to a thermohaline stratification favoured by an intense continental input (161 m^3 s^{-1}, data provided by Aguas de Galicia) during the second half of this period. High nitrate levels supplied by continental input enhanced phytoplankton increase at the halocline (>4 mg chl *a* m^{-3}). The June–July cruise captured the characteristic summer situation when strong upwelling-favourable winds alternate with relaxation periods. Thus, upwelling of cold (<14°C) and nutrient-rich water (>5 µmol kg^{-1} for nitrate and >3 µmol kg^{-1} for ammonium) stimulated phytoplankton growth (>5 mg m^{-3}) at the sea surface. Once upwelling subsided, the surface chl *a* was redistributed throughout the water column. After that, wind relaxation left nutrient-poor water with low chl *a* at the sea surface.

Thermohaline conditions and nitrate concentrations at RaS (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m498p043_supp.pdf) were not

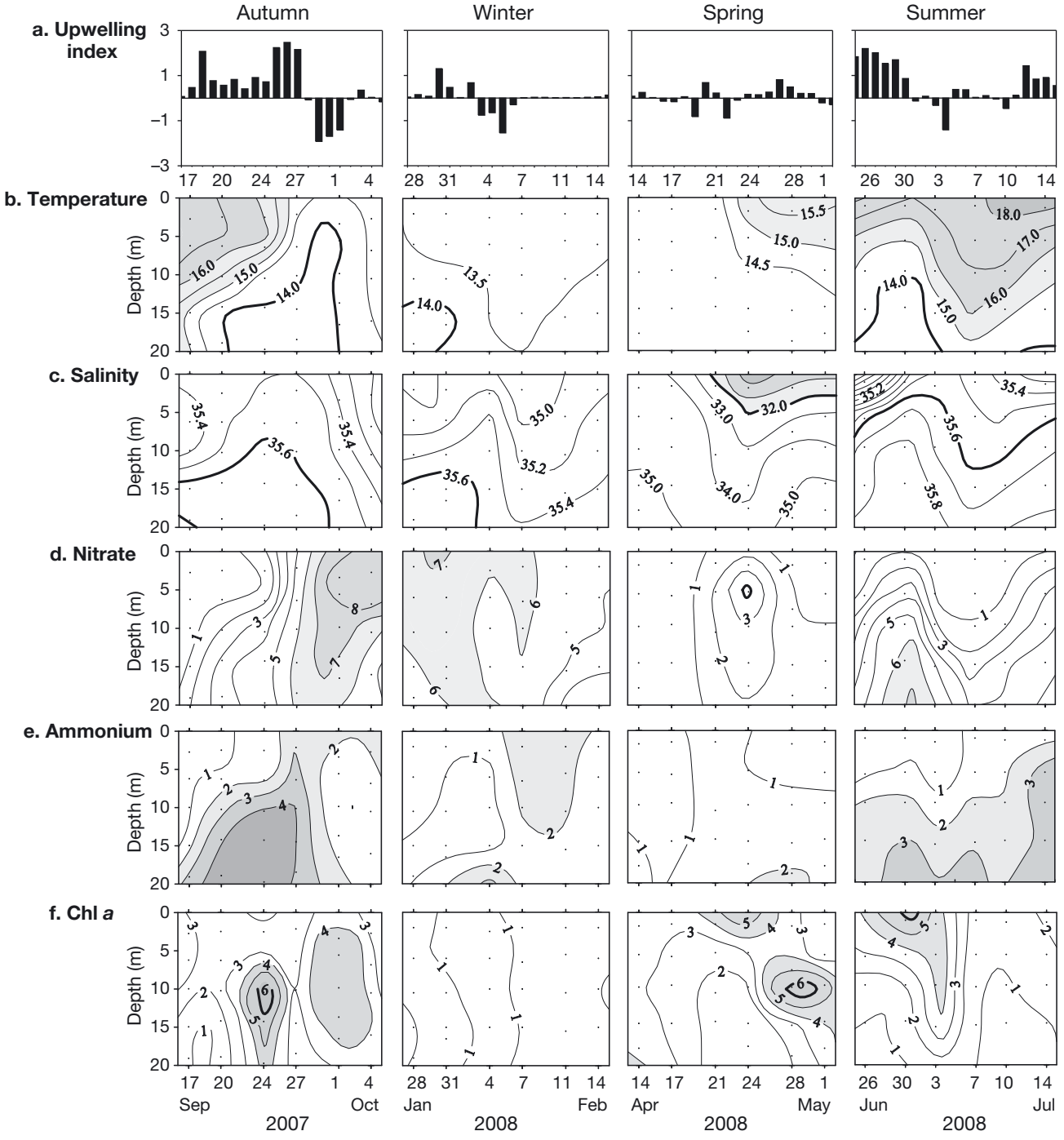

Fig. 2. (a) Upwelling index (×10^3 m^3 s^{-1} km^{-1}), (b) temperature (°C), (c) salinity, (d) nitrate concentration (µmol kg^{-1}), (e) ammonium concentration (µmol kg^{-1}) and (f) chl *a* concentration (mg m^{-3}) at the reference station (ReS) during 4 seasons

different from those at ReS (Fig. 2), as there were no significant differences between temperature, salinity and nitrate concentrations measured at the 2 locations (Kruskal-Wallis test, n = 240, p ≥ 0.57). However, differences were significant (Kruskal-Wallis test, n = 240, p < 0.001) in ammonium and chl *a* concentrations at RaS (Fig. 3) and at ReS (Fig. 2). Integrated (12 m depth) ammonium concentrations were higher at RaS (33 ± 14 mmol m^{-2}) than at ReS (20 ± 9 mmol m^{-2}), and integrated chl *a* concentration was lower at RaS (24 ± 17 mg m^{-2}) than at ReS (36 ± 22 mg m^{-2}).

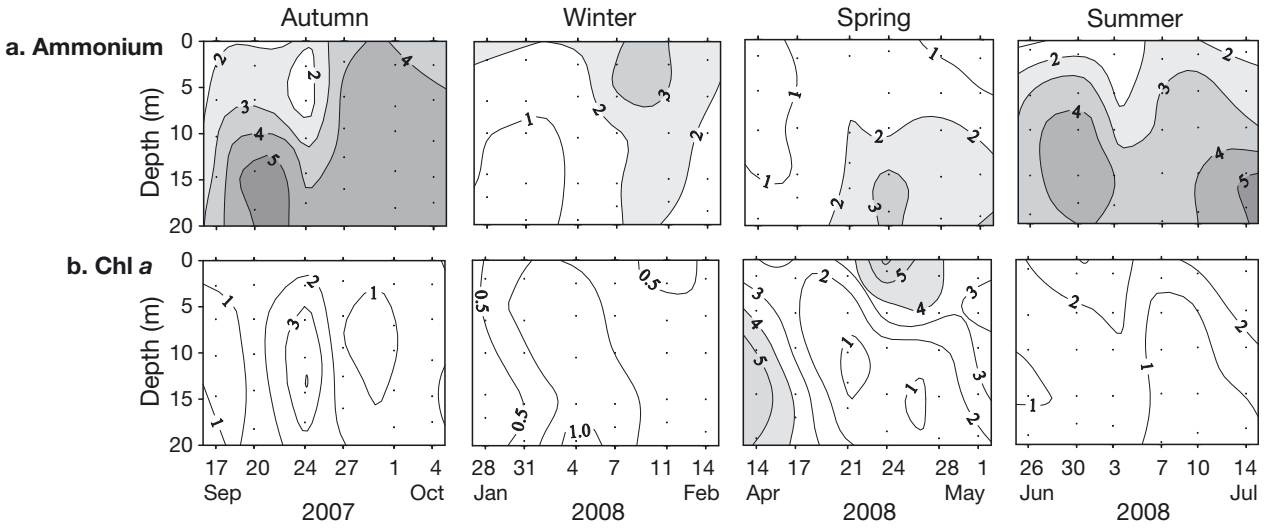

Fig. 3. (a) Ammonium (µmol kg^{-1}) and (b) chl *a* (mg m^{-3}) concentrations at the raft station (RaS) during 4 seasons

Variability in microbial plankton biomass: trophic and size structure

The observed variability in microbial plankton biomass at ReS (Fig. 4a, Table 1) was linked to hydrographic variability (Fig. 2). Total microbial plankton biomass expressed as total carbon (TC) remained around 2.5 g C m^{-2} during autumn, spring and summer, contrasting with the winter situation when TC (0.69 ± 0.18 g C m^{-2}) was substantially lower. Integrated autotrophic carbon biomass (AC) experienced continuous fluctuations over time, reaching their highest values in autumn and spring. In contrast, integrated heterotrophic carbon biomass (HC) was characterised by lower variability, and only exceeded AC in summer (Fig. 4a, Table 1) due to the presence of the large heterotrophic dinoflagellate (HD) *Noctiluca scintillans*, which accounted for 43 ± 15% of HC. Even though HC and AC were not related (r^2 = 0.029, $p > 0.05$), both biomasses showed a significant linear relationship when the summer sampling, with the dominance of *N. scintillans*, was not considered (HC = [0.30 ± 0.11] + [0.17 ± 0.06] × AC; r^2 = 0.32, $p < 0.05$).

Changes in the size structure of the microbial plankton community were also evident (Fig. 4b). Microplankton dominated during 3 of the 4 periods: autumn, spring and summer. This dominance was especially noticeable in spring, with microplankton accounting for 72 ± 8% of TC. However, microplank-

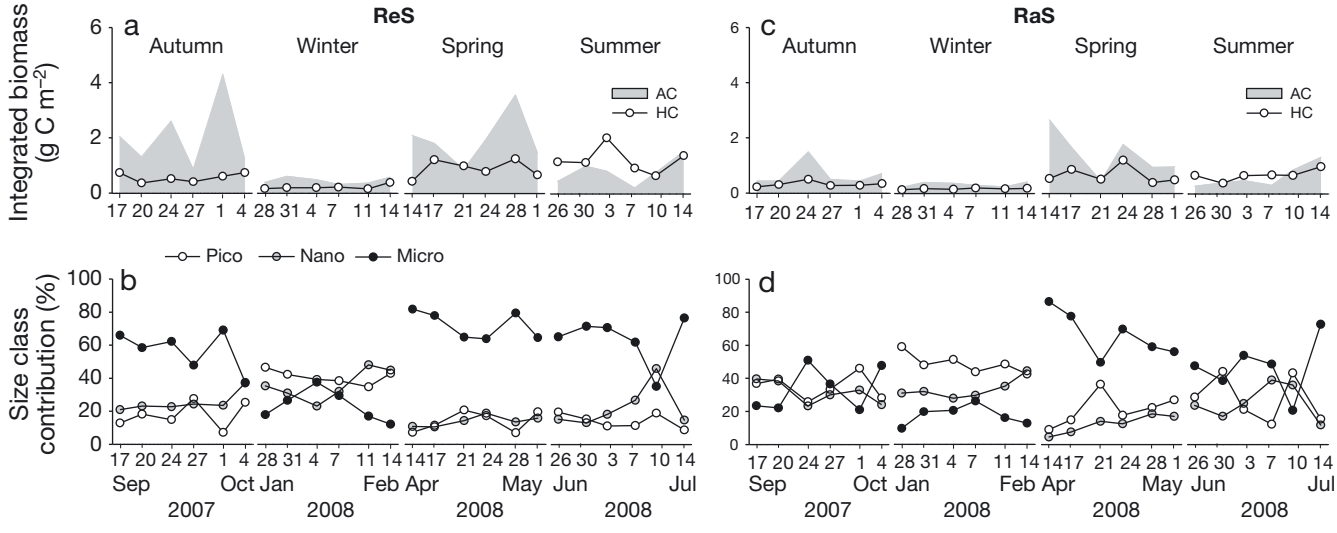

Fig. 4. Autotrophic (AC) and heterotrophic (HC) carbon biomass integrated in the upper 12 m of the water column, and contribution of picoplankton (Pico), nanoplankton (Nano) and microplankton (Micro) to total microbial plankton biomass at the (a,b) reference station (ReS) and (c,d) raft station (RaS) during 4 seasons

Table 1. Average (±1 SD) (g C m^{-2}) biomass of total microbial plankton (TC), autotrophic plankton (AC), heterotrophic plankton (HC) and chl *a* concentration (mg m^{-2}) integrated in the upper 12 m of the water column, for 4 seasonal periods at reference (ReS) and raft (RaS) stations

Stn	Season	TC	AC	HC	Chl *a*
ReS	Autumn	2.63 ± 1.31	2.07 ± 1.27	0.56 ± 0.16	47 ± 23
	Winter	0.69 ± 0.18	0.46 ± 0.12	0.23 ± 0.09	13 ± 4
	Spring	2.82 ± 1.05	1.94 ±0.91	0.88 ± 0.32	49 ± 18
	Summer	2.02 ± 0.75	0.80 ± 0.46	1.22 ± 0.47	34 ± 21
RaS	Autumn	0.99 ± 0.51	0.67 ± 0.42	0.32 ± 0.1	21 ± 15
	Winter	0.49 ± 0.09	0.33 ± 0.08	0.17 ± 0.02	8 ± 2
	Spring	2.04 ± 0.95	1.39 ± 0.79	0.65 ± 0.31	42 ± 19
	Summer	1.30 ± 0.56	0.62 ± 0.4	0.68 ± 0.19	23 ± 11

ton only accounted for 23 ± 9% of TC in winter, when picoplankton grew in importance, representing 41 ± 4% of TC. Variability was less evident in nanoplankton, which represented 24 ± 11% of TC over time.

Differences were also detected regarding the trophic structure in each size fraction across seasons (Fig. 5a–c). Within picoplankton, APP and heterotrophic picoplankton (HPP) biomasses showed low variability. HPP biomass slightly exceeded APP (Fig. 5a). In contrast, autotrophic nanoplankton (ANP) biomass exceeded heterotrophic nanoplankton (HNP) biomass in all samplings (Fig. 5b). Microplankton exhibited high variability (Fig. 5c), with HMP biomass lower than AMP biomass in autumn, winter and spring. The situation was completely different in summer, with HMP representing 73 ± 18% of total microplankton biomass due to the presence of *Noctiluca scintillans*.

A significantly lower value of TC was detected at RaS compared to TC at ReS (Fig. 4c, Table 1; Table S1 in the Supplement). Especially important was the difference observed in autumn, when TC was 57 ± 20% less at RaS, with both AC and HC being significantly lower (Table S1 in the Supplement). HC and AC showed a significant linear relationship at this RaS location (HC = [0.25 ± 0.08] + [0.27 ± 0.08] × AC; $r^2 = 0.33$, $p < 0.01$).

The size structure of the microbial community was different at RaS as compared to ReS (Fig. 4d). Thus, the dominance of microplankton was not so evident at the mussel-influenced site, though it remained the main component of the microbial community in spring and summer (57 ± 18% of TC). Picoplankton exceeded nanoplankton in the 4 samplings, with nanoplankton being the fraction of the microbial plankton with the lower contribution to TC.

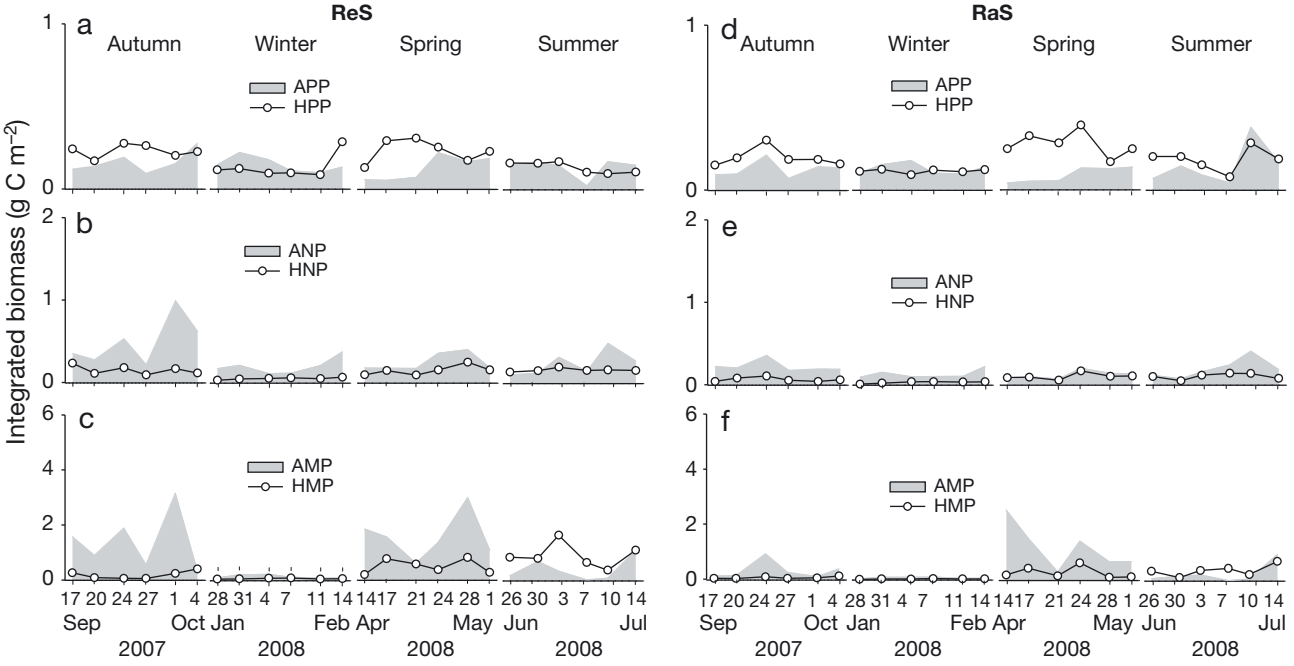

Fig. 5. Autotrophic (APP) and heterotrophic (HPP) picoplankton biomass, autotrophic (ANP) and heterotrophic (HNP) nanoplankton biomass and autotrophic (AMP) and heterotrophic (HMP) microplankton biomass integrated in the upper 12 m of the water column at the (a–c) reference station (ReS) and (d–f) raft station (RaS) during 4 seasons. Note the different *y*-axis scales

Within picoplankton, both APP and HPP did not show significant differences (Table S1 in the Supplement) at RaS (Fig. 5d) in relation to the distribution and values registered at ReS (Fig. 5a). However, within nanoplankton, HNP biomass was significantly lower at RaS (Fig. 5e) than at ReS (Fig. 5b) in autumn, winter and summer, whereas ANP biomass was significantly lower only in autumn and spring (Table S1 in the Supplement). Concerning microplankton (Fig. 5f), AMP experienced a significant reduction at RaS in autumn, when its biomass was 61 ± 41% lower than AMP biomass recorded at ReS. HMP biomass was also significantly lower in winter and summer at RaS, with a reduction of 54 ± 23% in summer due to the lower abundance of *Noctiluca scintillans* (Table S1 in the Supplement). At RaS, total nanoplankton (ANP + HNP) and total microplankton (AMP + HMP) showed decreases of 35 ± 22% and 46 ± 32% compare to ReS.

Structure and variability of autotrophic microbial plankton community

The autotrophic microbial plankton community at ReS was dominated by diatoms (49 ± 26% of AC), ANP (30 ± 19% of AC) and APP (17 ± 12% of AC) (Fig. S2 in the Supplement; Table 2). Total APP biomass was relatively stable over time, whereas variability was higher in ANP, with mean biomass in autumn doubling the values registered in the other 3 samplings (Table 2). Nevertheless, the greatest variability was observed in diatoms (Fig. S2b in the Supplement; Table 2), with biomass values being particularly high in autumn and spring. APF dominated within APP, accounting for 89 ± 10% of APP biomass. *Synechococcus* were only relatively important in autumn and winter. Much of the ANP biomass during winter (87 ± 8%) and summer (76 ± 13%) could be attributed to ANF (Fig. S2c in the Supplement). However, ANF only represented 48 ± 21% of ANP in autumn, when the presence of an unidentified small centric diatom was important. This centric diatom accounted for 75% of all ANP biomass on October 1, and was also an important part of the ANP community during the second half of the spring sampling.

The autotrophic microbial community at RaS was also dominated by diatoms, ANP and APP, together accounting for 98 ± 3% of all AC (Fig. S2 in the Supplement; Table 2). Nonetheless, the diatom biomass found at RaS was significantly lower (by 60 ± 43%) than the diatom biomass recorded at ReS during the autumn sampling (Table S1 in the Supplement). On the other hand, ANP biomass was significantly lower at RaS during autumn (by 44 ± 25%) and spring (by 50 ± 16%) (Table 2; Table S1 in the Supplement). During these 2 sampling periods, the small centric diatom found at ReS was not observed at RaS, and ANF were always the major component of the ANP biomass at RaS (Fig. S2f in the Supplement).

Water column-integrated AC and chl *a* (Table 1) were linearly related at ReS (AC = [−0.12 ± 0.2] + [40 ± 5] × chl *a*; $r^2 = 0.76$, $p < 0.001$) and at RaS (AC = [0.07 ± 0.12] + [29 ± 4] × chl *a*; $r^2 = 0.69$, $p < 0.001$), but in the latter case with a lower slope (ratio of AC:chl *a* = 29 ± 4).

Structure and variability of heterotrophic microbial plankton community

Within the heterotrophic community, HNP and HPP, the latter mainly composed of HB (81 ± 7%; Table 3), showed low temporal variability at ReS (Fig. S3a in the Supplement). HB biomass was especially important in autumn and spring (Table 3),

Table 2. Average (±1 SD) (g C m^{-2}) biomass of autotrophic plankton integrated in the upper 12 m of the water column for 4 seasonal periods at reference (ReS) and raft (RaS) stations. APF: autotrophic picoflagellates, ANP: autotrophic nanoplankton, AD: large (>20 µm) autotrophic dinoflagellates, ACil: large (>20 µm) autotrophic ciliates

Stn	Season	*Synechococcus*	APF	ANP	Diatoms	AD	ACil
ReS	Autumn	0.03 ± 0.02	0.13 ± 0.05	0.50 ± 0.29	1.32 ± 1.03	0.07 ± 0.11	0.002 ± 0.003
	Winter	0.02 ± 0.01	0.13 ± 0.04	0.20 ± 0.10	0.11 ± 0.06	0.003 ± 0.002	0.001 ± 0.001
	Spring	0.001 ± 0.001	0.12 ± 0.07	0.24 ± 0.10	1.50 ± 0.81	0.01 ± 0.01	0.06 ± 0.09
	Summer	0.008 ± 0.002	0.12 ± 0.05	0.24 ± 0.14	0.38 ± 0.41	0.01 ± 0.003	0.017 ± 0.017
RaS	Autumn	0.02 ± 0.01	0.11 ± 0.04	0.23 ± 0.07	0.31 ± 0.31	0.01 ± 0.01	0.0001 ± 0.0003
	Winter	0.02 ± 0.01	0.10 ± 0.03	0.13 ± 0.05	0.06 ± 0.03	0.003 ± 0.002	0.001 ± 0.001
	Spring	0.001 ± 0.001	0.09 ± 0.04	0.12 ± 0.05	1.15 ± 0.82	0.005 ± 0.003	0.03 ± 0.04
	Summer	0.01 ± 0.003	0.15 ± 0.12	0.20 ± 0.11	0.25 ± 0.35	0.01 ± 0.005	0.005 ± 0.004

Table 3. Average (±1 SD) (g C m^{-2}) biomass of heterotrophic microbial plankton integrated in the upper 12 m of the water column for 4 seasonal periods at reference (ReS) and raft (RaS) stations. HB: heterotrophic bacteria, HPF: heterotrophic picoflagellates, HPP: heterotrophic picoplankton, HNP: heterotrophic nanoplankton, HD: large (>20 µm) heterotrophic dinoflagellates, HCil: large (>20 µm) heterotrophic ciliates

Stn	Season	HB	HPF	HPP	HNP	HD	HCil
ReS	Autumn	0.20 ± 0.04	0.03 ± 0.01	0.23 ± 0.04	0.15 ± 0.05	0.08 ± 0.06	0.10 ± 0.13
	Winter	0.11 ± 0.07	0.03 ± 0.005	0.14 ± 0.08	0.05 ± 0.01	0.02 ± 0.01	0.02 ±0.01
	Spring	0.20 ± 0.07	0.03 ± 0.01	0.23 ± 0.07	0.15 ± 0.06	0.08 ±0.04	0.42 ± 0.24
	Summer	0.10 ± 0.02	0.03 ± 0.01	0.13 ± 0.03	0.16 ±0.02	0.77 ± 0.40	0.15 ±0.06
RaS	Autumn	0.17 ± 0.06	0.02 ±0.01	0.20 ± 0.05	0.06 ± 0.03	0.02 ± 0.01	0.03 ±0.03
	Winter	0.09 ± 0.01	0.02 ± 0.01	0.11 ± 0.01	0.03 ± 0.01	0.01 ±0.003	0.01 ± 0.01
	Spring	0.24 ± 0.08	0.03 ± 0.01	0.28 ± 0.08	0.10 ± 0.04	0.06 ± 0.04	0.21 ± 0.21
	Summer	0.15 ± 0.06	0.04 ±0.02	0.19 ± 0.07	0.11 ± 0.04	0.29 ± 0.20	0.10 ± 0.06

whereas HNP biomass, which was low in winter, remained relatively stable during the other 3 samplings (Table 3). HB and HNP jointly accounted for >65% of total HC in autumn and winter, but heterotrophic ciliates (HCil) in spring (45 ± 12% of HC) and HD in summer (61 ± 10% of HC) became more relevant (Fig. S3b in the Supplement). An unidentified peritrich ciliate and *Noctiluca scintillans* accounted for 54 ± 36% of the HCil biomass and 72 ± 23% of the HD biomass in spring and summer, respectively.

Although biomass values of HB registered at RaS (Fig. S3c in the Supplement) and ReS (Fig. S3a in the Supplement) were not significantly different (Table S1 in the Supplement), the biomass values of HNP and HD were significantly lower at RaS in autumn, winter, and summer (Fig. S3, Table S1 in the Supplement; Table 3). The decline in HD biomass was particularly evident in summer (59 ± 21%). As observed at ReS for this summer period, *Noctiluca scintillans* accounted for a substantial fraction (83 ± 8%) of the total HD biomass at RaS. During the spring sampling, HCil experienced a remarkable reduction at RaS (Fig. S3b,d in the Supplement), when their mean biomass was half of that recorded at ReS (Table 3).

DISCUSSION

Microbial plankton community in Ría de Vigo: importance of microplankton

The size structure of the microbial plankton community reported in the present study follows a similar pattern to that previously described for phytoplankton in the Ría de Vigo in studies based only on chl *a* fractionation (Cermeño et al. 2006, Arbones et al. 2008). Microplankton clearly dominated in the microbial plankton community during 3 (autumn, spring and summer) of the 4 hydrographic situations sampled, representing 64 ± 13% of microbial plankton biomass. Pico- and nanoplankton were part of the planktonic community throughout the year, with a relatively constant biomass of 0.32 ± 0.09 and 0.42 ± 0.23 g C m^{-2}, respectively. These 2 fractions become more relevant during winter, when microplankton abundance decreased. This size structure contrasts with that described for the microbial plankton community in shelf waters in front of the Ría de Vigo (Espinoza-González et al. 2012) and in shelf waters of other upwelling systems (Böttjer & Morales 2007), where small plankton (<20 µm) dominates and microplankton (mainly diatoms) is added in periods of intense upwelling. As small-sized plankton (<20 µm) is also present in the microbial plankton community of the Ría de Vigo, the main difference between the Ría and the adjacent continental shelf waters lies in the greater importance and continuous presence of microplankton in the Ría.

Concerning the trophic structure (pigmented vs. unpigmented plankton), the microbial plankton community in the Ría de Vigo can be considered fundamentally autotrophic, particularly due to the contribution of AMP, which considerably increased the autotrophic plankton biomass in autumn, spring and summer, when AC averaged 1.60 ± 1.06 g C m^{-2} and AMP accounted for 62 ± 23%. Although diatoms, ANP and APP were always present (Table 2), the largest variations in autotrophic biomass were due to changes in diatoms in spring and during intense upwelling events (autumn). This variability in the trophic structure throughout the year supports previous studies based on oxygen production and respiration measurements (Moncoiffé et al. 2000, Cermeño et al. 2006, Arbones et al. 2008). Such studies show that the microbial com-

munity of Ría de Vigo is net autotrophic all year round, but approaches metabolic balance in winter, when the autotrophic and heterotrophic plankton biomass are balanced, diatoms are scarce (Table 2; Fig. S2b in the Supplement) and pico- and nanoplankton attain higher importance (Fig. 4b). Again, this situation contrasts with that reported for the adjacent shelf (Espinoza-González et al. 2012), where the microbial community is net heterotrophic but shifting to autotrophy during periods of intense upwelling (Teira et al. 2001). The summer sampling deserves a specific mention, since during this sampling the heterotrophic biomass exceeded autotrophic biomass (Fig. 4a) due to the presence of *Noctiluca scintillans*. This species occurred in a period of upwelling relaxation (Fig. 2), a time of the year (summer) when is common to find HD within the microbial plankton community (Figueiras et al. 2002). Upwelling relaxation causes a considerable slowing down in the circulation of the Ría that frequently favours the accumulation of dinoflagellates with swimming or floating capacity in surface waters (Fermín et al. 1996), as probably was the case for *N. scintillans*.

The importance of microplankton and particularly the relevance of diatoms within the microbial community in the Ría de Vigo can be attributed to the high impact (frequency and intensity) that upwelling has on this coastal system. The estuarine circulation of the Ría and its bathymetric configuration, with depth continuously decreasing from its mouth towards the interior (Fig. 1), favours the intrusion of upwelled waters on the shelf along the bottom and the uplift of these waters at the inner part of the Ría (Figueiras et al. 2002, Crespo et al. 2007). In this way, even a weak upwelling that does not cause detectable response in the plankton on the continental shelf provides, however, the nutrients needed to trigger an appreciable response of the plankton community inside the Ría — a response that is mainly characterised by an increase in diatom abundance (Figueiras et al. 2002, Teixeira et al. 2011). Hence, the positive estuarine circulation of the Ría de Vigo, together with its unique topography, contributes to the intensifying of the effects of upwelling, promoting diatom growth and the export of organic matter to the adjacent shelf during the upwelling season (Tilstone et al. 2000, Crespo et al. 2007). Conversely, the dominance of smaller plankton cells in the Ría during winter indicates that the microbial loop prevails in this season (Teixeira et al. 2011), favouring the *in situ* remineralisation of photosynthesised organic matter.

Impact of mussel culture on microbial plankton community

Our results show, through comparing chl *a* and TC values at RaS and ReS, that mussel culture significantly affects the microbial community in the Ría de Vigo. The significant decrease that we recorded in chl *a* concentration at RaS lies within the range observed by Petersen et al. (2008) during a previous study conducted in the Ría de Vigo under summer stratification conditions and it is also comparable to that described for other culture areas (Ogilvie et al. 2000, Strohmeier et al. 2008). However, this result contrasts with that obtained by Trottet et al. (2008) in the Grande-Entrée Lagoon (Canada), who reported a not-significant mussel impact on the phytoplankton and microbial heterotrophic plankton community. Trottet et al. (2008) considered that the low bivalve production (180 t yr^{-1}) in the lagoon was responsible for the lack of impact on the microbial plankton community. In the Ría de Vigo, where we detected a significant impact on the microbial plankton community, mussel production (34 500 t yr^{-1}) is substantially higher (Labarta et al. 2004).

Our study also shows that in areas with mussels (RaS), there was a significant decrease in the biomasses of nano- and microplankton, but not picoplankton (Fig. 5), which led us to assume that the smallest plankton seems to be less efficiently retained on the gills of mussels and does not constitute a suitable food for them (Norén et al. 1999, Newell 2004, Petersen et al. 2008). This selective effect on microbial plankton was also observed in mesocosm experiments (Prins et al. 1998), where mussel feeding caused changes in phytoplankton composition, leading to the predominance of the smallest fraction. Therefore, it can be concluded that the reduction in plankton biomass that we observed at RaS resulted in a modification in the size structure of the microbial planktonic community (Fig. 4b,d). In all cases, the reduction in biomass affected the main components of the population, regardless of its trophic nature (pigmented or unpigmented): diatoms during upwelling, diatoms and ANP in spring (Fig. S2e in the Supplement), and the HD *Noctiluca scintillans* in summer (Fig. S3d in the Supplement). Furthermore, HCil also experienced a remarkable reduction in spring, just when their contribution to HMP was the highest (Fig. S3d in the Supplement). This fact would support the idea that HCil could constitute an important food source for mussels (Trottet et al. 2007).

According to these results, we can infer that mussel culture exerts a top-down control over the microbial

plankton population (Dame 1996), modifying its structure by consuming micro- and nanoplankton, without affecting picoplankton. At the same time, mussel farming could be exerting a bottom-up control on phytoplankton populations that escape mussel consumption (Prins et al. 1998, Newell 2004) by means of supplying regenerated nutrients, as suggested by the significantly higher ammonium levels recorded at RaS (Figs. 2 & 3). Zúñiga et al. (2013) found that ammonium excretion rates by *Mytilus galloprovincialis* generated ammonium excess in the mussel-farming zone. In addition, the lower AC:chl *a* ratio at RaS than at ReS points to a stimulation of phytoplankton growth at RaS, a view that supports our interpretation about a certain degree of bottom-up control of phytoplankton in the mussel zones. This view also agrees well with the results obtained in the Grande-Entrée Lagoon (Trottet et al. 2008), which showed that the rates of primary production in the culture area were significantly higher than outside this zone. Similarly, the increase in nutrient availability due to mussel feeding activity probably allowed the relatively higher phytoplankton growth rates recorded in mussel areas in Beatrix Bay in New Zealand (Ogilvie et al. 2000), when outside the farming area there was nitrogen limitation. Even though this bottom-up control of phytoplankton is expected to be more effective in oligotrophic environments (Asmus & Asmus 1993), our results suggest that it could also exists in upwelling zones without nutrient limitation.

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