# Microzooplankton feeding impact in a coastal upwelling system on the NW Iberian margin: the Ría de Vigo

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ABSTRACT

The dilution technique, combined with identification and enumeration of pico-, nano- and microplankton by microscopy, was used to estimate microzooplankton impact on the microbial community in surface waters of a coastal embayment on the NW Iberian upwelling system. Microzooplankton were important consumers of autotrophic and heterotrophic plankton in this system, feeding up to 93% of standing stock and more than 100% of production of several groups. Heterotrophic bacteria and heterotrophic picoflagellates experienced the highest and constant impact, with 75-84% of their standing stocks and 85-102% of their production being channelled through the microbial food web. Pico- and nanophytoplankton were also consumed, although maximum grazing occurred on diatoms during upwelling events, coinciding with highest primary production. Predation on pico-nanoheterotrophs was especially relevant under downwelling conditions, when consumption of total carbon and particularly autotrophic carbon was considerably lower than during upwelling. The results suggest that the existence of a multivorous food web, extending from the microbial loop to the herbivorous food web, could be a major feature in this coastal upwelling system. The microbial loop, which occurs as a permanent background in the system, would contribute to sustain the microbial food web during downwelling, whereas the herbivorous food web could coexist with a microbial food web based on large diatoms during upwelling. The multivorous food web would partially divert diatoms from sinking and hence favour the retention of organic matter in the water column. This could enhance the energy transfer to higher pelagic trophic levels in coastal upwelling systems.

*Keywords:* Microzooplankton, microbial food web, dilution technique, microscopic enumeration, coastal upwelling systems, Ría de Vigo

## 1. Introduction

The role of heterotrophic nano- and microplankton (hereafter microzooplankton) is widely recognised across marine systems, exerting a fundamental ecological function recycling nutrients and transferring matter and energy from the small-sized organisms to large consumers (Calbet and Landry, 2004). Nonetheless, in coastal upwelling systems, the function of microzooplankton has classically been mistreated (Ryther, 1969). Typically, it has been accepted that short food chains prevail in these productive areas, with large phytoplankton directly passing to zooplankton and then to larger animals. However, microzooplankton are abundant in upwelling regions, and evidence of their importance continuously increases (Painting et al., 1992; Neuer and Cowles, 1994; García-Pámanes and Lara-Lara, 2001; Vargas and González, 2004). Thus, it is well known that microzooplankton not only feed on small phytoplankton, they also impact on communities dominated by large phytoplankton (Calbet, 2008), often abundant in coastal upwelling areas. Particularly, heterotrophic dinoflagellates are now considered as major herbivores of large and chain-forming diatoms (Sherr and Sherr, 2007). Microzooplankton also consume heterotrophic plankton, such as bacteria and other phagotrophic organisms (Azam et al., 1983; Rassoulzadegan and Sheldon, 1986; Jeong, 1999), and so modulate biogeochemical fluxes through complex interactions within the microbial food web.

Short food chains, owing to the few steps involved, are more efficient than microbial food webs transferring energy to higher trophic levels. Nonetheless, a significant amount of material can be removed from the photic layer, via rapid sinking of large diatoms and/or faecal material from large metazoans (Turner, 2002), in areas or moments with predominance of short food chains. Consequently, the co-occurrence of the two trophic ways or the existence of a “multivorous food web” (Legendre and Rassoulzadegan, 1995), in which microzooplankton are a key player, could contribute

to reduce carbon losses from the photic layer while still retaining enough efficiency in the energy transfer to high pelagic trophic levels. Knowledge of the role that microzooplankton play in coastal upwelling systems is hence fundamental to advance in our understanding of carbon fluxes in these highly productive oceanic areas.

Reports on the importance of microzooplankton in the Iberian upwelling are scarce. Although some studies, through indirect approaches, suggest that microzooplankton activity must be important in this upwelling area (Figueiras and Ríos, 1993; Bode and Varela, 1994; Bode et al., 2004), microzooplankton grazing activity was only determined in shelf and oceanic waters (Fileman and Burkill, 2001). On the contrary, microzooplankton activity in the highly productive coastal bays known as Rías Baixas (Fig. 1a) has never been determined. In the Rías Baixas, coastal upwelling, induced by northerly winds, introduces subsurface nutrient-rich water through the bottom from spring to autumn. During the rest of the year, the dominant southerly winds cause downwelling (Fraga, 1981). Relaxation and even opposite events can however occur within each season, in response to short-time variations in the wind regime driven by small fluctuations in the large-scale climatology of the North Atlantic. Plankton composition in these systems is typical of temperate coastal regions, but it is also influenced by the hydrographic variability imposed by upwelling-downwelling events (Figueiras et al., 2002). Thus, large diatoms are abundant in spring, whereas the plankton community in summer is composed of heterotrophic and autotrophic organisms, with autotrophy (diatoms) dominating during upwelling events and heterotrophy (dinoflagellates and ciliates) attaining greater importance during relaxations. Large pigmented dinoflagellates, sometimes forming harmful blooms, are common in autumn, while small flagellates dominate in winter. Pico- and nanophytoplankton are present in the system all through the year, though their contribution to the phytoplankton community is higher in winter, because peaks of

autotrophic biomass during upwelling are caused by diatoms (Figueiras et al., 2002; Arbones et al., 2008). Therefore, biogeochemical fluxes in this coastal upwelling system could be affected by the high variability in plankton composition and size structure.

The aim of this work was to quantify for the first time in the coastal upwelling system of the Ría de Vigo (the southernmost of the Rías Baixas, Fig. 1b), the feeding impact of microzooplankton on the several autotrophic and heterotrophic plankton groups (≤200 μm) during different hydrographic conditions. It was achieved by performing dilution experiments (Landry and Hassett, 1982) associated with identification and enumeration of plankton components by microscopy.

## 2. Materials and methods

### *2.1. Sampling and experimental set up*

Sampling took place at dawn in a station located in the main channel at the central part of the Ría de Vigo (Fig. 1b) in February, April, July and September 2002 on board of the R/V *Mytilus*.

For the hydrographic survey, the station was sampled four times each month (see Fig.2). Salinity and temperature were recorded with a SBE 9/11 CTD probe attached to a rosette sampler. Water samples were collected in plastic bottles (~ 75 ml) from the CTD upcasts to determine nitrate concentrations in the water column. These samples were kept refrigerated until their analysis in the laboratory within 2 h of their collection.

Mortality and growth rates of autotrophic and heterotrophic plankton ≤200 μm at the surface layer were estimated using the dilution technique (Landry and Hassett, 1982) on two days during each sampling month (see Fig. 2). All experimental containers, bottles, filters and tubing were soaked in 10% HCl and rinsed with Milli-Q water before each experiment. Surface water was collected from 2 dips of a 30 l Niskin bottle. Water from the first dip was gravity filtered through a 0.2 μm Gelman Suporcap to a polycarbonate

container and water from the second dip was directly and gently transferred to another polycarbonate container. Both containers were kept in the dark while being transported to the laboratory within 2 h of their collection.

At the laboratory, the filtered water from the first dip and the unfiltered seawater obtained from the second dip were gently combined into carboys to obtain dilution levels of ~ 10, 20, 40, 60, 80 and 100% of unfiltered seawater. The exact dilution levels were checked from chlorophyll *a* (chl *a*) concentrations determined in triplicate samples (see below). Two clear polycarbonate bottles of 2.3 l were completely filled from each dilution level and incubated for 24 h at simulated *in situ* light and temperature conditions in an incubator placed in the laboratory's terrace. Temperature was controlled by flowing seawater directly pumped from the sea, whereas a grey mesh was placed on top of the incubator to allow the passage of ~60% of incident irradiance. This is a light level similar to that found in the surface layer of the Ría de Vigo.

Nutrient addition, often performed in this type of experiments, can however affect phytoplankton growth negatively (Lessard and Murrell, 1998; Worden and Binder, 2003). Additionally, changes in the feeding behaviour of microzooplankton within the dilution series have also been reported (Worden and Binder, 2003). Because microzooplankton feeding behaviour is particularly relevant determining mortality patterns in this system (Teixeira and Figueiras, 2009) and like other authors (e.g. García-Pámanes and Lara-Lara, 2001; Landry et al., 2008, 2009), we did not add nutrients to our incubation bottles. The aim was to maintain the plankton community as close as possible to *in situ* conditions.

Triplicate 250 ml subsamples were taken from all dilution levels at the beginning and at the end of the incubation time for the determination of chlorophyll a (chl a) concentrations. These subsamples were filtered through 25 mm Whatman GF/F filters and these filters were then stored frozen at -20º C until their analysis.

Subsamples for the determination of the carbon biomass (mg C $m^{-3}$) of autotrophic and heterotrophic pico- (0.2 to 2 µm), nano- (2 to 20 µm) and microplankton (20 to 200 µm), were taken from the unfiltered seawater at the beginning and from all dilution bottles at the end of the incubation time. The initial concentrations of these organisms for each dilution level were estimated taking into account the dilution factor. For pico- and nanoplankton biomass, subsamples of 10 ml were fixed with buffered 0.2 µm filtered formaldehyde (2% final concentration) and stained with DAPI at 0.1 µg $ml^{-1}$ final concentration (Porter and Feig, 1980). After 10 minutes in the dark, these samples were filtered through 0.2 µm black Millipore-Isopore filters. These filters were then stored frozen in the dark until their analysis. For microplankton, subsamples of 250-500 ml were preserved in Lugol's iodine and stored in the dark.

*2.2. Analyses*

Nitrate concentrations were analysed using Alpkem autoanalysers according to Hansen and Grasshoff (1983).

Chl *a* concentration (mg $m^{-3}$) was determined by fluorometry after pigments extraction in 90% acetone at 4º C in the dark during 24 h.

Pico- and nanoplankton were examined at x1000 magnification using an epifluorescence microscope, after immersing the filters in low fluorescence immersion oil. Autotrophic organisms were enumerated under blue light excitation and heterotrophic organisms were counted under excitation with UV light. Although *Prochlorococcus* cannot be accurately counted with this technique, their abundance is not important in this coastal system (Rodríguez et al., 2003). Bacterial biomass was estimated according to Lee and Furhmann (1987). Dimensions of at least 30 cells of the other groups were taken and cell volumes were calculated assuming spherical shape.

Cell carbon was estimated following Verity et al. (1992) for pico- and nanoflagellates and Bratbak and Dundas (1984) for *Synechococcus*-type cyanobacteria.

For microplankton, a variable volume of 10-200 ml (depending on chl *a* concentration and the number of organisms counted) was sedimented in composite sedimentation chambers and observed through an inverted microscope. The organisms were counted and identified to the species level or to the nearest taxonomic level that morphological characteristics and settled position allowed. Distinction between phototrophic and heterotrophic species of dinoflagellates was made following bibliographic records (e.g. Lessard and Swift, 1986; Larsen and Sournia, 1991) and also using epifluorescence microscopy. Dimensions were taken to calculate cell biovolumes after approximation to the nearest geometrical shape (Hillebrand et al., 1999) and cell carbon was calculated following Strathmann (1967) for diatoms and dinoflagellates, Verity et al. (1992) for flagellates other than dinoflagellates (>20 µm) and Putt and Stoecker (1989) for ciliates.

Pigmented pico-, nano- and microflagellates were assumed to be autotrophic, even though mixotrophy is common within the microbial community (Stoecker, 1999; Unrein et al., 2007; Zubkov and Tarran, 2008).

*2.3. Growth and mortality rates*

Instantaneous growth (µ, $d^{-1}$) and mortality ($m$, $d^{-1}$) rates for each plankton group, chl *a*, total carbon biomass (TC), total autotrophic carbon biomass (AC) and total heterotrophic carbon biomass (HC) were estimated by linear regression of the net growth rates $k$ ($d^{-1}$) against the dilution factor $D$ (Landry and Hassett, 1982):

$$k = \mu - m \cdot D \qquad (1)$$

Net growth rates $k$ ($d^{-1}$) are:

$$k = \frac{1}{t} \cdot \ln\left(\frac{C_t}{C_0}\right) \quad (2)$$

where $t$ is the duration of the experiment (1 day) and $C_0$ and $C_t$ are the initial and final carbon or chl *a* concentrations, respectively.

In cases of non-linear feeding responses, μ was obtained by regression of the linear part of the response and $m$ was calculated by the difference between μ and the net growth rate in the undiluted sample. A complete description of these types of non-linear responses can be found in Teixeira and Figueiras (2009).

The quantity of carbon and chl *a* consumed ($G$, mg $m^{-3}$ $d^{-1}$) and produced ($P$, mg $m^{-3}$ $d^{-1}$) were calculated as:

$$G = m \times C_m \qquad P = \mu \times C_m \quad (3)$$

where $C_m$ (mg $m^{-3}$) is:

$$C_m = C_0 \left[e^{(\mu - m)t} - 1\right] / (\mu - m)t \quad (4)$$

Therefore, the daily impact on production ($\%P$, $d^{-1}$) can be estimated as:

$$\%P = \frac{G}{P} \times 100 = \frac{m}{\mu} \times 100 \quad (5)$$

The impact on the standing stock ($\%SS$, $d^{-1}$) was obtained as:

$$\%SS = (1 - e^{-m}) \times 100 \quad (6)$$

## 3. Results

### *3.1. Hydrography*

The rapidly changing hydrographic conditions commonly observed in the region were found during the four sampling periods. Thus, the upwelling that characterised the first two days of sampling in February (Figs. 2a-c), quickly reverted to downwelling on the third day (February 25) to persist until the end of sampling. The two dilution experiments of this month were done under these two contrasting conditions. The opposite situation occurred in April (Figs. 2d-f), when the water column at the beginning of sampling was still responding to a previous downwelling event. Then, after a weak upwelling event, which did not reach the surface, the water column became stratified. Again, the two dilution experiments were performed under these two different environmental conditions. July (Figs. 2g-i) showed a stratified water column with a short relaxation separating two upwelling events, during which the dilution experiments were done. Downwelling was the main feature during the sampling of September (Figs. 2j-l). Although nitrate concentrations in the surface layer were <1 μM in April and September, concentrations of total inorganic nitrogen <1 μM were only recorded in April (Teixeira and Figueiras, 2009). Further details on the hydrographic conditions can be found in Piedracoba et al. (2005).

### *3.2. Plankton biomass and composition*

Total plankton (≤200 μm) biomass, although variable, showed a clear seasonal trend (Fig 3a), with low values in winter (February) and higher values in summer (July) and

the beginning of autumn (September). The highest biomass (819 mg C $m^{-3}$) was recorded during the first upwelling event of July, whereas the lowest (84 mg C $m^{-3}$) coincided with the downwelling of the end of February. The plankton community was clearly dominated by autotrophs (Fig. 3a). However, this dominance was more evident in summer and autumn (July and September), when autotrophic carbon (AC) accounted for 80 ± 8% of total planktonic carbon (TC). In winter and spring (February and April) the contribution of AC to TC (58 ± 5%) was appreciably lower. Changes in total plankton biomass were largely due to variations in autotrophic biomass [TC = (78.39 ± 28.78) + (1.07 ± 0.08)AC, $r^2 = 0.96$, $p < 0.001$]. Variations in heterotrophic plankton biomass were considerably lower (35 mg C $m^{-3}$ on February 28; 159 mg C $m^{-3}$ on April 18) and these variations did not significantly contribute to the changes recorded in TC (Fig. 3a).

Diatoms together with autotrophic nanoflagellates (ANF) and autotrophic dinoflagellates (ADF) accounted for the largest fraction (94 ± 5%) of AC (Fig. 3b). Diatoms, always present (Fig. 3b), were especially abundant in July, when they accounted for >90% of AC. In contrast, the biomass of ANF and their contribution to AC was higher in February and April (30 ± 17 mg C $m^{-3}$ and 27 ± 5%, respectively) than in July and September (13 ± 5 mg C $m^{-3}$ and 4 ± 3%, respectively). The presence of ADF was only significant during the downwelling of September (Fig. 3b), when they accounted for 34 ± 14% of AC. *Synechococcus*-type cyanobacteria and autotrophic picoflagellates (APF) represented a very small fraction of the total autotrophic biomass, 2 ± 2% and 4 ± 5% respectively (data not shown). Although chl *a* followed a similar evolution to that of AC (Fig. 3b), both variables were not significantly correlated, reflecting the variable AC : chl *a* ratios, which fluctuated between 19 on April 11 and 106 on July 18.

In addition to seasonal variations in biomass, the composition of the diatom community (Table 1) also showed changes related to the short-term hydrographic variability (Fig. 2). This short-time variability was especially evident for the two upwelling-downwelling sequences registered in February and September (Table 1). Thus, *Skeletonema* cf. *costatum*, which dominated during the first sampling of February virtually disappeared form the water column a week later, being replaced by larger chain-forming species (*Thalassiosira rotula* and *Chaetoceros* spp.). Similarly, the dominance of *Proboscia alata* on September 19 vanished on September 26, when the diatom community turned to be more diverse with significant contributions of other species (*Skeletonema* cf. *costatum*, *Chaetoceros* spp., *Leptocylindrus danicus* and *Thalassiosira nana*). However, species substitution was not observed during the samplings of April and July. Changes during these two sampling periods were limited to variations in abundance (Table 1). *Chaetoceros* spp., *Pseudo-nitzschia* cf. *seriata*, *Detonula pumila* and *T. rotula* were the more abundant species in April, whereas small chain-forming diatoms (*Leptocylindrus danicus* and small *Chaetoceros* spp.) dominated in July. The large pigmented dinoflagellates *Ceratium fusus* and *C. furca* were especially abundant in September, dominating the ADF community (Table 1). *Dinophysis acuminata* was also important on September 19, the first sampling of this month (Table 1).

Seasonal variability also occurred within the heterotrophic community, although this variability was not as evident as that recorded for the autotrophic community. Variations in the heterotrophic community were mainly caused by heterotrophic nanoflagellates (HNF), heterotrophic dinoflagellates (HDF) and ciliates (Fig. 3c), because biomass ($17 \pm 9$ mg C $m^{-3}$) and contribution ($21 \pm 8\%$) of heterotrophic bacteria (HB) to HC, as well as biomass ($13 \pm 5$ mg C $m^{-3}$) and contribution ($15 \pm 4\%$) of heterotrophic picoflagellates (HPF) remained relatively constant (data not shown). HNF

were more important in winter and spring (February and April), when they reached the highest biomass (116 mg C $m^{-3}$ on April 18), than in summer and autumn (Fig. 3c). On the contrary, the contribution of HDF to HC was higher in summer and autumn (22 ± 6%), attaining the highest biomass (31 mg C $m^{-3}$) on September 26 (Fig. 3c). The HDF community was mostly composed of small naked species (<50 µm) in spring, whereas large species (*Noctiluca scintillans*, *Gyrodinium* spp. and *Protoperidinium* spp.) were more abundant in summer and autumn (Table 1). The biomass of heterotrophic ciliates (Fig. 3c) was positively correlated with the biomass of HDF ($r = 0.84$; $p < 0.01$), varying between ~0.20 mg C $m^{-3}$ during the two samplings of February and 24 mg C $m^{-3}$ on September 19. Aloricate choreotrichs >20 µm (Table 1) were the major components, accounting for 77 ± 1% of the total biomass of ciliates.

Metazoa ≤200 µm were only present in very few samples at low abundance and their contribution to microzooplankton biomass and the dynamics of the microbial food web was not considered.

*3.3. Growth and mortality rates*

Growth and mortality rates derived from the dilution experiments for the several plankton groups were highly variable (Fig. 4; see Tables 2 and 3 in Teixeira and Figueiras, 2009 for levels of significance). Although non significant responses were found in several experiments, smaller forms (APF and *Synechococcus*) generally showed the highest growth and mortality rates among autotrophs (Figs. 4a, d). A seasonal increase in the growth rates of APF was especially evident, reaching values (~3 $d^{-1}$) at the end of summer (Fig. 4a) which were the highest recorded among all plankton groups and experiments (Fig. 4). Significant responses were also obtained for diatoms in the experiments of February and July (Figs. 4a, d), with both growth and mortality rates being higher in July. HB and HPF showed significant responses in all experiments

(Figs. 4b, e). Mortality rates of HPF ($m$ = 1.88 ± 0.44 $d^{-1}$) were slightly higher than those of HB ($m$ = 1.46 ± 0.30 $d^{-1}$), but they were not significantly different from the corresponding growth rates ($p$ = 0.91 for HPF, μ = 1.91 ± 0.46 $d^{-1}$; $p$ = 0.16 for HB, μ = 1.79 ± 0.51 $d^{-1}$, t-test for two samples). Significant results for HNF were only obtained during the experiments of April and July (Fig. 4b), with $m > \mu$ in April and the opposite in July. Rates for chl *a* and AC, both representing changes in the autotrophic community, were only similar in February (Fig. 4c, f). Although mortality rates were not correlated, growth rates of chl *a* and AC showed positive correlation ($r^2$ = 0.71, $p <$ 0.01).

Growth rates of autotrophs were usually higher than their mortality rates (Fig. 5a), whereas growth and mortality rates of heterotrophs were more tightly coupled (Fig. 5b), particularly those of HB and HPF.

*3.4. Microzooplankton impact on the microbial plankton community*

Carbon consumption and production (Figs. 6, 7) derived from the rates obtained for the bulk properties (TC, AC and HC) were not significantly different ($0.32 \leq p \leq 0.99$, t-test for two samples) from the corresponding estimates obtained by the addition of carbon consumed and produced by the several plankton components with significant responses in the dilution experiments. This suggests that the plankton groups with significant responses in the experiments (Fig. 4) were those actually consumed and growing in the Ría de Vigo at that time.

The highest consumption of microbial plankton biomass occurred during the upwelling of July, with 987 mg C $m^{-3}$ $d^{-1}$ being consumed in the first experiment and 383 mg C $m^{-3}$ $d^{-1}$ in the second (Fig. 6a). Most of this carbon was autotrophic, mostly diatoms (89% and 73% of TC on July 18 and July 26, respectively) (Fig. 6b). Consumption of TC was considerably lower in the other experiments, varying between

125 mg C $m^{-3}$ $d^{-1}$ on April 18 and 66 mg C $m^{-3}$ $d^{-1}$ on February 21 and September 26 (Fig. 6a). Diatoms were also grazed during the upwelling of February 21 (45 mg C $m^{-3}$ $d^{-1}$) (Fig. 6b), when they accounted for 90% and 68% of the AC and TC consumed, respectively. In the other experiments, the AC consumed (1 - 25 mg C $m^{-3}$ $d^{-1}$) corresponded to pico- and nanophytoplankton, which in general occurred at very low rates (13 ± 8 mg C $m^{-3}$ $d^{-1}$). Consumption of chl *a* did not follow that of AC (Fig. 6b), showing apparent deviations in April and July. These deviations were not only due to variations in AC : Chl *a* ratios, but also caused by differences in the mortality rates (Figs. 4c, f).

Below the high variability recorded in carbon consumption due to grazing on diatoms (Figs. 6a, b), there was a rather constant predation (67 ± 27 mg C $m^{-3}$ $d^{-1}$) on heterotrophic carbon (Figs. 6a, c). It mainly occurred on HB (29 ± 10 mg C $m^{-3}$ $d^{-1}$) and HPF (24 ± 10 mg C $m^{-3}$ $d^{-1}$). HNF were only apparently consumed in April and July (Fig. 6c). Predation on HC was especially relevant during non-upwelling conditions, when it represented >60% of the TC consumed and >90% in some occasions (April 18, Fig. 6a).

Consumption of TC (Fig. 6a) was strongly correlated ($r^2$ = 0.94, p < 0.001) with TC production (Fig. 7a), and it was basically due to the correlation between production and consumption of AC ($r^2$ = 0.97, $p$ < 0.001), which in fact was due to the coupling between consumption and production of diatoms ($r^2$ = 0.97, p < 0.001). Thus, the upwelling of July showed the highest TC production (1251 mg C $m^{-3}$ $d^{-1}$ on July 18 and 643 mg C $m^{-3}$ $d^{-1}$ on July 26) (Fig. 7a), with diatoms (Fig. 7b) accounting for 82% and 76% of the TC production, respectively. The contribution of diatoms (165 mg C $m^{-3}$ $d^{-1}$) to AC production (179 mg C $m^{-3}$ $d^{-1}$) and TC production (273 mg C $m^{-3}$ $d^{-1}$) was also important during the upwelling of February 21 (Figs. 7a, b). Production of chl *a* and AC

were correlated ($r^2$ = 0.86, p < 0.001) (Fig. 6b), because of the correlation between both growth rates.

Regardless of the strong correlation between AC production and herbivory, the impact of microzooplankton on AC production was highly variable (Table 2). Grazing, although low (Fig. 6b), largely exceeded phytoplankton production during the downwelling of April (Table 2), when AC production (1-8 mg C $m^{-3}$ $d^{-1}$) was extremely low (Figs. 7a,b). Excluding these two experiments of April, microzooplankton consumed between 28% and 83% (average 47 ± 22%) of the AC produced. The highest impact took place on July 18 (Table 2) when AC production was also the highest (Figs. 7a, b). The impact on the production of chl *a* (Table 2) showed a similar pattern. Consumption largely exceeded production in April, whereas microzooplankton consumed between 19-103% (average 47 ± 32%) of the chl *a* produced in the other months (Table 2). Like AC, chl *a* production experienced the highest impact on July, but in this case on July 26 (Table 2).

Between 58% and 209% (average 95 ± 51%) of the HC production was consumed by microzooplankton (Table 2). The highest impact on HC production (209%) was recorded on April 18, when consumption on AC (1 mg C $m^{-3}$ $d^{-1}$) was the lowest (Fig. 6b) and almost all consumption (125 mg C $m^{-3}$ $d^{-1}$) took place on HC (Fig. 6a). Between 63 and 135% (average 102 ± 26%) of the HPF production and between 59 and 123% (average 85 ± 25%) of the HB production were daily removed by microzooplankton (Table 2). Microzooplankton consumed between 24 and 299% (average 92 ± 87%) of TC produced.

The impact on standing stocks showed lower variability (Table 3), with ~40% of the AC, chl *a* and TC stocks being removed by microzooplankton. The standing stock of HC was slightly more affected (57 ± 11%) and again heterotrophic picoplankton (HPF and HB) experienced the highest impact.

## 4. Discussion

The high hydrographic variability that characterizes the Ría de Vigo, ideally requires a survey with high temporal and spatial resolution to fully capture all scenarios that can be found in this system during the year. Even though our sampling was relatively scarce to catch all variability in this high dynamic system, the hydrographic conditions and the associated plankton communities observed during the four sampling periods were characteristic of the typical seasonal cycle in the Ría de Vigo (Figueiras and Ríos, 1993). Therefore, our 8 experiments can be considered as representative of the several environmental conditions regularly found in this coastal system. Overall, major variations in plankton biomass occurred due to variations in autotrophic biomass (Fig. 3). Specifically, diatoms were responsible for the peaks of biomass and primary production recorded in response to upwelling events. This agrees with the established picture for phytoplankton dynamics in this area and other upwelling regions, where nutrient inputs cause major variations in phytoplankton through the addition of large size classes (Chisholm, 1992, Cermeño et al., 2006; Arbones et al., 2008). Among heterotrophs, picoheterotrophic organisms showed a rather constant background of biomass, whereas microzooplankton presented a seasonal succession also typical of this system (Figueiras and Ríos, 1993). Thus, large forms of heterotrophic dinoflagellates and ciliates appeared throughout summer and autumn, while small flagellates were relatively more important in winter and spring (Fig. 3, Table 1). This distribution also compares with other upwelling regions with the abundance of the larger forms associated with the productive seasons when large size preys are more abundant (e.g. Neuer and Cowles, 1994; Vargas et al., 2007; Calbet, 2008).

*4.2. Microzooplankton impact on phytoplankton*

Microzooplankton impact on phytoplankton estimated by changes on chl *a* and AC provided different results, despite these variables are both estimates of phytoplankton biomass. Specifically, growth rates of chl *a* and AC followed the same pattern but mortality rates were not correlated at all. Several reasons can be behind these differences, and it is difficult to undoubtedly explain their occurrence. First, they could result from the different AC : chl *a* ratios observed during samplings (Fig. 3b). Second, phytoplankton species can contribute with different percentages to the AC and chl *a* pools, and the selective grazing on some phytoplankton groups or species (Teixeira and Figueiras, 2009) could decouple the overall mortality estimates derived from both variables. Finally, the use of pigments as an index for changes in phytoplankton community has some inherent problems, mainly related to the incomplete degradation of chl *a* inside predators at the beginning or the end of the incubation (e.g. Barlow et al., 1988; Waterhouse and Welschmeyer, 1995) or to changes in chl *a* concentration caused by light acclimation during incubation (McManus, 1995). Nevertheless, acclimation should have been of minor importance in this case because collected surface water was incubated at similar irradiance levels.

Despite the obvious differences between the rates estimated through changes in chl *a* and AC, the mean impact of microzooplankton for all experiments on the phytoplankton standing stock (~40%) and primary production (47%, excluding the April experiments) were very similar. These values compare well with other estimates reported for coastal waters (e.g. Gallegos, 1989; Calbet and Landry, 2004) and coastal upwelling systems (Neuer and Cowles, 1994; Vargas and González, 2004; Vargas et al., 2007), including those estimates found during an upwelling/relaxation event along the NW Iberian shelf (Fileman and Burkill, 2001).

Pico- and nanoplankton were predated upon, but the total carbon biomass consumed by microzooplankton was tightly coupled with primary production, which in the Ría de Vigo was basically due to diatoms (Fig. 7). Consequently, highest consumption occurred during upwelling conditions and on diatoms (Fig. 6), a feature that apparently is common in coastal upwelling systems (Neuer and Cowles, 1994; Vargas et al., 2007) and contradicts traditional views of these systems that point to prevalence of short and very efficient food webs (Ryther, 1969). Grazing on diatoms, mainly on large and chain-forming species has been attributed to large heterotrophic dinoflagellates, which are capable of consuming organisms larger than themselves (Sherr and Sherr, 2007). The high grazing on diatoms during the upwelling of July (Fig. 6b) coincided with the presence of large heterotrophic dinoflagellates (e.g. *Noctiluca*, *Protoperidinium* spp., *Gyrodinium* spp.) in the microplankton community (Table 1); an association found when grazing on chain-forming diatoms occurred (e.g. Neuer and Cowles, 1994, Strom and Strom, 1996; Kim et al., 2007). However, the high consumption of diatoms observed in July could also be due to ciliates since large aloricate choreotrichs, known to feed on diatoms (e.g. Paranjape, 1990; Aberle et al., 2007), were present in the microzooplankton at that time (Table 1).

*4.3. Microzooplankton impact on heterotrophic plankton*

The role of microzooplankton as consumers of heterotrophic plankton has long been recognized (Azam et al., 1983). The dissolved organic compounds released into the medium by biological processes and assimilated by HB are transferred through the food web by bacterivory (and predation of small bacterivores). Regardless of the importance of this process for the cycling of matter in marine systems, the microzooplankton impact on heterotrophs is not frequently quantified. Previous studies in other coastal upwelling systems, using a modelling approach (Vargas and González, 2004; Vargas et

al., 2007) or the dilution technique (Linacre et al., 2010), suggest that microzooplankton can consume a significant fraction of heterotrophs. Here, also direct measurements of this consumption through the dilution technique effectively showed that microzooplankton consumed heterotrophs in the coastal upwelling system of the Ría de Vigo. Predation on HB and HPF was relatively important and constant (Fig. 6c), indicating that large fractions of their productions were channelled through the microbial food web (Table 2). In fact, this tight coupling could explain the relative constant biomasses of HB and HPF found over the year in this upwelling system, which suggests an efficient top-down control on these organisms by microzooplankton. Small flagellates are considered the main bacterivores, but ciliates and heterotrophic dinoflagellates can also consume HB (Fenchel, 1982; Lessard and Swift, 1985; Rassoulzadegan and Sheldon, 1986). As at least one of these bacterivores was present in the microzooplanktonic community of the Ría de Vigo (Fig. 3c), HB could be always consumed. HB can also be controlled by HPF (Rassoulzadegan and Sheldon, 1986; Calbet et al., 2001), but in the Ría de Vigo both groups were heavily consumed, hindering definitive conclusions as to what extent HB were consumed by HPF. Alternatively, the small size of HPF would permit their control by the same groups controlling HB (Lessard and Swift, 1985; Rassoulzadegan and Sheldon, 1986; Calbet et al., 2001). In addition, HNF were consumed in April and July, coinciding with the increase in the biomass and size of ciliates and heterotrophic dinoflagellates (Fig. 3c, Table 1), two groups that ingest small flagellates (Verity, 1991; Jeong, 1999). In fact, predation on HNF was important during April (Fig. 6c), when consumption of phytoplankton was extremely low (Fig. 6b), and the microbial food web was largely sustained by heterotrophs (Fig. 6a).

## 5. Conclusion

The results obtained during this study clearly demonstrate the importance of microzooplankton in the Ría de Vigo. Microzooplankton not only feed on pico- and nanoplankton, they also consume large diatoms and, in this way, contribute to establish a multivorous food web (Legendre and Rassoulzadegan, 1995) in this coastal upwelling system. The multivorous food web probably extends from the microbial loop to the herbivorous or classical food web. A rather constant carbon flow through the microbial loop was present as a permanent background in the system. Consequently, the microbial loop was relatively more important during non-upwelling conditions, when predation on autotrophs was very low or nil, and the microbial food web was basically maintained by pico- and nanoheterotrophs. A microbial food web based on large diatoms could coexist with the classical food web and the microbial loop during upwelling. Despite the fate of phytoplankton blooms in coastal upwelling systems being largely controlled by hydrodynamics, the multivorous food web should facilitate the retention of organic matter in the water column through limiting sinking, which should in turn enhance energy transfer to higher pelagic trophic levels. As the multivorous food web seems to be a common feature in coastal upwelling systems (Neuer and Cowles, 1994; Vargas and González, 2004; Vargas et al., 2007; Linacre et al., 2010), the microzooplankton-associated pathway, which channels a significant part of microbial plankton biomass, should be considered as an important component of the pelagic food web in these eutrophic systems.

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**FIGURE CAPTIONS**

Fig. 1. (a) NW Iberian margin showing the location of the four Rías Baixas. (b) Map of the Ría de Vigo showing the position of the sampled station.

Fig. 2. Variations in salinity (psu), temperature (ºC) and nitrate concentration (µM) in the water column during the four sampling periods: February, April, July and September 2002. The days when the dilution experiments were performed are in bold.

Fig. 3. Initial concentrations in the dilution experiments of (a) total heterotrophic and autotrophic carbon, (b) chl *a* and carbon of the main autotrophic plankton groups, and (c) carbon of the main heterotrophic plankton groups. HC, total heterotrophic carbon; AC, total autotrophic carbon; ADF, autotrophic dinoflagellates; ANF, autotrophic nanoflagellates; HNF, heterotrophic nanoflagellates; HDF, heterotrophic dinoflagellates.

Fig. 4. Growth (a-c) and mortality (d-e) rates for the several plankton components. ANF, autotrophic nanoflagellates; APF, autotrophic picoflagellates; HB, heterotrophic bacteria; HPF, heterotrophic picoflagellates; HNF, heterotrophic nanoflagellates; AC, total autotrophic plankton; HC, total heterotrophic carbon; TC, total carbon. See Teixeira and Figueiras (2009) for more details on these rates.

Fig. 5. Growth (µ) *versus* mortality (*m*) rates for (a) autotrophic and (b) heterotrophic organisms. ANF, autotrophic nanoflagellates; APF, autotrophic picoflagellates; HB, heterotrophic bacteria; HPF, heterotrophic picoflagellates; HNF, heterotrophic nanoflagellates. The lines represent the 1:1 relationship.

Fig. 6. Consumption of (a) total carbon, (b) chl *a* and autotrophic carbon and (c) heterotrophic carbon. HC, total heterotrophic carbon; AC, total autotrophic plankton; TC, total carbon; APF, autotrophic picoflagellates; ANF, autotrophic nanoflagellates; HB, heterotrophic bacteria; HPF, heterotrophic picoflagellates; HNF, heterotrophic nanoflagellates.

Fig. 7. Production of (a) total carbon, (b) chl *a* and autotrophic carbon and (c) heterotrophic carbon. HC, total heterotrophic carbon; AC, total autotrophic plankton; TC, total carbon; APF, autotrophic picoflagellates; ANF, autotrophic nanoflagellates; HB, heterotrophic bacteria; HPF, heterotrophic picoflagellates; HNF, heterotrophic nanoflagellates.

**Figure 1**

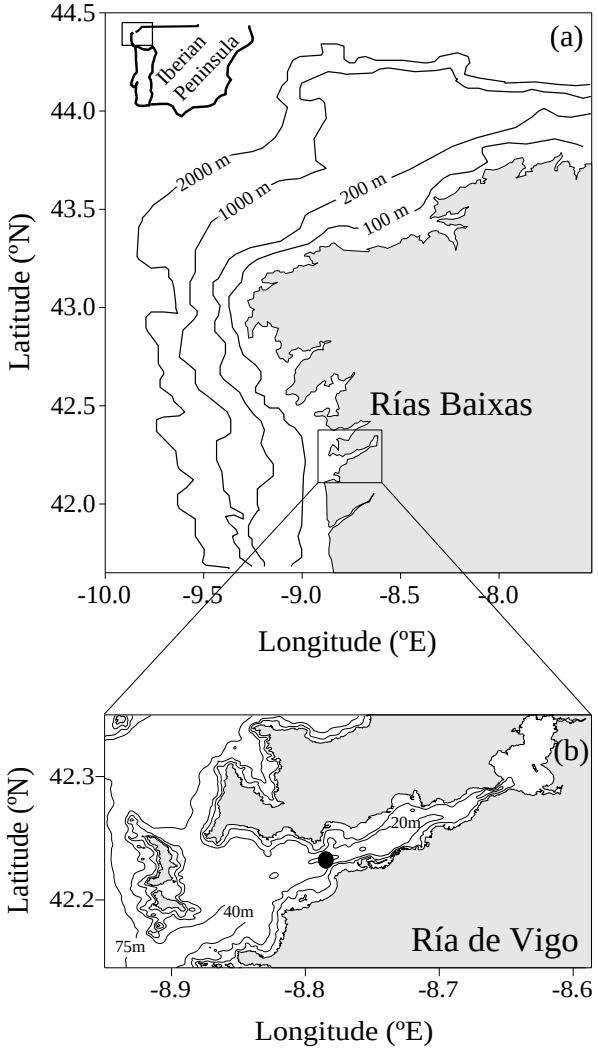


Fig. 1
Teixeira et al.

**Figure 2**

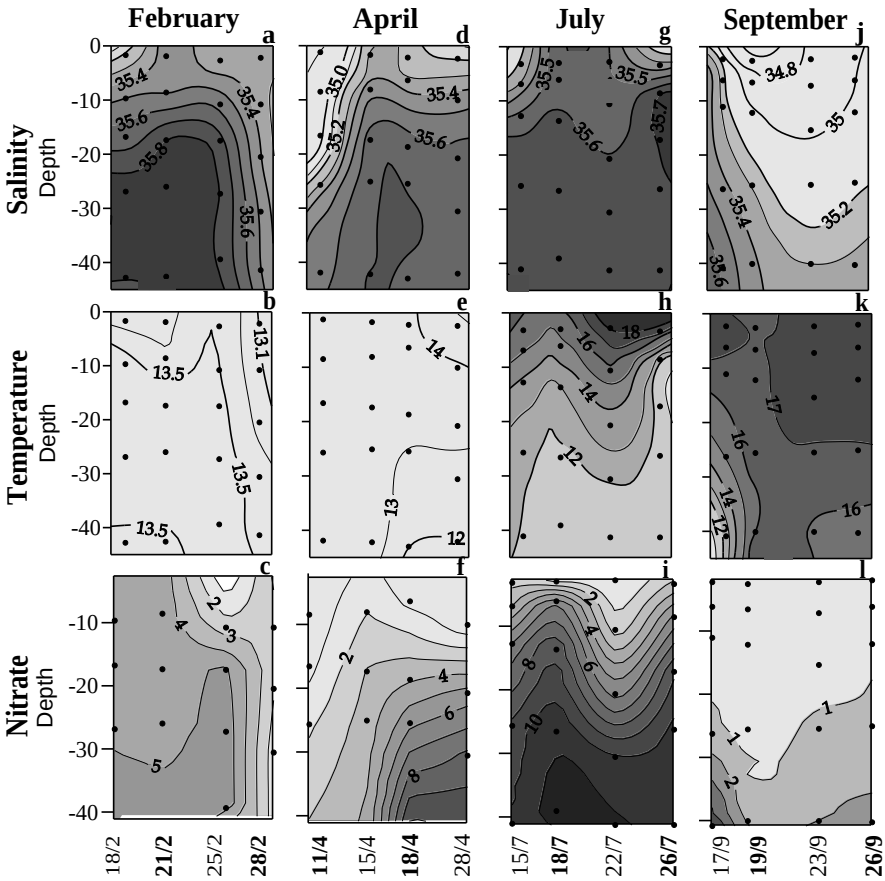


Fig. 2
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**Figure 3**

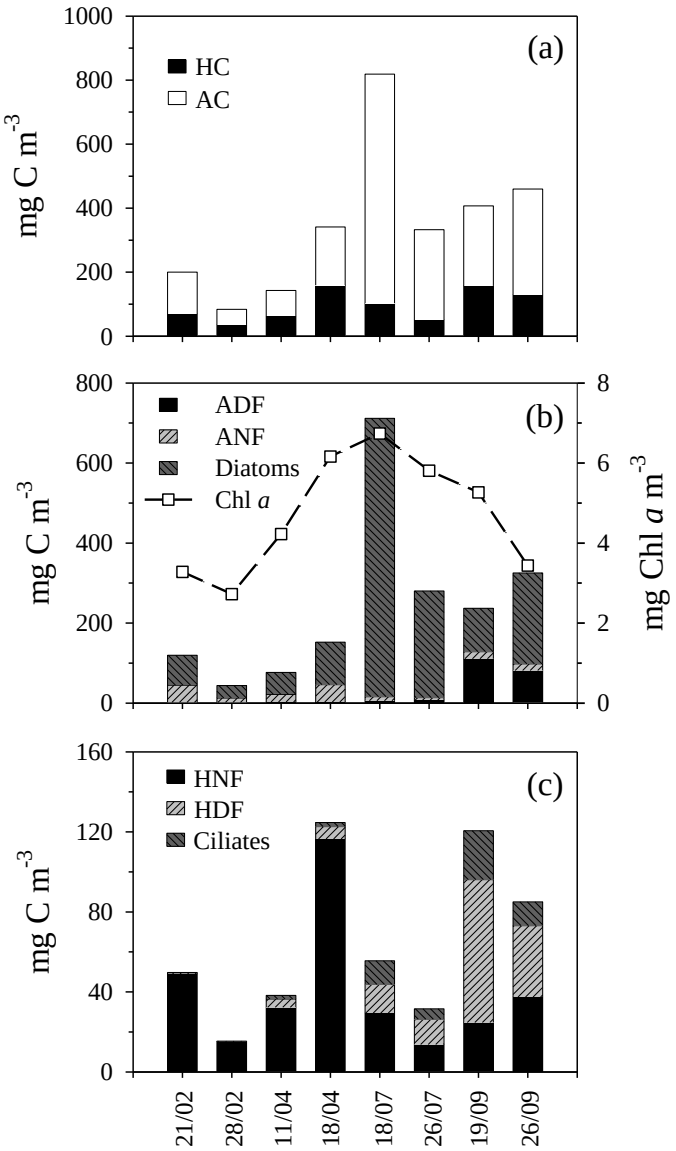


Fig. 3
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**Figure 4**

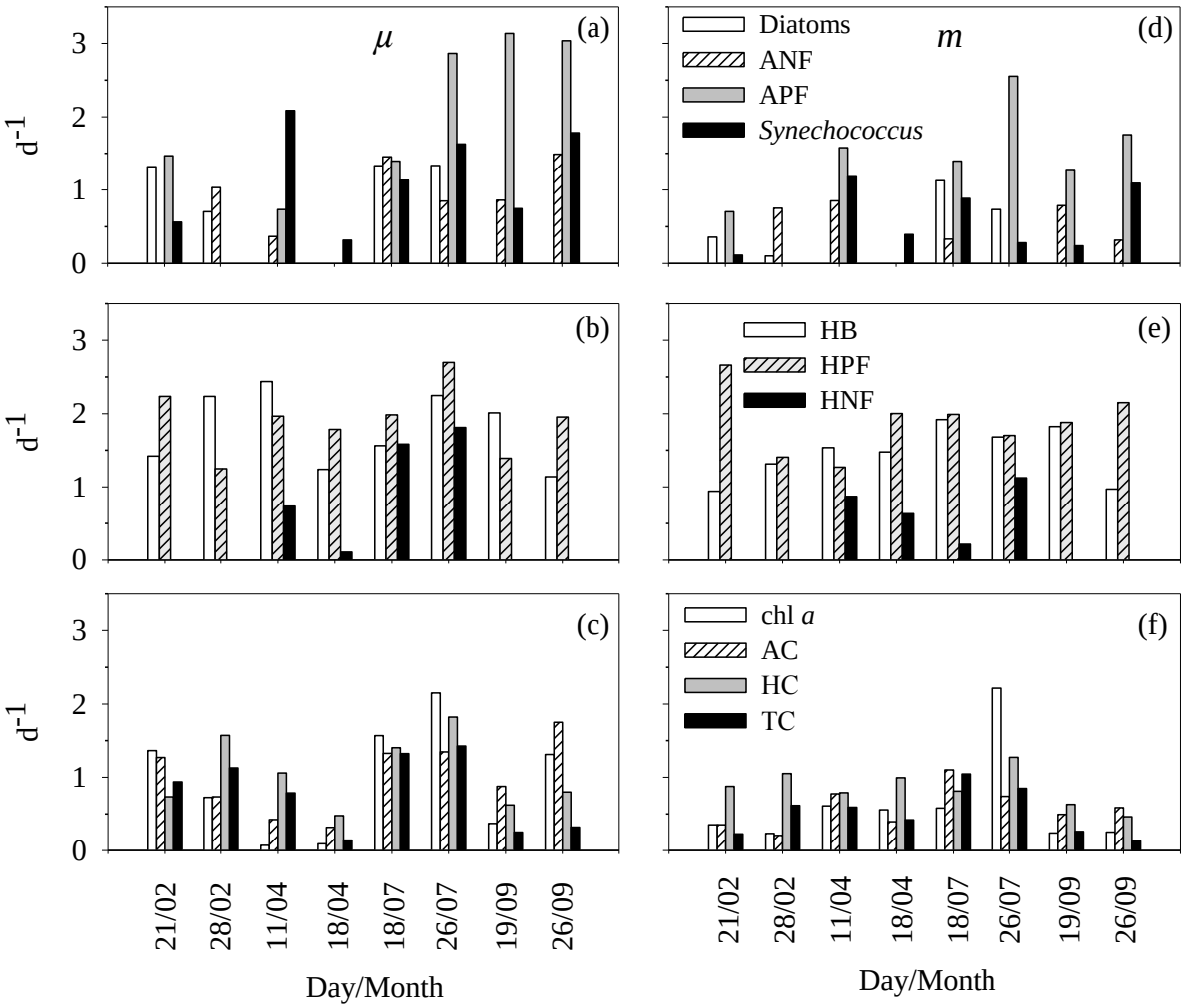


Fig. 4
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**Figure 5**

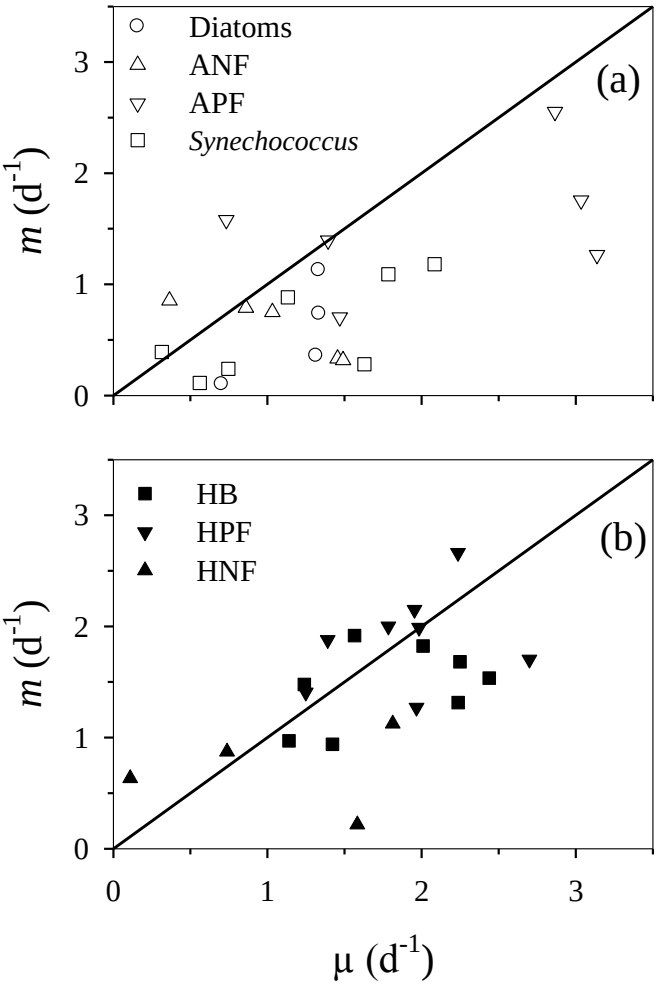


Fig. 5
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**Figure 6**

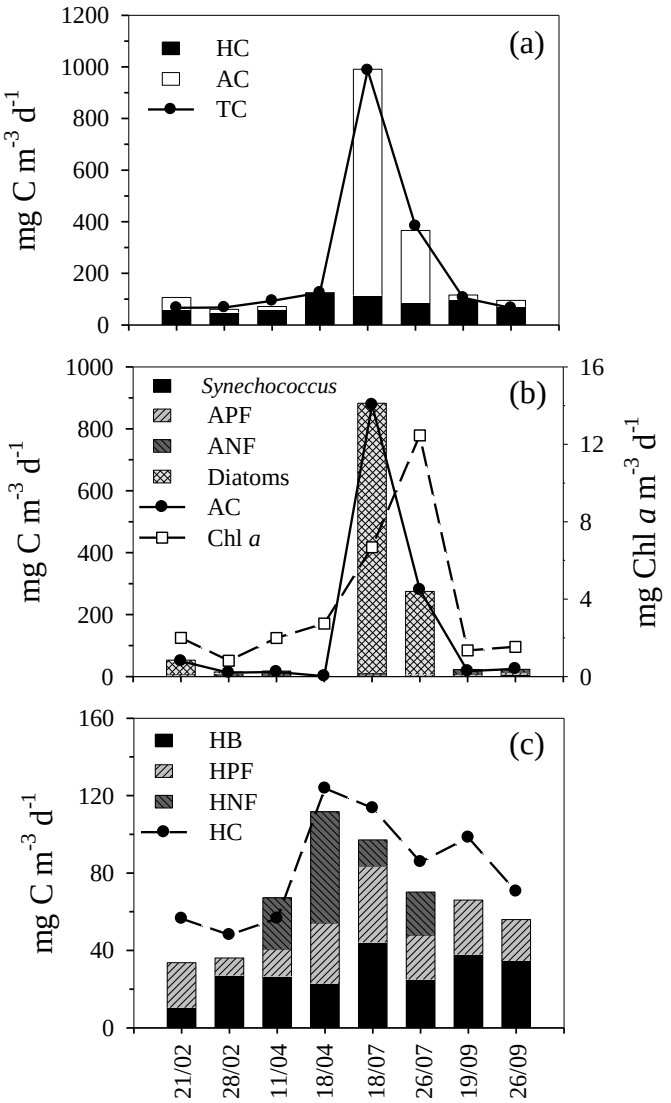


Fig. 6
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**Figure 7**

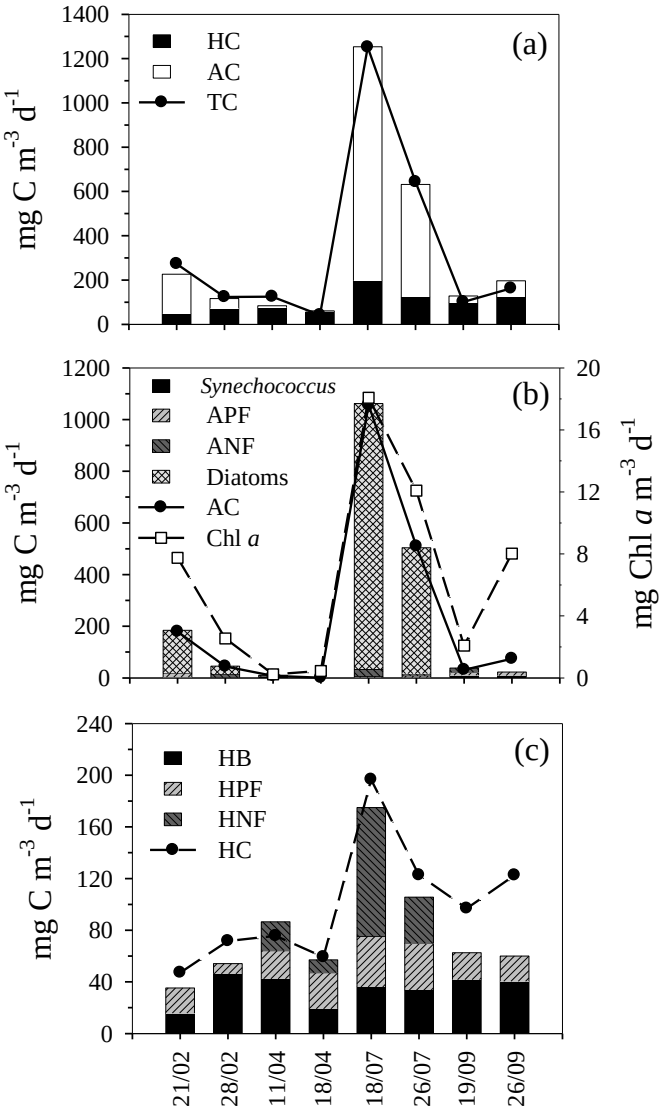


Fig. 7
Teixeira et al

Table 1. Initial biomass (mg C $m^{-3}$) of plankton species or groups more abundant for each experiment. ADF, autotrophic dinoflagellates; HDF, heterotrophic dinoflagellates; Un., unidentified.

| Plankton species or groups | 21/02 | 28/02 | 11/04 | 18/04 | 18/07 | 26/07 | 19/09 | 26/09 |
|---|---|---|---|---|---|---|---|---|
| **Diatoms** | | | | | | | | |
| *Chaetoceros* spp. | | 4.19 | 15.11 | 54.71 | 283.11 | 219.39 | 2.84 | 53.09 |
| *Detonula pumila* | | | 10.92 | 8.01 | | | | |
| *Guinardia delicatula* | | | 0.96 | 1.21 | 68.44 | 3.23 | | 0.95 |
| *Leptocylindrus danicus* | | | 0.90 | 2.67 | 319.92 | 20.51 | 17.43 | 41.02 |
| *Proboscia alata* | | | | | 0.99 | 0.99 | 79.82 | 26.32 |
| *Pseudo-nitzschia* cf. *seriata* | | | 12.10 | 12.26 | | | | 0.70 |
| *Skeletonema* cf. *costatum* | 71.81 | 0.64 | | | | | | 56.16 |
| *Thalassiosira nana* | 2.02 | | 0.77 | 1.96 | 1.78 | | 1.49 | 41.91 |
| *Thalassiosira rotula* | | 25.56 | 8.69 | 19.36 | | 0.88 | | 0.73 |
| **ADF** | | | | | | | | |
| *Amphidoma caudata* | | 0.11 | | | | | | |
| *Ceratium furca* | | 0.04 | | | | | 68.08 | 31.24 |
| *Ceratium fusus* | | | 0.06 | | 0.30 | 0.07 | 28.43 | 35.74 |
| *Dinophysis acuminata* | | | | | 1.11 | | 46.01 | 4.45 |
| *Goniodoma sphaericum* | 0.07 | | | | | | | |
| *Gymnodinium agiliforme* | 0.08 | | 0.33 | | 0.84 | 5.85 | 0.05 | 0.05 |
| *Gymnodinium* cf. *varians* | | | | 1.79 | 0.83 | 0.80 | 3.06 | 1.76 |
| *Scrippsiella trochoidea* | | | 0.29 | 0.05 | 1.52 | 0.19 | 0.34 | 0.10 |
| Un. naked dinoflagellate <50µm | 0.12 | 0.04 | 1.37 | 0.33 | 0.83 | | | 6.63 |
| **HDF** | | | | | | | | |
| *Gymnodinium* spp. <20µm | 0.46 | 0.41 | 1.70 | 2.63 | 0.77 | | 8.84 | 2.32 |
| *Gymnodinium* spp. 20-50µm | | 0.01 | 0.05 | 2.41 | | | | |
| *Gyrodinium* spp. | 0.03 | 0.01 | 0.36 | 0.13 | 1.41 | 0.81 | 0.09 | 2.12 |
| *Noctiluca scintillans* | | | | | 7.82 | 8.69 | | 5.21 |
| *Protoperidinium* spp. | 0.06 | 0.02 | 0.31 | 0.10 | 1.04 | 0.83 | 3.37 | 8.02 |
| Un. naked dinoflagellate <20µm | | | 0.97 | 0.27 | | 0.93 | 1.62 | |
| **Ciliates** | | | | | | | | |
| Un. aloricate choreotrichs >50µm | | | 0.41 | 0.37 | 3.48 | | | 1.02 |
| Un. aloricate choreotrichs 20-50µm | | | 0.10 | 0.94 | 2.30 | 0.59 | 5.82 | 1.94 |
| Un. aloricate choreotrichs <20µm | 0.10 | 0.10 | 0.09 | 0.39 | 1.75 | 3.24 | 1.69 | 2.07 |
| *Strombidium* spp. | 0.02 | 0.08 | 0.68 | 0.25 | 1.25 | 0.84 | 12.16 | 5.50 |
| Tintinnida | 0.03 | | | | | | 0.83 | |

Table 2. Percentages of production daily removed by microzooplankton. ANF, autotrophic nanoflagellates; APF, autotrophic picoflagellates; chl *a*, chlorophyll *a*; AC, total autotrophic carbon; HNF, heterotrophic nanoflagellates; HPF, heterotrophic picoflagellates; HB, heterotrophic bacteria; HC, total heterotrophic carbon; na, not applicable.

| Plankton group | 21 Feb | 28 Feb | 11 Apr | 18 Apr | 18 Jul | 26 Jul | 19 Sep | 26 Sep | Mean ± SD |
|---|---|---|---|---|---|---|---|---|---|
| **Autotrophs** | | | | | | | | | |
| Diatoms | 27 | 14 | na | na | 85 | 55 | na | na | 45 ± 31 |
| ANF | Na | 73 | 234 | na | 23 | na | 92 | 21 | 74 ± 86 |
| APF | 48 | na | 216 | na | 100 | 89 | 40 | 58 | 79 ± 69 |
| *Synechococcus* | 20 | na | 57 | 124 | 78 | 17 | 32 | 61 | 56 ± 38 |
| chl *a* | 26 | 32 | 871 | 617 | 37 | 103 | 65 | 19 | 221 ± 331 |
| AC | 28 | 28 | 184 | 124 | 83 | 55 | 56 | 33 | 74 ± 55 |
| **Heteterotrophs** | | | | | | | | | |
| HNF | Na | na | 118 | 578 | 14 | 62 | na | na | 193 ± 260 |
| HPF | 119 | 113 | 65 | 112 | 100 | 63 | 135 | 110 | 102 ± 26 |
| HB | 66 | 59 | 63 | 119 | 123 | 75 | 91 | 85 | 85 ± 25 |
| HC | 120 | 67 | 75 | 209 | 58 | 70 | 102 | 58 | 95 ± 51 |
| **Total Carbon** | 24 | 55 | 75 | 299 | 79 | 60 | 104 | 41 | 92 ± 87 |

Table 3. Percentages of the standing stocks daily removed by microzooplankton. ANF, autotrophic nanoflagellates; APF, autotrophic picoflagellates; chl *a*, chlorophyll *a*; AC, total autotrophic carbon; HNF, heterotrophic nanoflagellates; HPF, heterotrophic picoflagellates; HB, heterotrophic bacteria; HC, total heterotrophic carbon; na = not applicable.

| Plankton group | 21 Feb | 28 Feb | 11 Apr | 18 Apr | 18 Jul | 26 Jul | 19 Sep | 26 Sep | Mean ± SD |
|---|---|---|---|---|---|---|---|---|---|
| **Autotrophs** | | | | | | | | | |
| Diatoms | 30 | 9 | na | na | 68 | 52 | na | na | 40 ± 25 |
| ANF | na | 53 | 57 | na | 28 | 0 | 55 | 27 | 37 ± 23 |
| APF | 50 | na | 79 | na | 75 | 92 | 72 | 83 | 65 ± 31 |
| *Synechococcus* | 11 | na | 69 | 32 | 59 | 24 | 21 | 66 | 40 ± 24 |
| chl *a* | 30 | 21 | 46 | 43 | 44 | 89 | 21 | 22 | 39 ± 23 |
| AC | 30 | 19 | 54 | 32 | 67 | 52 | 39 | 44 | 42 ± 15 |
| **Heteterotrophs** | | | | | | | | | |
| HNF | na | na | 58 | 47 | 19 | 68 | na | na | 48 ± 21 |
| HPF | 93 | 76 | 72 | 86 | 86 | 82 | 85 | 88 | 84 ± 7 |
| HB | 61 | 73 | 78 | 77 | 85 | 81 | 84 | 62 | 75 ± 9 |
| HC | 58 | 65 | 55 | 63 | 56 | 72 | 47 | 37 | 57 ± 11 |
| **Total Carbon** | 20 | 46 | 45 | 34 | 65 | 57 | 23 | 12 | 38 ± 19 |