View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Digital.CSIC

1 # Role of microzooplankton during a *Phaeocystis* sp. bloom in the

2 # Oosterschelde (SW Netherlands)

3

4 I. G. Teixeira[a,*], B. G. Crespo[a,1], T. G. Nielsen[b,2], F. G. Figueiras[a]

5

6 [a] Instituto de Investigacións Mariñas (CSIC), Eduardo Cabello 6, 36208 Vigo, Spain

7

8 [b] Department of Marine Ecology, The National Environmental Research Institute, University

9 of Aarhus, Frederiksborgvej 399, PO Box 358, 4000 Roskilde, Denmark.

10

11 [1] Present address: Departament de Biologia Marina I Oceanografia, Institut de Ciències del

12 Mar (CSIC), Pg Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain

13

14 [2] Present addres: National Institute of Aquatic Resources, DTU Aqua Section for

15 Oceanecology and Climate, Technical University of Denmark, Kavalergården 6, 2920

16 Charlottenlund, Denmark

17

18

19 *Corresponding author:

20 Email: isabeltx@iim.csic.es

21 Telephone: +34 986231930

22 Fax: +34 986 292767

23

24 RUNNING HEAD: Role of microzooplankton during a *Phaeocystis* sp. bloom

ABSTRACT

The impact of microzooplankton on the plankton community was assessed during a bloom of *Phaeocystis* sp. in the Oosterschelde basin (SW Netherlands). Chlorophyll *a* concentration and *Phaeocystis* sp. abundance reached maxima values in the middle of the sampling period (~27 mg chl *a* m^{-3} and 3.4 x 10^{10} cells m^{-3}, respectively). The bloom collapse coincided with the last sampling day, when accentuated decreases in chlorophyll *a* concentrations (~11 mg chl *a* m^{-3}) and *Phaeocystis* sp. cells numbers (~1.3 x 10^{10} cells m^{-3}) were recorded. Microzooplankton organisms were significant consumers of both phytoplankton and heterotrophic plankton. Although *Phaeocystis* sp. was the most consumed organism (336 ± 71 mg C m^{-3} d^{-1}), microzooplankton impact on its standing stock was lower than on the stocks of other less abundant organisms. This impact was also lower during the peak of the bloom, when colonial forms of *Phaeocystis* sp. presumably predominated, than during the bloom collapse, when free-living cells were supposedly more abundant. The impact of microzooplankton on heterotrophic organisms was higher than on phytoplankton, and increased when the bloom collapsed. Picoheterotrophs experienced the highest impact on their standing stock (~75% d^{-1}) and production (~90% d^{-1}). These results demonstrate that during a *Phaeocystis* sp. bloom, the microbial food web was responsible for channelling a significant fraction of plankton biomass, either from direct consumption of *Phaeocystis* sp. cells or through consumption of heterotrophs, which would have been favoured by the high quantities of organic matter released during the bloom collapse.

KEY WORDS: *Phaeocystis* sp., microzooplankton, microbial food web, Oosterschelde, SW Netherlands

1 **1. Introduction**

2 *Phaeocystis* (Prymnesiophyceae, Haptophyta) is a worldwide distributed phytoplankton
3 genus with a significant role in biogeochemical processes, primarily due to the production of
4 high amounts of organic carbon and the production of sulphur compounds involved in climate
5 regulation (e.g. Schoemann et al., 2005). The major particularity of this organism is related to
6 its polymorphic life cycle, in which the solitary stage characterised by flagellate or non-motile
7 free-living small cells (3–9 μm) can alternate with the colonial stage composed of non-motile
8 cells embedded in a mucilaginous matrix (e.g. Rousseau et al., 1994, 2007). When in the form
9 of these mucilaginous colonies, which can reach several millimetres in diameter, *Phaeocystis*
10 spp. frequently forms nearly mono-specific blooms of huge biomass in polar and temperate
11 regions during the spring (e.g. Schoemann et al., 2005). The magnitude of these blooms is
12 such that locally they can account for up to 70% of the annual primary production (Joiris et
13 al., 1982).

14 Despite numerous investigations, several details on the life cycle of *Phaeocystis* spp. are
15 still not totally understood. This must be due to its morphological and physiological
16 variability and the contrasting environmental conditions of the different systems where this
17 alga can temporally dominate. High nutrient concentrations and high irradiance have been
18 assigned as responsible for promoting the transformation of solitary cells into colonial forms
19 (Veldhuis et al., 1986; Verity et al., 1988a; Peperzak, 1993). Conversely, the bloom demise,
20 habitually occurring within a short time period, has been attributed to nutrient limitation
21 and/or changes in temperature, both causing the colony disruption and the release of the
22 colonial cells into the water column (Veldhuis et al., 1986; Verity et al., 1988b). Pelagic
23 consumption, cell lysis and sedimentation are other processes that can be responsible for the
24 sudden disappearance of biomass from the water column (Wassmann, 1994; Weisse et al.,
25 1994; Brussaard et al., 1996). Though sedimentation has been observed to occur on several

1 occasions, cell lysis mediated by viral infection and pelagic consumption are frequently
2 identified as the main responsible factors for the termination of *Phaeocystis* spp. blooms
3 (Rousseau et al., 2000; Brussaard et al., 2005a; Baudoux et al., 2006).

4 The magnitude of the pelagic consumption of *Phaeocystis* spp. cells and derived material,
5 which determines the fraction of biomass that is transferred through the food web, has major
6 implications for the fluxes of matter and energy in the systems where this organism
7 dominates. Colonial forms, which are protected by a tough skin (Hamm et al., 1999) that aids
8 them to avoid viral infection and grazing on individual cells, apparently are suitable for
9 consumption by copepods and larger animals. Thus, colonial forms could contribute to sustain
10 the classical short food chain. In fact, it has been recently reported that colony formation and
11 enlargement in *Phaeocystis* spp. is enhanced by the presence of micrograzers, which could
12 indicate that this strategy corresponds to a mechanical defence against predation by
13 microzooplankton (Jakobsen and Tang, 2002; Tang, 2003). However, the trophic significance
14 of colonial biomass for metazoans is uncertain, and divergent reports regarding ingestion
15 rates, the nutritive value of preys and adverse effects on consumers are widespread through
16 literature (Weisse et al., 1994). These contrasting results seem to be related to the variability
17 in the size ratio between predators and colonies, the physiological state of preys and whether
18 experiments were performed in laboratory with *Phaeocystis* spp. as single preys or *in situ*
19 (Nejstgaard et al., 2007). In general, crustacean grazers depict a much lower grazing impact in
20 the field than in the laboratory, which could be due to the presence of alternative preys *in situ*,
21 and changes in the chemical properties of *Phaeocystis* spp. in the laboratory conditions which
22 could favour their consumption. Rather than consuming *Phaeocystis* spp. biomass during
23 bloom periods, copepods have been observed to feed on alternative preys, namely
24 microzooplankton (Hansen et al., 1993; Gasparini et al., 2000). In contrast, microzooplankton
25 apparently shows preference for solitary cells of only a few micrometers (Admiraal and

Venekamp, 1986; Weisse and Scheffel-Möser, 1990; Tang et al., 2001; Brussaard et al., 2005a), although large naked dinoflagellates, such as *Noctiluca scintillans* and *Gyrodinium spirale*, have been observed to feed on colonies (Jakobsen and Tang, 2002; Stelfox-Widdicombe et al., 2004). In addition, both colony matrix disintegration and cell lysis at the end of the bloom period, which increase the quantities of dissolved organic matter in the water column (Alderkamp et al., 2007), should enter the microbial food web through enhanced bacterial production and microzooplankton bacterivory (Fernández et al., 1992; van Boeckel et al., 1992; Brussaard et al., 1995; Brussaard et al., 1996; Rousseau et al., 2000).

As the fate of the high biomass produced during *Phaeocystis* spp. blooms has a major implication for biogeochemical cycles in marine systems, further assessment of the role of microzooplankton during these blooms is fundamental to reach a better understanding of the functioning of the marine systems dominated by these organisms. In this context, an intensive and multidisciplinary research was conducted in the Oosterschelde (SW Netherlands) (Fig. 1) between 4 and 13 May 2004, coinciding with the development and decay of a *Phaeocystis* sp. bloom when phytoplankton biomass, primary production and the microzooplankton feeding impact were studied.

2. Materials and methods

2.1. Experimental procedure

Sampling took place on board of R/V *Luctor* between 4 and 13 May 2004. Seawater samples to determine chlorophyll *a* (chl *a*) concentration, phytoplankton biomass and primary production were taken at three depths (surface, middle and bottom) during high tide in a location close to a mussel bed (Fig. 1) using Niskin bottles. The sampling depths (1-6 m) depended on the tidal range, which varied according to the sampling days. On 9 and 12 May samples were not taken. Temperature, practical salinity and light penetration in the water

1 column were measured with a CTD to which a spherical PAR quantum sensor was attached.
2 Practical salinity was converted to absolute salinity (g kg^{-1}) according to TEOS-10 Primer,
3 where $\delta S_A = 0$ in this shallow water system.
4
5 *2.2. Analyses*
6 For chl *a*, three replicates of 50 ml were filtered onto GF/F filters. Pigments were extracted
7 overnight in 5 ml 96% ethanol in the dark and chl *a* concentration was determined against a
8 chl *a* standard after reading fluorescence before and after acidification on a Turner Designs
9 Model 700 Fluorometer.
10 Samples for microplankton counts were preserved in Lugol's iodine solution and
11 sedimented in composite sedimentation chambers. Diatoms, dinoflagellates, other flagellates
12 >20 µm and ciliates were identified and counted at species level, when possible, using an
13 inverted microscope. Differentiation of phototrophic species of dinoflagellates, other
14 flagellates (hereafter, flagellates) and ciliates was basically done following Lessard and Swift
15 (1986) and Larsen and Sournia (1991); however, epifluorescence microscopy (see below) was
16 also used to assign phototrophy within these plankton groups. The biovolumes of each taxon
17 were calculated from the dimensions and shapes according to Hillebrand et al. (1999). The
18 plasmatic volumes of diatoms and total cell volume of dinoflagellates, flagellates >20 µm and
19 ciliates were converted to cell carbon following Strathmann (1967) for diatoms and
20 dinoflagellates, Verity et al. (1992) for flagellates and Putt and Stoecker (1989) for ciliates.
21 Fixation procedure did not allowed us to quantify the number of colonies of *Phaeocystis* sp.,
22 and consequently the carbon biomass calculated for this species does not include the carbon
23 content of the colonial matrix (Rousseau et al., 1990; van Rijssel et al., 1997).
24 Primary production was measured as carbon assimilation after 24h *in situ* incubations.
25 Samples were placed in tissue culture flasks (3 light flasks and 2 dark flasks) and immediately

1 inoculated with ca. 3.7 x 10^5 Bq of $NaH^{14}CO_3$. Then, samples were placed in a rope with
2 specially constructed holders. A weight kept the line vertical, whereas the surface of the line
3 was maintained afloat by a large buoy. After 24h of incubation samples were recovered and
4 filtered through 25 mm GF/F filters under low vacuum pressure (<20 kPa). Filters were
5 placed in scintillation vials and left in HCl fumes atmosphere for 12h to remove unassimilated
6 ^{14}C. Radioisotope incorporation was determined with a liquid scintillation counter using the
7 external standard and the channel ratio methods to correct for quenching.
8
9 *2.3. Microzooplankton feeding impact*
10 The feeding impact of microzooplankton on the several organisms of the plankton
11 community was determined using the dilution technique (Landry and Hassett, 1982) on 4, 6,
12 8, 10 and 13 May 2004. Water was collected from the surface with a Niskin bottle as soon as
13 possible in the morning. All experimental containers, bottles and tubing were soaked in
14 10% HCl and rinsed with Milli Q water before each experiment. Filtered seawater was
15 obtained by gravity filtration through 0.8/0.2 µm Suporcap filter into a 25 l container. Sample
16 water was transferred to another 25 l container, very gently through a tube. In the laboratory,
17 dilutions were prepared directly in 2 l polycarbonate bottles (Nalgene). A total of 6 dilutions,
18 in the proportions of 100, 80, 60, 40, 20 and 10% of sample relatively to filtered seawater
19 were performed, with two replicates each. These bottles were incubated for 24h in running
20 seawater and at *in-situ* temperature and surface light conditions. During incubation period,
21 these bottles were regularly gently hand-rotated to avoid massive sedimentation.
22 Samples to determine the biomasses of plankton organisms ≤200 µm were taken from the
23 natural seawater at the beginning of the incubation and from each incubation bottle at the end
24 of the incubation. Samples for microplankton biomass determinations were processed as
25 described above. Metazooplankton <200 µm and mesozooplankton were found occasionally

1 and at very low concentrations in the dilution bottles. So, their importance as grazers in these
2 experiments was not considered. Pico- and nanoplankton were determined in subsamples of
3 10 ml fixed with buffered 0.2 μm filtered formaldehyde (2% final concentration) and stained
4 with DAPI at 0.1 μg ml^{-1} final concentration (Porter and Feig, 1980). After 10 minutes in the
5 dark, samples were filtered through 0.2 μm black Millipore-Isopore filters. The filters were
6 then immersed in low fluorescence immersion oil and examined at x1000 magnification using
7 an epifluorescence microscope. Autotrophic organisms were enumerated under blue light
8 excitation and heterotrophic organisms were counted under excitation with UV light.
9 Bacterial biomass was estimated according to Lee and Furhmann (1987). Dimensions of
10 several individuals of the other groups were taken and cell volumes were calculated assuming
11 spherical shape. Cell carbon was estimated following Verity et al. (1992) for pico- and
12 nanoflagellates and Bratbak and Dundas (1984) for *Synechococcus*-type cyanobacteria.
13 Changes in total plankton (≤200 μm) biomass and in the several plankton groups
14 occurring between the beginning (C_0) and the end (C_t) of the incubation time (t = 1 day) were
15 used to calculate the net growth rates (k, d^{-1}):
16
$$k = \frac{1}{t} \cdot \ln\left(\frac{C_t}{C_0}\right) \quad (1)$$
17 Mortality rates due to microzooplankton (m, d^{-1}) were calculated as the slope and the
18 specific growth rates (μ, d^{-1}) as the y-axis intercept of the linear regression between the net
19 growth rates and the fraction of unfiltered seawater (X) (Landry and Hassett, 1982):
20
$$k = \mu - m \cdot X \quad (2)$$
21 In the cases of non-linear responses (saturated and saturated-increased, see Teixeira and
22 Figueiras, 2009), the specific growth rate (μ) was obtained by the y-axis intercept of the
23 regression of the linear part of the response. The mortality rate (m) was then obtained by

equation 2, replacing the specific growth rate calculated and using the net growth rate obtained in the undiluted sample (Gallegos, 1989; Teixeira and Figueiras, 2009).

The daily mortality impact on the standing stock (%SS, %d^{-1}) and on production (%P, %d^{-1}) were calculated as:

$$\%SS = \left(1 - e^{-m}\right) \times 100 \tag{3}$$

$$\%P = \frac{m}{\mu} \times 100 \tag{4}$$

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

$$G = m \times C_m \tag{5}$$

$$P = \mu \times C_m \tag{6}$$

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

$$C_m = C_0\left[e^{(\mu - m)t} - 1\right] / (\mu - m)t \tag{7}$$

3. Results

3.1. Hydrographic conditions

The water column (maximum depth = 6.5m) was well mixed from surface to bottom during all days (Figs. 2a & b). There was a slight increase in the salinity of the water column, which varied between 30.8 g kg^{-1} on the first 3 days and values higher than 31 g kg^{-1} on the following days (Fig. 2a). Temperature did not show any tendency, varying between 12.6 and 13.3 ºC (Fig. 2b).

The light attenuation coefficient in the water column (Fig. 2c) varied between 0.66 and 1.03 m^{-1}, with the highest value occurring on 8 May and the lowest on 13 May, at the end of

1 the sampling. Relatively low values (0.71 m^{-1}) were also recorded at the beginning of the
2 sampling, on 4 May (Fig. 2c).
3
4
5 *3.2. Chl a and Phaeocystis sp.*
6 Chl *a* concentration increased from 20.4 (± 1.0) to 26.8 (± 1.3) mg chl *a* m^{-3} during the
7 first 5 days, falling to 11.1 (± 0.2) mg chl *a* m^{-3} on the last day (Fig. 3a). Differences in chl *a*
8 concentrations between the 3 sampling depths were not significant ($0.40 < p < 0.88$; t-test for
9 paired samples).
10 Chl *a* concentration was positively correlated with phytoplankton abundance ($r = 0.91$, $p <$
11 0.01) and carbon biomass ($r = 0.89$, $p < 0.01$), two variables that showed a similar evolution
12 during the sampling period (Figs. 3b & c). The phytoplankton community was dominated by
13 *Phaeocystis* sp., which accounted for ~98% of the total phytoplankton abundance and ~90%
14 of the total phytoplankton biomass (Figs. 3b & c). This species reached an abundance of 3.4 x
15 10^{10} (± 1.3 x 10^{10}) cells m^{-3} and a biomass of 871 (± 343) mg C m^{-3} during the peak of the
16 bloom on 8 May, and then dropped to 1.3 x 10^{10} (± 2.5 x 10^{9}) cells m^{-3} and 345 (± 63) mg C
17 m^{-3} on the last day. Differences between depths in abundance and biomass of *Phaeocystis* sp.
18 and total phytoplankton were not significant ($0.40 < p < 0.95$; t-test for paired samples).
19 Despite *Phaeocystis* sp. was mainly forming colonies (as it was observed in fresh samples and
20 deduced from the high accumulation of gelatinous structures on filters), the lugol's iodine that
21 was added to preserve the phytoplankton samples destroyed these structures. This resulted in
22 a much higher abundance of *Phaeocystis* sp. as solitary cells (99.4%) than as colonial forms
23 (0.6%) in the counted samples.
24 As a result of the correlation between phytoplankton carbon biomass and chl *a*
25 concentration, the autotrophic carbon to chl *a* (AC:chl *a*) ratio (Fig. 3d) remained relatively

constant around a mean value of 34 ± 3 during the sampling period. Nevertheless, the AC:chl *a* ratio was slightly but significantly lower ($p < 0.05$, t-test for two samples) when chl *a* and phytoplankton biomass were increasing (31 ± 2, 4 to 7 May) than when these two variables were decreasing (37 ± 2, 8 to 13 May).

3.3. Primary production

Primary production decreased with depth (Fig. 4) in accordance with the light attenuation coefficient in the water column (Fig. 2c). Thus, primary production varied between 102 and 608 mg C m^{-3} d^{-1} at the surface, 76 and 444 mg C m^{-3} d^{-1} at the middle depth and between 0 and 89 mg C m^{-3} d^{-1} at the bottom layer (Fig. 4). Highest differences between depths were observed on 8 May. The zero value recorded at the bottom layer that day coincided with the strongest light attenuation in the water column (Fig. 2c), which led to a photic layer shallower than the depth at the station. Depth integrated primary production varied between 353 and 1626 mg C m^{-2} d^{-1}, with lowest values at the beginning and the end of the sampling period (Fig. 4) and highest values in between, on 8 and 10 May. Integrated primary production was positively correlated with integrated phytoplankton carbon biomass ($r = 0.74$; $p < 0.05$).

3.4. Microzooplankton feeding impact

In addition to *Phaeocystis* sp., which represented ~90% of total phytoplankton biomass (Fig. 5a), diatoms were also present in the initial plankton populations of dilution experiments. Nevertheless, diatoms accounted for a minor fraction (3-7%) of the total autotrophic carbon (AC). The biomass of diatoms increased from ~25 mg C m^{-3} during the first days to ~40 mg C m^{-3} on the last days. Among this group, *Rhizosolenia imbricata*, *Guinardia delicatula*, *Cerataulina pelagica* and small and large *Pseudo-nitzschia* spp. were the most abundant species. The phototrophic ciliate *Mesodinium rubrum*, autotrophic

dinoflagellates and picoautotrophs (*Synechococcus*-like cyanobacteria and picoflagellates) were also found, but their contribution to phytoplankton biomass was very low (Fig. 5a). The evolution of heterotrophs was different from that of autotrophs (Fig. 5b), showing a peak on 6 May (155 mg C m^{-3}) due to the high biomass of heterotrophic nanoflagellates (HNF) on that day (121 mg C m^{-3}). HNF, which dominated within microzooplankton community at the beginning of the sampling period, accounting almost for 90% of the microzooplankton biomass, decreased from 6 May onwards. On the last day of sampling, HNF with a biomass of 12 mg C m^{-3} only represented 20% of microzooplankton carbon. Heterotrophic picoflagellates (HPF) showed also a decreasing tendency during the sampling period, but their biomass was much smaller, varying between 3.8 and 1.3 mg C m^{-3}. On the contrary, the importance of heterotrophic dinoflagellates (mainly naked forms >20 µm), ciliates (mainly aloricate choreotrichs >50 µm) and heterotrophic bacteria (HB) increased through the sampling period (Fig. 5b); heterotrophic dinoflagellates from 3 to 26 mg C m^{-3}, ciliates from 7 to 23 mg C m^{-3} and HB from 19 to 37 mg C m^{-3}.

According to the outcome of the dilution experiments (Table 1), heterotrophic pico- and nanoplankton (HB, HPF and HNF), S*ynechococcus* and *Phaeocystis* sp. were consumed during all the sampling period. Diatoms were also consumed, but only on May 13. Growth and mortality rates were highly variable amongst the several organisms, with growth rates varying between 0.15 and 3.08 d^{-1} and mortality rates between 0 and 2.64 d^{-1}. The rates were generally higher for total heterotrophic carbon (HC) ($1.03 \leq \mu \leq 1.48$ d^{-1} and $0.69 \leq m \leq 1.48$ d^{-1}) than for total AC ($0.16 \leq \mu \leq 0.91$ d^{-1} and $0.23 \leq m \leq 0.58$ d^{-1}). Growth and grazing rates of AC were very similar to those obtained for *Phaeocystis* sp. ($0.15 \leq \mu \leq 0.96$ d^{-1} and $0.26 \leq m \leq 0.62$ d^{-1}).

On average, 40 ± 5% d^{-1} of the whole plankton standing stock ≤200 µm was consumed by microzooplankton (Fig. 6a). HC was more impacted (Fig. 6a, 67 ± 11% d^{-1}) than AC (Fig. 6a,

1 34 ± 9% d^{-1}). Moreover, there was an increase in the consumption of the standing stock of HC
2 from 50% d^{-1} being ingested at the beginning of the sampling to almost 80% d^{-1} at the end
3 (Fig. 6a). Among autotrophs, the lowest grazing impact (21% d^{-1}) was found on 8 May (Fig.
4 6a), coinciding with the maximum peak in the abundance of *Phaeocystis* sp. (Fig. 3b & c).
5 The impact on the standing stock of *Phaeocystis* sp. (37 ± 9% d^{-1}) was relatively low, always
6 below 50% d^{-1} (Fig. 6b). Regarding the several plankton groups, the highest impact occurred
7 on the standing stock of picoheterotrophs (75 ± 6% d^{-1} for HB and 74 ± 19% d^{-1} for HPF),
8 followed by HNF (62 ± 36% d^{-1}), though HNF were not consumed on 4 May (Fig. 6c).
9 Concerning the microzooplankton impact on production, a sudden increase was observed
10 for AC and TC on the last day of sampling (Fig. 6d), when consumption shifted from 63 ±
11 18% d^{-1} of AC and 74 ± 23% d^{-1} of TC produced during the previous days to 373% d^{-1} of the
12 AC and 265% d^{-1} of the TC produced on 13 May. This evolution closely resembled that
13 observed for the impact on the production of *Phaeocystis* sp. (Fig. 6e), which derived from a
14 consumption of 67 ± 20% d^{-1} of the production between 4 and 10 May to a value of 422% d^{-1}
15 on 13 May. The impact on the production of the other two phytoplankton groups with
16 significant responses in the dilution experiments was highly variable, ranging from 17 to
17 100% d^{-1} for *Synechococcus* and from 52 to 98% d^{-1} for autotrophic picoflagellates (APF)
18 (Fig. 6e). In contrast, the microzooplankton impact on the production of HC gradually
19 increased over the sampling period, varying from 56% d^{-1} on the first day to 113% d^{-1} on the
20 last day (Fig. 6d), showing a relatively constant and significant impact (96 ± 16% d^{-1}) on the
21 production of HB (Fig. 6f). The other two heterotrophic groups, HPF and HNF, also
22 experienced a high impact (87 ± 33% d^{-1} and 73± 45% d^{-1}, respectively) on their production,
23 although it was highly variable (Fig. 6f).
24 In spite of these percentages, consumption of autotrophic biomass (255–420 mg C m^{-3} d^{-}
25 ^{1}) was always higher than consumption of heterotrophic biomass (90–161 mg C m^{-3} d^{-1}) (Fig.

7a). *Phaeocystis* sp. was virtually the only phytoplankton species consumed from 4 to 10 May and represented 95% of the total AC removed on 13 May, when diatoms were also consumed (Fig. 7b). Among heterotrophs (Fig. 7c), HNF, which were not consumed on the first day of sampling, were highly consumed on 6 May (131 mg C m^{-3} d^{-1}), showing after that a decreasing tendency. In contrast, the HB biomass daily removed by microzooplankton increased over the sampling period, from 28 mg C m^{-3} d^{-1} on the first day to 45 mg C m^{-3} d^{-1} in the last dilution experiment. The autotrophic biomass produced (68 - 980 mg C m^{-3} d^{-1}) during the sampling period showed higher variations than the biomass consumed (Figs. 7a & d). *Phaeocystis* sp. was the phytoplankton species with the highest production (Figs. 7e), representing >96% of the total autotrophic carbon produced. In contrast, consumption (90-161 mg C m^{-3} d^{-1}) and production (120-164 mg C m^{-3} d^{-1}) of heterotrophic biomass were more tightly coupled (Figs. 7a,d,c,f), with HNF showing higher production during the first 3 days of sampling and HB acquiring more importance at the end.

Growth and microzooplankton grazing rates derived from dilution experiments (Table 1) were used to predict the expected *Phaeocystis* sp. biomass in the water column on the following day (Fig. 8). The observed and predicted biomasses were in reasonable agreement at the beginning of the bloom, with predicted biomass exceeding the observed biomass on 6 and 49 mg C m^{-3} on May 5 and 7, respectively. However, this difference was much higher when the bloom started to decay (May 11), with observed biomass being 403 mg C m^{-3} lower than expected biomass estimated considering only growth and microzooplankton grazing rates obtained in dilution experiments.

4. Discussion

4.1. The Phaeocystis sp. bloom

Blooms of *Phaeocystis* spp. in the Oosterschelde (Laanbroek et al., 1985; Bakker et al., 1990) and in the adjacent North Sea (e.g. Veldhuis et al., 1986; Weisse & Scheffel-Möser, 1990; Rousseau et al., 2000) are frequent in spring. Here, we followed an almost monospecific *Phaeocystis* sp. bloom in the Oosterschelde well reflected in the evolution of chl *a* concentration and cell abundance (Figs. 3a & b). The bloom was also discernible in the evolution of primary production (Fig. 4), which was correlated with *Phaeocystis* sp. biomass (Fig. 3c), and in the evolution of the light attenuation coefficient (Fig. 2c). Even though the progress of this bloom was observed inside the Oosterschelde, its advection from the North Sea cannot be disregarded, because the increase in salinity recorded during the sampling period (Fig. 2a) points to the possible input of water from the North Sea, saltier than the water in the Oosterschelde (Laanbroek et al., 1985).

The high chl *a* concentration (20 mg chl *a* m^{-3}) and the high cell abundance (~23 x 10^9 cells m^{-3}) of *Phaeocystis* sp. (Figs. 3a & b) recorded on the first day of sampling were above the threshold reported for the formation of colonies (Schoemann et al., 2005). This indicates that our sampling started after the onset of the bloom, when *Phaeocystis* sp. was already in the colonial form. The bloom rapidly evolved to reach its maximum 4 days later, and then declined. Changes in temperature and/or nutrient limitation have been assigned as triggering factors for the beginning of bloom collapse (Veldhuis et al., 1986; Verity et al., 1988b). In this case temperature did not show any significant change (Fig. 2b), but nutrient limitation could have been responsible for finishing this event. Although not having a nutrient record during the sampling period, we do have nutrient data before (on April 29) and after (May 17) the survey. Thus, on April 29 nitrate concentration was 25.44 µmol l^{-1} while on May 17 was 8.53 µmol l^{-1} (T. Ysebaert, personal communication), indicating a high nutrient decrease during that period. By its turn, phosphate concentrations were very low on both days (0.1 and 0.23 µmol l^{-1}, respectively) and this nutrient is usually limiting *Phaeocystis* blooms in the

North Sea (Veldhuis et al., 1986; Peperzak et al., 1998). Nonetheless, the decrease in nitrate concentrations could lead to a reduction in *Phaeocystis* biomass that can be viewed as the bloom collapse Also the changes observed in the AC:chl *a* ratios (Fig. 3d), with slightly but significant higher ratios during the demise phase, could point to nutrient limitation as the possible factor triggering the bloom decline. However, this could also be a consequence of a decrease in the light attenuation coefficient in the water column with the progress of the bloom (Fig. 2c). Other loss factors, as sedimentation, have also been reported (Wassmann, 1994), but sinking seems unusual in this region where tidal currents are strong enough to prevent it. In fact, in this work we did not observe accumulation of phytoplankton in bottom layers at the end of sampling (Fig. 4).

4.2. Microzooplankton feeding impact

Our results showed that microzooplankton organisms were significant consumers in the pelagic system, but their impact varied during the bloom period (Fig. 6). The highest quantity of carbon biomass consumed by microzooplankton corresponded to *Phaeocystis* sp. cells (Figs. 7a & b). However, this pattern was forced by the dominance of this species during the sampling period resulting in higher quantities consumed even though mortality rates were lower than those of the other plankton groups (Table 1). Despite these high quantities consumed, microzooplankton was unable to control the bloom development, consuming only a fraction of the *Phaeocystis* sp. standing stock and production (Figs. 6b & e). However, on the last sampling day, when the bloom decayed, microzooplankton consumption exceeded 4 times the production of this haptophyte (Fig. 6e). These variations in consumption must be related to the structural changes occurring in *Phaeocystis* sp. through the sampling period. With exception of few dinoflagellate species, such as *Noctiluca scintillans* (Jakobsen and Tang, 2002) or *Gyrodinium spirale* (Stelfox-Widdicombe et al., 2004), microzooplankton is

1 not an efficient consumer either of the whole colonial structure or the colonial single cells
2 protected by the colony skin (Hamm et al., 1999). However, when *Phaeocystis* sp. is in the
3 form of single cells in the water column, microzooplankton organisms are able to ingest them
4 (Admiraal and Venekamp, 1986; Weisse and Scheffel-Möser, 1990; Tang et al., 2001). Here
5 we found a higher microzooplankton grazing on *Phaeocystis* sp. on the last day (Figs. 6b &
6 e), when colonies presumably had collapsed and cells were free-living. As on the previous
7 days *Phaeocystis* sp. was probably forming colonies, the impact of microzooplankton would
8 have occurred on the less abundant solitary cells that usually are present in the water column
9 coexisting with colonial structures (Rousseau et al. 1994). Consumption of some colonial
10 forms could also have occurred, because large naked forms predominated among
11 heterotrophic dinoflagellates and these organisms could feed on *Phaeocystis* sp. colonies as
12 *Gyrodinium spirale* do it (Stelfox-Widdicombe et al., 2004). It must be noted however that
13 the accentuated impact on *Phaeocystis* sp. production on May 13 was not due to a higher
14 grazing mortality, but to the low growth rate of *Phaeocystis* sp. on this day, several times
15 lower than the mortality rate (Table 1). This low growth rate can indicate nutrient limitation,
16 as stated before, but also that microzooplankton was not the only mortality factor for
17 *Phaeocystis* sp. on this day. When viral lysis is significant, the conventional dilution protocol
18 can under-estimate the specific growth rates (Evans et al., 2003; Baudoux et al., 2006). Cell
19 lysis mediated by viral attack has been shown to be very important in the termination of the
20 *Phaeocystis* spp. blooms (Rousseau et al., 2000; Brussaard et al., 2005a; Baudoux et al.,
21 2006). Thus, the high microzooplankton grazing on *Phaeocystis* sp. production at the end of
22 the bloom must be taken carefully, as probably both viral lysis and grazing by micro-
23 heterotrophs were acting on this alga simultaneously. This can also explain the results shown
24 in Fig. 8, where other loss factors than microzooplankton grazing were apparently responsible
25 for the disappearance of *Phaeocystis* sp. biomass from the water column on May 11, when the

1 bloom started to decay. Besides viral lysis and grazing by micro-heterotrophs, other loss
2 processes can also include pelagic consumption by mesozooplankton (Weisse et al., 1994) or
3 even benthic consumption by mussels (Smaal and Twisk, 1997), which are abundant in this
4 system. Colony destruction occurring when the bloom started to collapse probably made
5 *Phaeocystis* sp. more susceptible to all consumers.

6 Consumption by microzooplankton during the *Phaeocystis* sp. bloom was not restricted to
7 this alga. Picoautotrophs (APF and *Synechococcus*) were also grazed (Table 1), although the
8 quantities of carbon consumed of these organisms were insignificant due to their low
9 biomasses (Fig. 7b). Grazing on diatoms, which only occurred on the last day (Table 1), could
10 be related to the presence of *Pseudo-nitzschia* spp. Pennate diatoms, including these species,
11 are known to be attached to *Phaeocystis* sp. colonies (Peperzak et al., 1998; Sazhin et al.,
12 2007). Thus, *Pseudo-nitzschia* spp. could have been attached to the colonial forms during the
13 bloom and hence be protected against grazing. On the last day, with the disintegration of
14 colonies, *Pseudo-nitzschia* spp. could became free-living, and as *Phaeocystis* sp. free cells, be
15 more susceptible to pelagic consumption.

16 In addition to phytoplankton, heterotrophs were also an important nutritional source for
17 microzooplankton (Figs. 7a & c). In relative terms of biomass and abundance,
18 microzooplankton impact on heterotrophs was actually higher than on phytoplankton (Figs.
19 6a). Among this group, picoheterotrophs (HPF and HB) were highly impacted by
20 microzooplankton, with ~75% of their standing stock and ~90% of their production being
21 channelled to the microbial food web (Figs. 6c & f). The microzooplankton consumption was
22 not enough to fully control HB, which showed slight increases in biomass (Fig. 5b) and
23 production (Fig. 7f) over the sampling period, reaching maximum values during the bloom
24 decay. This increase can be associated with the response of HB to the increase in dissolved
25 organic matter concentrations due to colony disintegration (Laanbroek et al., 1985; Veldhuis

et al., 1986; Verity et al., 1988b; Noordkamp et al., 2000; Rousseau et al., 2000; Brussaard et al., 2005b). HNF were also consumed (Fig. 7c), in agreement with other studies indicating consumption of these organisms by microzooplankton during *Phaeocystis* sp. blooms (Brussaard et al., 1995, 1996; Rousseau et al., 2000).

Noteworthy, microzooplankton impact increased towards the end of the sampling period (Fig. 6). This was not only observed for grazing on phytoplankton, but also for predation on heterotrophic components. This increasing importance of the microbial food web in the transfer of biomass with the progress of the bloom adds new evidences to previous reports (Admiraal and Venekamp, 1986; Weisse and Scheffel-Möser, 1990; Brussaard et al., 1995; Peperzak et al., 1998; Rousseau et al., 2000) supporting the view that *Phaeocystis* sp. blooms stimulate the dominance of the microbial food web.

4.3. Concluding remarks

These results indicate that a significant fraction of the *Phaeocystis* sp. bloom was channelled through the microbial food web, with two pathways in which microzooplankton is a direct intervenient. Firstly, direct consumption of *Phaeocystis* sp. cells by microzooplankton occurred in all experiments and increased during the bloom decay. Secondly, microzooplankton consumption of small heterotrophs allowed the return to the food web of the high amounts of organic matter released during the collapse of the bloom (Alderkamp et al., 2007): HB taking advantage of the released organic matter, and HNF being favoured by the increase in bacterial production. Thus, microzooplankton, which is consumed by copepods during *Phaeocystis* blooms (Hansen et al., 1993; Gasparini et al., 2000), transfers the photosynthesised organic matter to higher trophic levels, linking the microbial and classical food webs and therefore enhancing the efficiency of the pelagic food web.

1 **Ackowledgements**

2 We thank our MABENE colleagues and to the crew from R/V *Luctor* for their help during the
3 field campaign. This work was funded by the EU project MABENE (grant: EKV3-2001-
4 00144). Special thanks to Dr. T. Ysebaert for providing nutrient data and Fig.1 and Dr. P.J.
5 Herman, Dr. Paffenhofer and 2 anonymous reviewers for the comments on an earlier version
6 of the manuscript. I. G. T. was supported by a FCT (Portuguese Foundation for Science and
7 Technology) doctoral fellowship (SFRH/BD/11309/2002) and B.G.C. by a CSIC-ESF I3P
8 fellowship.

9

1 **References**

2 Admiraal, W. and Venekamp, L.A.H., 1986. Significance of tintinnid grazing during blooms
3 of *Phaeocystis pouchetii* (Haptophyceae) in Dutch coastal waters. Neth. J. Sea Res.
4 20(1), 61-66.
5 Alderkamp, A.-C., Buma, A.G.J. and Van Rijssel, M., 2007. The carbohydrates of
6 *Phaeocystis* and their degradation in the microbial food web. Biogeochemistry 83, 99-
7 118.
8 Bakker, C., Herman, P.M.J. and Vink, M., 1990. Changes in seasonal succession of
9 phytoplankton induced by the storm-surge barrier in the Oosterschelde
10 (S.W.Netherlands). J. Plankton Res. 12(5), 947-972.
11 Baudoux, A.-C., Noordeloos, A.A.M., Veldhuis, M.J.W. and Brussaard, C.P.D., 2006. Virally
12 induced mortality of *Phaeocystis globosa* during two spring blooms in temperate
13 coastal waters. Aquat. Microb. Ecol. 44, 207-217.
14 Bratbak, G. and Dundas, I., 1984. Bacterial dry matter content and biomass estimation. Appl.
15 Environ. Microb. 48(4), 755-757.
16 Brussaard, C.P.D., Gast, G.J., van Duyl, F.C. and Riegman, R., 1996. Impact of
17 phytoplankton bloom magnitude on a pelagic microbial food web. Mar. Ecol. Prog.
18 Ser. 144, 211-221.
19 Brussaard, C.P.D., Kuipers, B. and Veldhuis, M.J.W., 2005a. A mesocosm study of
20 *Phaeocystis globosa* population dynamics I. Regulatory role of viruses in bloom
21 control. Harmful Algae 4, 859-874.
22 Brussaard, C.P.D., Mari, X., Van Bleijswijk, J.D.L. and Veldhuis, M.J.W., 2005b. A
23 mesocosm study of *Phaeocystis globosa* (Prymnesiophyceae) population dynamics II.
24 Significance for the microbial community. Harmful Algae 4, 875-893.

Brussaard, C.P.D., Riegman, R., Noordeloos, A.A.M., Cadée, G.C., Witte, H., Kop, A.J., Nieuwland, G., van Duyl, F.C. and Bak, R.P.M., 1995. Effects of grazing, sedimentation and phytoplankton cell lysis on the structure of a coastal pelagic food web. Mar. Ecol. Prog. Ser. 123, 259-271.

Evans, C., Archer, S.D., Jacquet, S. and Wilson, W.H., 2003. Direct estimates of the contribution of viral lysis and microzooplankton grazing to the decline of a *Micromonas* spp. population. Aquat. Microb. Ecol. 30, 207-219.

Fernández, E., Serret, P., Madariaga, I.d., Harbour, D.S. and Davies, A.G., 1992. Photosynthetic carbon metabolism and biochemical composition of spring phytoplankton assemblages enclosed in microcosms: the diatom - *Phaeocystis* sp. succession. Mar. Ecol. Prog. Ser. 90, 89-102.

Gallegos, C.L., 1989. Microzooplankton grazing on phytoplankton in Rhode River, Maryland: nonlinear feeding kinetics. Mar. Ecol. Prog. Ser. 57(1), 23-33.

Gasparini, S., Daro, M.H., Antajan, E., Tackx, M., Rousseau, V., Parent, J.-Y. and Lancelot, C., 2000. Mesozooplankton grazing during the *Phaeocystis globosa* bloom in the southern bight of the North Sea. J. Sea Res. 43, 345-356.

Hamm, C.E., Simson, D.A., Merkel, R. and Smetacek, V., 1999. Colonies of *Phaeocystis globosa* are protected by a thin but tough skin. Mar. Ecol. Prog. Ser. 187, 101-111.

Hansen, F.C., Reckermann, M., Klein Breteler, W.C.M. and Riegman, R., 1993. *Phaeocystis* blooming enhanced by copepod predation on protozoa: evidence from incubation experiments. Mar. Ecol. Prog. Ser. 102, 51-57.

Hillebrand, H., Dürselen, C., Kirschtel, D., Pollingher, U. and Zohary, T., 1999. Biovolume calculation for pelagic and benthic microalgae. J. Phycol. 35, 403-424.

1 Jakobsen, H.H. and Tang, K.W., 2002. Effects of protozoan grazing on colony formation in
2 *Phaeocystis globosa* (Prymnesiophyceae) and the potential costs and benefits. Aquat.
3 Microb. Ecol. 27, 261-273.
4 Joiris, C., Billen, G., Lancelot, C., Daro, M.H., Mommaerts, J.P., Bertels, A., Bossicart, M.,
5 Nijs, J. and Hecq, J.H., 1982. A budget of carbon cycling in the Belgian coastal zone:
6 relative roles of zooplankton, bacterioplankton and benthos in the utilization of
7 primary production. Neth. J. Sea Res. 16, 260-275.
8 Laanbroek, H.J., Verplanke, J.C., Visscher, P.R.M.d. and Vuyst, R.d., 1985. Distribution of
9 phyto- and bacterioplankton growth and biomass parameters, dissolved inorganic
10 nutrients and free amino acids during a spring bloom in the Oosterschelde basin, The
11 Netherlands. Mar. Ecol. Prog. Ser. 25, 1-11.
12 Landry, M.R. and Hassett, R.P., 1982. Estimating the grazing impact of marine
13 microzooplankton. Mar. Biol. 67, 283-288.
14 Lee, S. and Fuhrman, J.A., 1987. Relationships between biovolume and biomass of naturally
15 derived marine bacterioplankton. Appl. Environ. Microb. 53, 1298-1303.
16 Lessard, E.J. and Swift, E., 1986. Dinoflagellates from the North Atlantic classified as
17 phototrophic or heterotrophic by epifluorescence microscopy. J. Plankton Res. 8(6),
18 1209-1215.
19 Nejstgaard, J.C., Tang, K.W., Steinke, M., Dutz, J., Koski, M., Antajan, E. and Long, J.D.,
20 2007. Zooplankton grazing on *Phaeocystis*: a quantitative review and future
21 chalenges. Biogeochemistry 83, 147-172.
22 Noordekamp, D.J.B., Gieskes, W.W.C., Gottschal, J.C., Forney, L.J. and van Rijssel, M.,
23 2000. Acrylate in *Phaeocystis* colonies does not affect the surrounding bacteria. J. Sea
24 Res. 43, 287-296.

1 Peperzak, L., 1993. Daily irradiance governs growth rate and colony formation of *Phaeocystis*
2 (Prymnesiophyceae). J. Plankton Res. 15(7), 809-821.
3 Peperzak, L., Colijn, F., Gieskes, W.W.C. and Peeters, J.C.H., 1998. Development of the
4 diatom-*Phaeocystis* spring bloom in the Dutch coastal zone of the North Sea: the
5 silicon depletion versus the daily irradiance threshold hypothesis. J. Plankton Res.
6 20(3), 517-537.
7 Porter, K.G. and Feig, Y.S., 1980. The use of DAPI for identifying and counting aquatic
8 microflora. Limnol. Oceanogr. 25(5), 943-948.
9 Putt, M. and Stoecker, D.K., 1989. An experimental determined carbon:volume ratio for
10 marine "oligotrichous" ciliates from estuarine and coastal waters. Limnol. Oceanogr.
11 34(6), 1097-1103.
12 Rousseau, V., Becquevort, S., Parent, J.-Y., Gasparini, S., Daro, M.-H., Tackx, M. and
13 Lancelot, C., 2000. Trophic efficiency of the planktonic food web in a coastal
14 ecosytem dominated by *Phaeocystis* colonies. J. Sea Res. 43, 357-372.
15 Rousseau, V., Chrétiennot-Dinet, M.-J., Jacobsen, A., Verity, P. and Whipple, S., 2007. The
16 life cycle of *Phaeocystis*: state of knowledge and presumptive role in ecology.
17 Biogeochemistry 83, 29-47.
18 Rousseau, V., Mathot, S. and Lancelot, C., 1990. Calculating carbon biomass of *Phaeocystis*
19 sp. from microscopic observations. Mar. Biol. 107, 305-314.
20 Rousseau, V., Vaulot, D., Casotti, R., Cariou, V., Lenz, J., Gunkel, J. and Baumann, M.,
21 1994. The life cycle of *Phaeocystis* (Prymnesiophyceae): evidence and hypothesis. J.
22 Marine Syst. 5, 23-39.
23 Sazhin, A.F., Artigas, L.F., Nejstgaard, J.C. and Frischer, M.E., 2007. The colonization of
24 two *Phaeocystis* species (*Prymnesiophyceae*) by pennate diatoms and other protists: a
25 significant contribution to colony biomass. Biogeochemistry 83, 137-145.

1 Schoemann, V., Becquevort, S., Stefels, J., Rousseau, V. and Lancelot, C., 2005. *Phaeocystis*
2 blooms in the global ocean and their controlling mechanisms: a review. J. Sea Res. 53,
3 43-66.
4 Smaal, A.C. and Twisk, F., 1997. Filtration and absorption of *Phaeocystis* cf. *globosa* by the
5 mussel *Mytilus edulis* L. J. Exp. Mar. Biol. Ecol. 209, 33-46.
6 Stelfox-Widdicombe, C.E., Archer, S.D., Burkill, P.H. and Stefels, J., 2004.
7 Microzooplankton grazing in *Phaeocystis* and diatoms-dominated waters in the
8 southern North Sea in spring. J. Sea Res. 51, 37-51.
9 Strathmann, R., 1967. Estimating the organic carbon content of phytoplankton from cell
10 volume or plasma volume. Limnol. Oceanogr. 12(3), 411-418.
11 Tang, K.W., 2003. Grazing and colony size development in *Phaeocystis globosa*
12 (Prymnesiophyceae): the role of a chemical signal. J. Plankton Res. 25(7), 831-842.
13 Tang, K.W., Jakobsen, H.H. and Visser, A.W., 2001. *Phaeocystis globosa*
14 (Prymnesiophyceae) and the planktonic food web: Feeding, growth, and the trophic
15 interactions among grazers. Limnol. Oceanogr. 46(8), 1860-1870.
16 Teixeira, I.G. and Figueiras, F.G., 2009. Feeding behaviour and non-linear responses in
17 dilution experiments in a coastal upwelling system. Aquat. Microb. Ecol. 55, 53-63.
18 van Boekel, W.H.M., Hansen, F.C., Riegman, R. and Bak, R.P.M., 1992. Lysis-induced
19 decline of a *Phaeocystis* spring bloom and coupling with the microbial foodweb. Mar.
20 Ecol. Prog. Ser. 81, 269-276.
21 van Rijssel, M., Hamm, C.E. and Gieskes, W.W.C., 1997. *Phaeocystis globosa*
22 (Prymnesiophyceae) colonies: hollow structures built with small amounts of
23 polysaccharides. Eur. J. Phycol. 32, 185-192.
24 Veldhuis, M.J.W., Colijn, F. and Venekamp, L.A.H., 1986. The spring bloom of *Phaeocystis*
25 *pouchetii* (Haptophyceae) in Dutch coastal waters. Neth. J. Sea Res. 20(1), 37-48.

Verity, P., Robertson, C.Y., Tronzo, C.R., Andrews, M.G., Nelson, J.R. and Sieracki, M.E., 1992. Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton. Limnol. Oceanogr. 37(7), 1434-1446.

Verity, P.G., Villareal, T.A. and Smayda, T.J., 1988a. Ecological investigations of blooms of colonial *Phaeocystis pouchetii* - I. Abundance, biochemical composition, and metabolic rates. J. Plankton Res. 10(2), 219-248.

Verity, P.G., Villareal, T.A. and Smayda, T.J., 1988b. Ecological investigations of blooms of colonial *Phaeocystis pouchetii*. II. The role of life-cycle phenomena in bloom termination. J. Plankton Res. 10(4), 749-766.

Wassmann, P., 1994. Significance of sedimentation for the termination of *Phaeocystis* blooms. J. Marine Syst. 5, 81-100.

Weisse, T. and Scheffel-Möser, U., 1990. Growth and grazing loss rates in single-celled *Phaeocystis* sp. (Prymnesiophyceae). Mar. Biol. 106, 153-158.

Weisse, T., Tande, K., Verity, P., Hansen, F. and Gieskes, W., 1994. The trophic significance of *Phaeocystis* blooms. J. Marine Syst. 5, 67-79.

1 **Figure legends**

2

3 Fig. 1. Map of the Oosterschelde with the location of the sampling site (black dot) and its
4 position in the Dutch coast (inset).

5

6 Fig. 2. Vertical profiles of (a) absolute salinity (g kg^{-1}), (b) temperature (ºC), and (c) light
7 attenuation coefficient (m^{-1}) in the water column.

8

9 Fig. 3. Mean values in the water column of (a) chl *a* concentration (mg chl *a* m^{-3}), (b) cell
10 abundance of *Phaeocystis* sp. and total phytoplankton (cells m^{-3}), (c) carbon biomass of
11 *Phaeocystis* sp. and total phytoplankton (mg C m^{-3}) and (d) autotrophic carbon to chl *a* ratio.
12 Vertical bars in a, b, and c are standard deviations.

13

14 Fig. 4. Primary production rates at the surface, middle and bottom depths (mg C m^{-3} d^{-1}).
15 Values of integrated primary production (mg C m^{-2} d^{-1}) are also given.

16

17 Fig. 5. Initial carbon biomass (mg C m^{-3}) for each dilution experiment of (a) autotrophic and
18 (b) heterotrophic plankton community. APF: autotrophic picoflagellates, AC: autotrophic
19 carbon, HPF: heterotrophic picoflagellates, HB: heterotrophic bacteria, HNF: heterotrophic
20 nanoflagellates, HC: heterotrophic carbon. Left y-axis corresponds to bars and right y-axis
21 corresponds to lines.

22

23 Fig. 6. Percentages of the standing stock (a-c) and production (d-f) daily consumed by
24 microzooplankton of the bulk plankton groups (a & d), autotrophic plankton groups (b & e)
25 and heterotrophic plankton groups (c & f). AC, autotrophic carbon; HC, heterotrophic carbon;

TC, total carbon; APF, autotrophic picoflagellates, HPF, heterotrophic picoflagellates; HNF, heterotrophic nanoflagellates; HB, heterotrophic bacteria.

Fig. 7. Plankton carbon biomass (mg C m^{-3}) daily ingested by microzooplankton (left panels) and daily produced (right panels). (a & d) total autotrophic and heterotrophic plankton, (b & e), autotrophic plankton groups, (c & f) heterotrophic plankton groups. AC, autotrophic carbon; HC, heterotrophic carbon; APF, autotrophic picoflagellates, HPF, heterotrophic picoflagellates; HNF, heterotrophic nanoflagellates; HB, heterotrophic bacteria. Right y-axis in b & e corresponds to lines.

Fig. 8. Comparison between *Phaeocystis* sp. biomass (mg C m^{-3}) observed *in situ* and estimated using the growth and microzooplankton grazing rates obtained from the dilution experiments started the previous day.

Table 1
Click here to download Table(s): Table 1.doc

Table 1. Mortality (*m*, d^{-1}) and growth (μ, d^{-1}) rates obtained from the dilution experiments. AC: total autotrophic carbon, APF: autotrophic picoflagellates, HC: total heterotrophic carbon, HPF: heterotrophic picoflagellates, HNF: heterotrophic nanoflagellates, HB: heterotrophic bacteria, TC: total plankton carbon biomass (≤200µm). r^2: coefficient of determination for the linear regressions ($p < 0.05$) between the net growth rate and the fraction of unfiltered seawater. In cases of saturated or saturated-increased responses, r^2 was obtained for the linear part of the response (Teixeira and Figueiras, 2009). When r^2 is not given, the growth rate was obtained with only the two highest diluted bottles (Gallegos, 1989). a: non-linear responses. ns: not significant.

	04 May			06 May			08 May			10 May			13 May		
	m	μ	r^2	*m*	μ	r^2	*m*	μ	r^2	*m*	μ	r^2	*m*	μ	r^2
AC	0.39	0.66	0.84[a]	0.51	0.60	0.73[a]	0.23	0.36	a	0.39	0.91	0.76[a]	0.58	0.16	0.91
Diatoms		ns			ns			ns			ns		0.54	0.64	0.90[a]
Synechococcus	0.43	0.70	0.99[a]	0.21	1.26	0.93[a]	0.35	0.30	0.84	1.02	1.10	0.90	1.33	1.33	0.95[a]
APF	0.36	0.49	0.93		ns		1.02	1.04	0.71	0.87	1.67	0.70		ns	
Phaeocystis sp.	0.43	0.70	0.86[a]	0.57	0.61	0.74[a]	0.26	0.38	a	0.43	0.96	0.76[a]	0.62	0.15	0.89
HC	0.69	1.24	0.89	0.97	1.03	0.80	1.36	1.37	0.97	1.25	1.48	0.87[a]	1.48	1.31	0.95
HPF	0.78	1.48	0.84[a]	0.80	1.49	0.80[a]	1.42	1.09	0.85	2.42	2.45	0.88[a]	2.64	2.74	0.91
HNF	0.00	1.09	0.95[a]	1.20	1.00	0.93	1.15	1.59	0.91	1.60	1.70	0.72	2.36	3.08	0.85
HB	1.73	1.42	0.93	1.45	1.78	0.86[a]	1.15	1.20	0.81	1.52	1.86	0.96[a]	1.20	1.23	0.91[a]
TC	0.44	0.77	0.96[a]	0.52	0.53	0.67	0.48	0.53	a	0.51	1.00	0.75[a]	0.65	0.25	0.90

Figure 1

Click here to download Figure(s): Fig.1.doc

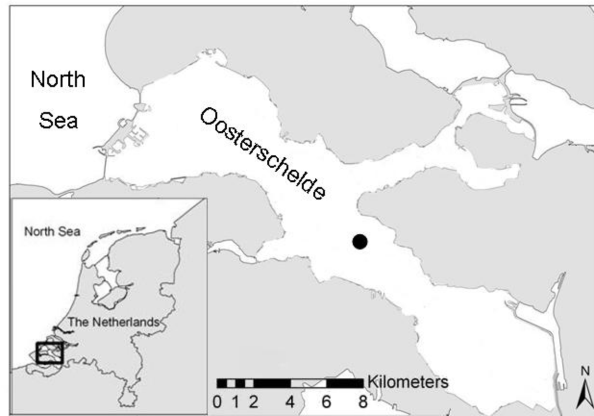

Fig. 1
Teixeira et al.

Figure 2
Click here to download Figure(s): Fig2.doc

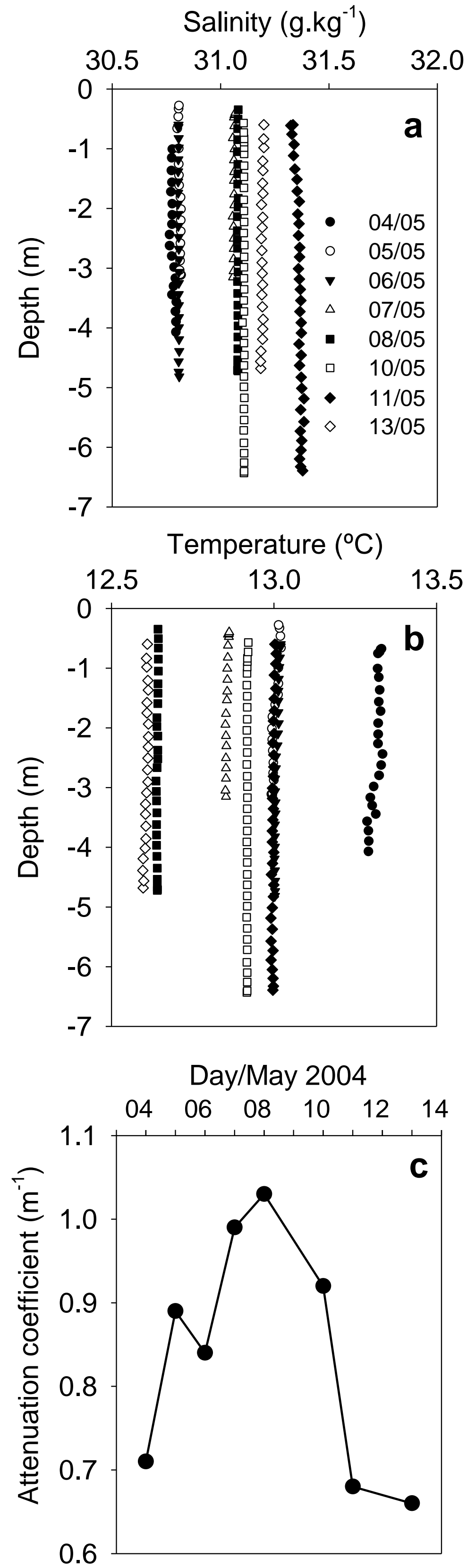

Fig. 2
Teixeira et al.

Figure 3
Click here to download Figure(s): Fig3.doc

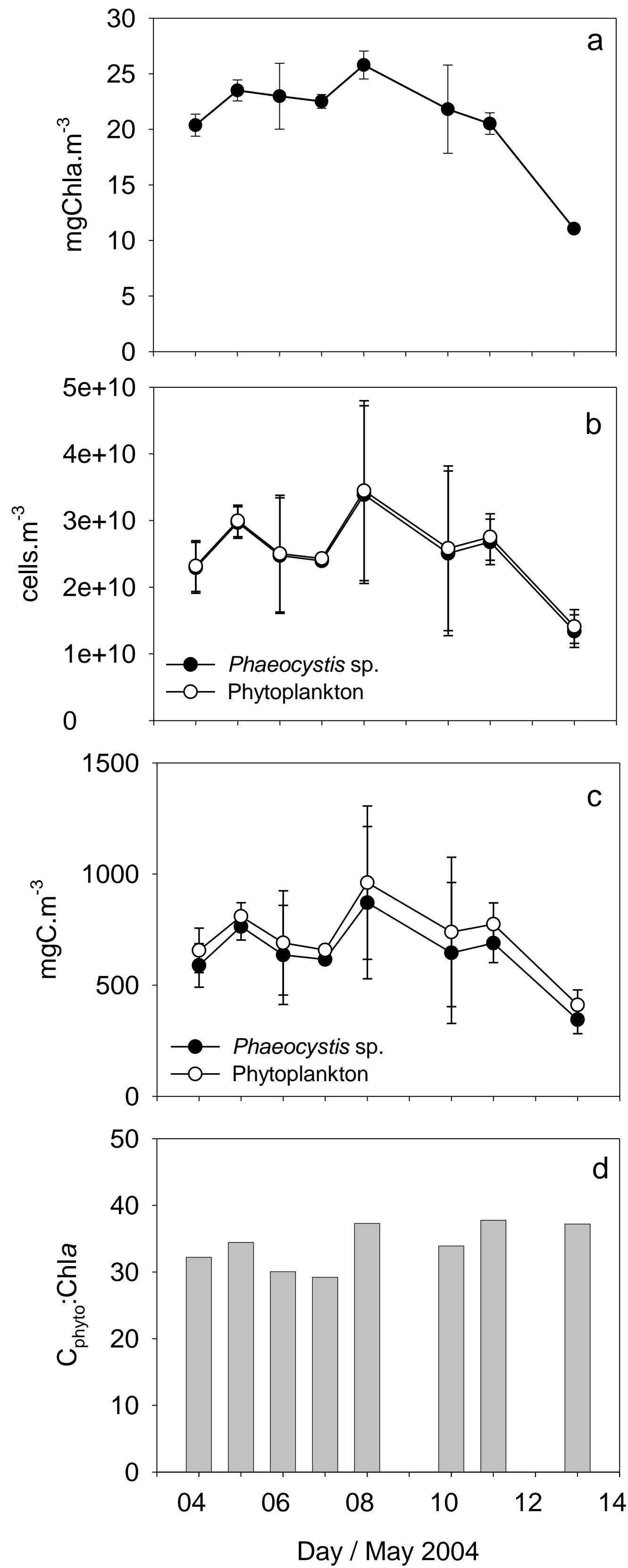

Fig. 3
Teixeira et al.

Figure 4
Click here to download Figure(s): Fig4.doc

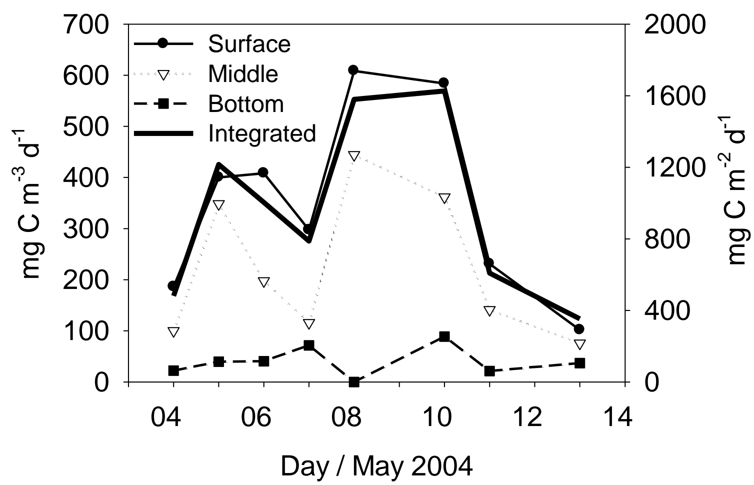

Fig. 4
Teixeira et al.

Figure 5
Click here to download Figure(s): Fig5.doc

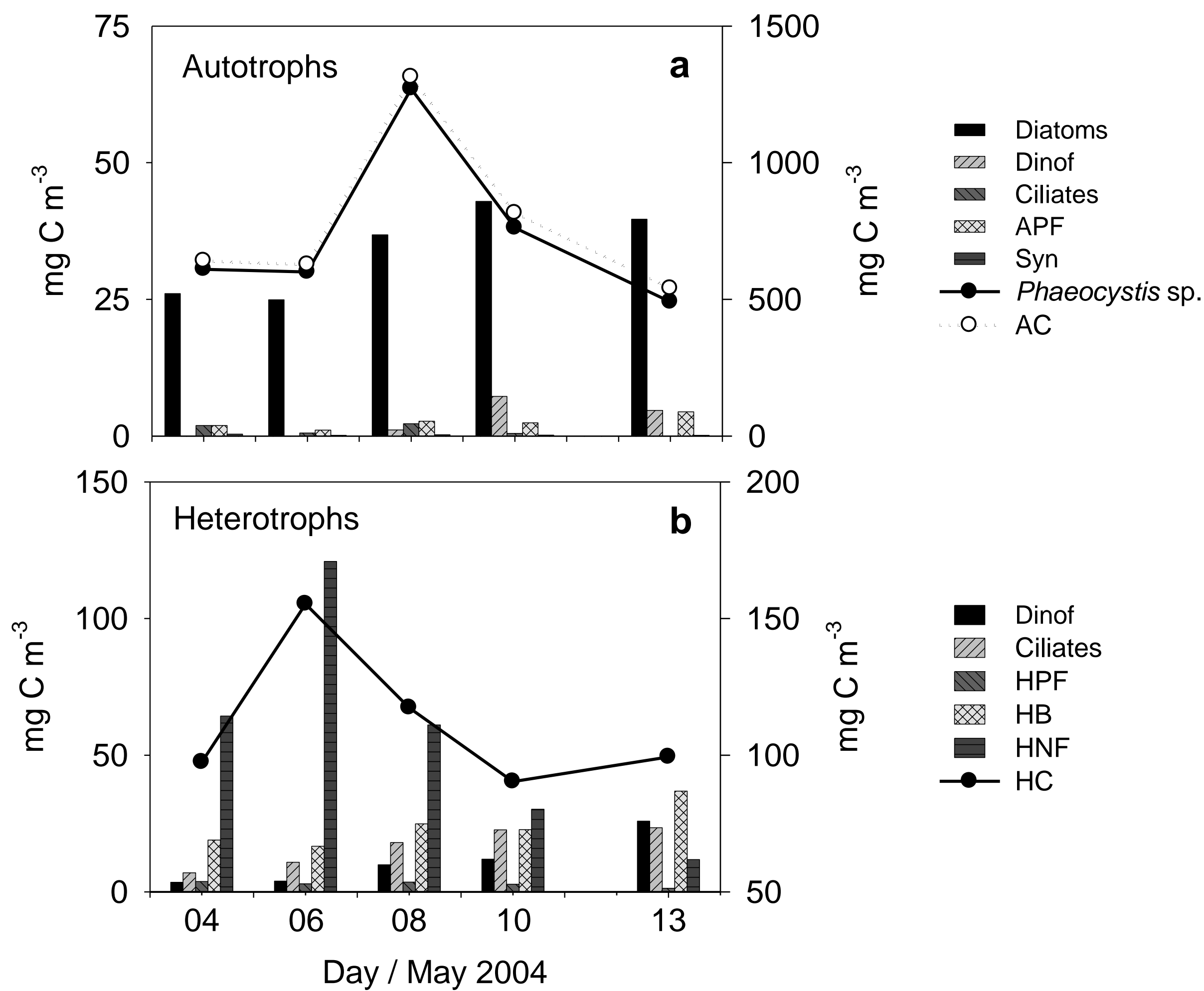

Fig. 5
Teixeira et al.

Figure 6
Click here to download Figure(s): Fig6.doc

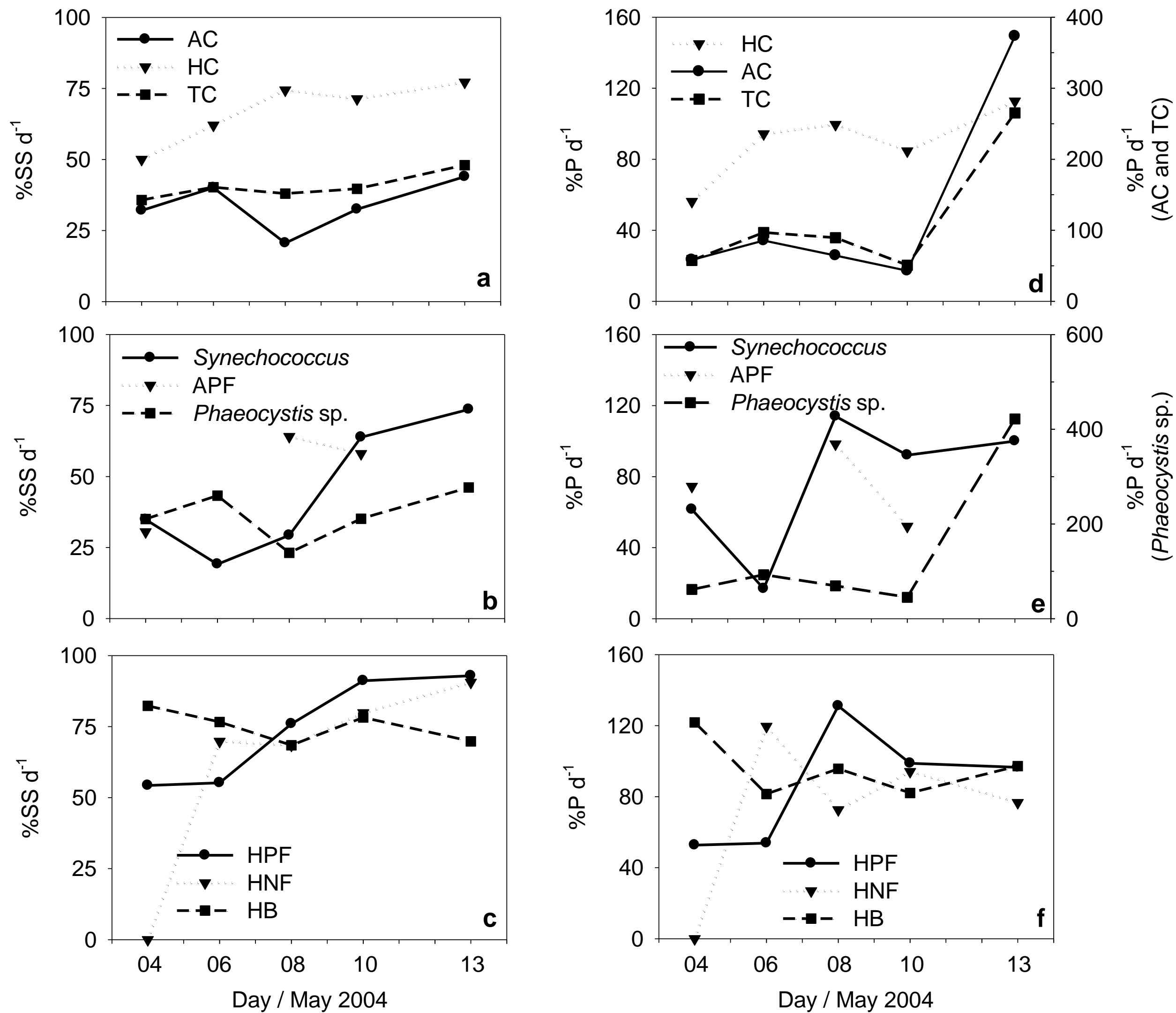

Fig. 6
Teixeira et al.

Figure 7

Click here to download Figure(s): Fig7.doc

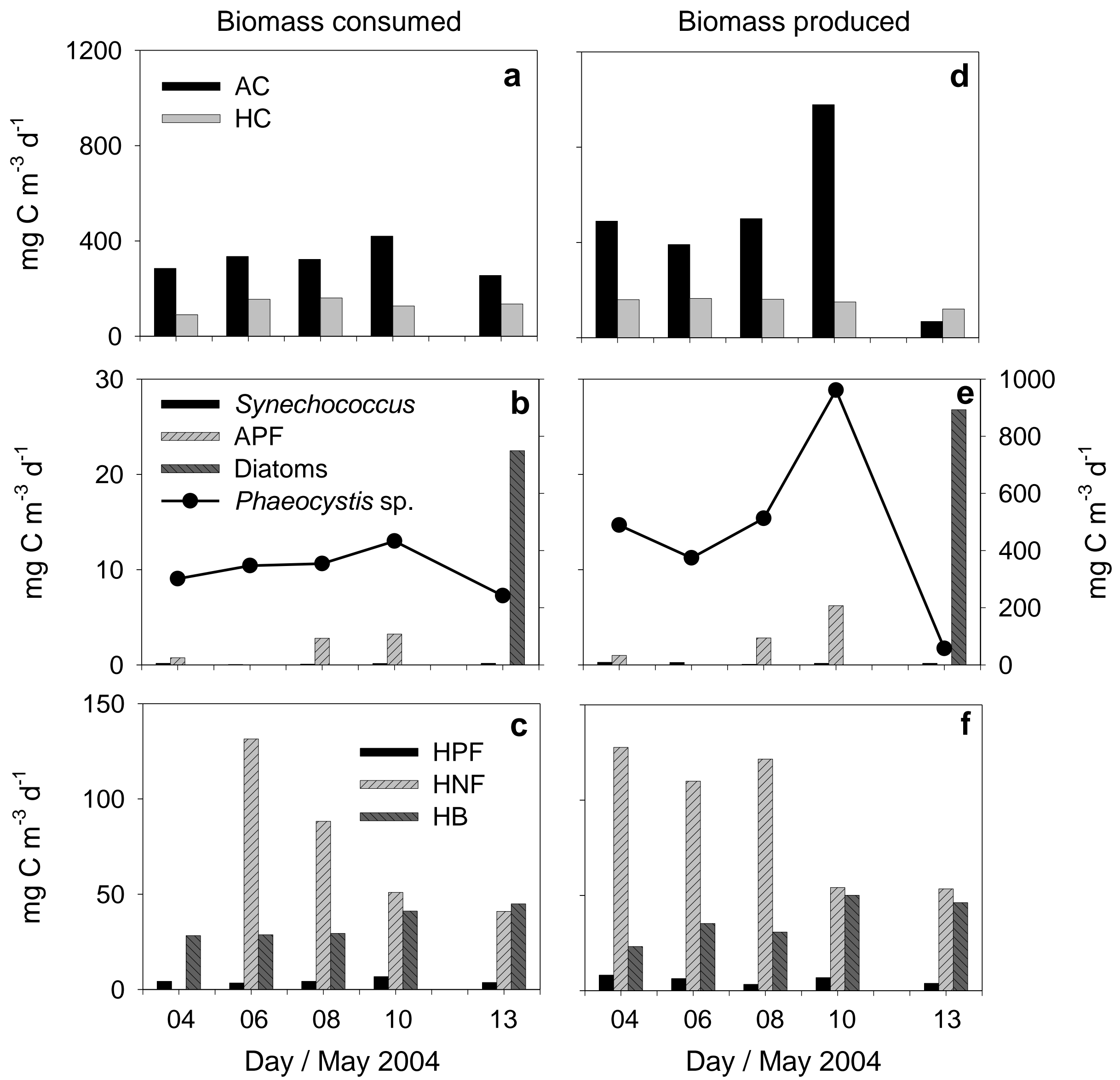

Fig.7
Teixeira et al.

Figure 8
Click here to download Figure(s): Fig8.doc

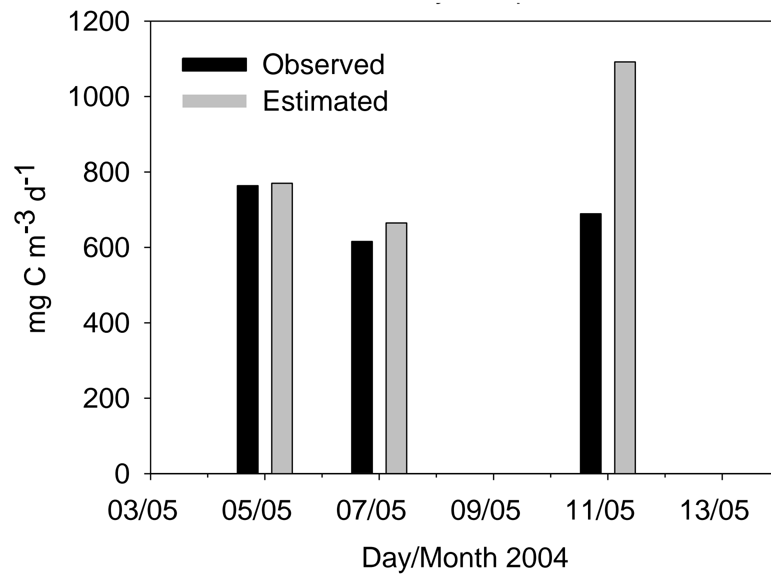

Fig. 8
Teixeira et al.

***Highlights**

Microzooplankton consumed a significant fraction of *Phaeocystis* sp. biomass.
Microzooplankton impact was higher during the bloom collapse, when cells were free-living.
Heterotrophs were also consumed by microzooplankton simultaneously.
We observed two main pathways for channelling *Phaeocystis* sp bloom biomass.