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Albert Calbet, Enric Saiz, Rodrigo Almeda, Juan Ignacio Movilla, Miguel Alcaraz. Low microzooplankton grazing rates in the Arctic Ocean during a *Phaeocystis pouchetii* bloom (Summer 2007): fact or artifact of the dilution technique?. *Journal of Plankton Research*, 2010, 10.1093/plankt/FBQ142 . hal-00642440

HAL Id: hal-00642440

<https://hal.science/hal-00642440>

Submitted on 18 Nov 2011

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Low microzooplankton grazing rates in the Arctic Ocean during a *Phaeocystis pouchetii* bloom (Summer 2007): fact or artifact of the dilution technique?

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|-------------------------------|---|
| Journal: | <i>Journal of Plankton Research</i> |
| Manuscript ID: | JPR-2010-197.R2 |
| Manuscript Type: | Original Article |
| Date Submitted by the Author: | 01-Oct-2010 |
| Complete List of Authors: | Calbet, Albert; Institut de Ciències del Mar (CSIC), Biologia Marina i Oceanografia Saiz, Enric; Institut de Ciències del Mar (CSIC), Biologia Marina i Oceanografia Almeda, Rodrigo; Institut de Ciències del Mar (CSIC), Biologia Marina i Oceanografia Movilla, Juan Ignacio; Institut de Ciències del Mar (CSIC), Biologia Marina i Oceanografia Alcaraz, Miguel; Institut de Ciències del Mar (CSIC), Biologia Marina i Oceanografia |
| Keywords: | Arctic Ocean, Microzooplankton, Dilution technique, Food web |
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4 **Low microzooplankton grazing rates in the Arctic Ocean during a *Phaeocystis***
5 ***pouchetii* bloom (Summer 2007): fact or artifact of the dilution technique?**
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Abstract

We studied the structure and dynamics of the microbial community of Arctic waters during July 2007 using a microzooplankton grazing dilution approach. The sampling covered a latitudinal transect along the East Greenland Sea, and a series of stations in the high Arctic (up to 80° 50' N), west and north of the Svalbard Islands. A main feature of the area was the presence of *Phaeocystis pouchetii*, which formed dense blooms. Despite the considerable biomass of microzooplankton (mostly large ciliates and dinoflagellates), their grazing impact on phytoplankton, assessed as total chlorophyll *a*, was significant in only 6 out of 16 experiments, which resulted in 8% of the standing stock being consumed on average. Overall, phytoplankton instantaneous growth rates were very low and even negative at times (range: -0.24 to 0.14; average: -0.04 for total chlorophyll), which could not be attributed to nutrient limitation nor the estimated microzooplankton grazing. We present 3 nonexclusive explanations for this fact: 1) we were facing a senescent community in which many organisms were dying either as a result of virus infections or for other natural causes, as corroborated by parallel estimates of natural cell mortality using membrane permeability probes; 2) the widespread and abundant *P. pouchetii* was probably deterring grazing and adversely affecting the entire planktonic community at the time of the study; and 3) the dilution technique failed to give a real estimate of grazing (i.e. either non significant or positive slopes), likely as a consequence of trophic cascades (decline of major grazers in the more concentrated treatments) combined with saturated-feeding responses. This last point calls for special attention when intending to use the dilution technique in productive environments, where grazing may be saturated.

Keywords: Arctic Ocean; Microzooplankton; *Phaeocystis pouchetii*; Dilution technique, Food web.

Running title: Food web interactions in the Arctic Ocean

Introduction

The fast melting of Arctic ice, a consequence of global warming (Cavalieri *et al.*, 2003; Johannessen *et al.*, 2004), has profound ecological consequences on Arctic wildlife, including polar bears, walruses, and ringed seals, which are threatened by the reduction of sea-ice cover. Not so evident are the changes that the inhabitants of the waters underneath and surrounding the Polar ice cap will suffer under progressive melting conditions. One of the key groups in marine food webs, which are potentially sensitive to these Arctic scenario changes, are the microzooplankton.

Microzooplankton are of paramount importance in controlling primary production (Calbet and Landry, 2004). Regrettably, their role in the high Arctic still remains uncertain and needs to be determined to fully understand and predict the consequences of the changes this ecosystem will experience in the future. For instance, the relevance of the trophic impact of this group in the Arctic Ocean food web has mostly been studied either in bays or in relatively low latitude sites (Paranjape, 1987; Gifford *et al.*, 1995; Olson and Strom, 2002; Verity *et al.*, 2002; Strom *et al.*, 2008), or it has been derived indirectly (Levinsen *et al.*, 1999; Rysgaard *et al.*, 1999; Levinsen and Nielsen, 2002). Most of these studies indicate a strong control of primary production by microzooplankton grazing. However, recent work in high Arctic waters by Sherr *et al.* (Sherr *et al.*, 2009) questions such strong control, likely due, according to the authors, to a strong top-down impact of copepods on microzooplankton (Levinsen and Nielsen, 2002; Campbell *et al.*, 2009).

For this reason, we undertook a study of the microbial interactions in Arctic waters during the melting season. Our research coincided with a bloom of *P. pouchetii* (Lasternas and Agustí, in press), which will add further value to our results because, despite being a successful species in Arctic waters (Schoemann *et al.*, 2005), the very few data dealing with the impact of microzooplankton feeding on these algae have resulted in contradictory conclusions. Weisse and Scheffel-Moser (Weisse and Scheffel-Moser, 1990) measured microzooplankton grazing in a *P. cf. pouchetii* bloom in the North Sea using the dilution technique (Landry and Hassett, 1982)

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3 finding grazing loss rates from 0.037 to 0.174 h⁻¹, grazing rates increasing in the
4 course of the bloom and exceeding phytoplankton growth rates at the end. Gifford *et*
5 *al.* (Gifford *et al.*, 1995), also using the dilution technique, did not detect any grazing
6 on phytoplankton in the high-latitude North Atlantic Ocean during a *P. pouchetii*
7 bloom, but they obtained increased microzooplankton grazing as the bloom declined.
8 Archer *et al.* (Archer *et al.*, 2000) measured microzooplankton grazing impact under
9 relatively low abundance of *P. pouchetii* in 3 fjords of the northern Norway, and
10 obtained significant grazing rates on total phytoplankton and on fluorescently labeled
11 algae of similar size to *P. pouchetii*. On the other hand, Wolfe *et al.* (2000) only found
12 significant grazing on chlorophyll and DMSP when *P. pouchetii* cells were in poor
13 condition in the Labrador Sea. In this regard, the ability of microzooplankton to ingest
14 *Phaeocystis* spp. has been widely discussed in the literature (see reviews by Whipple
15 *et al.*, 2005, and Nejstgaard *et al.*, 2007). It is usually accepted that microzooplankton
16 exerts their pressure mostly on single cells; however, there is evidence that *Noctiluca*
17 *scintillans* (Weisse *et al.*, 1994; Jakobsen and Tang, 2002) and *Gyrodinium cf. spirale*
18 (Stelfox-Widdicombe *et al.*, 2004) are able to ingest small colonies.

45 Method

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47 This study was part of a multidisciplinary project (ATOS: POL2006-00550/CTM),
48 and took place on board the research vessel BIO Hespérides from July 1 to 24, 2007.
49 The cruise departed Reykjavik (Iceland), and sampling started northward across the
50 Greenland Sea (Table 1, Fig. 1). In Arctic waters, we sampled a series of stations in
51 the vicinity of the ice-edge, alternating between several stations free of ice and two
52 coastal stations near the Svalbard Islands. During the study, we reached a historical
53 minimum of Arctic ice cover (Zhang *et al.* 2008), allowing samples to be taken from
54 areas up to 80° 50' N. The stations lasted 24 h, starting with profiles for the
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3 measurement of salinity, temperature, and fluorescence during the early morning
4 using a Seabird CTD911, followed by collection of water samples for the
5 determination of chlorophyll *a* concentration (hereafter Chl *a*) with 12 l Niskin bottles
6 fitted to a rosette during the ascending CTD casts.
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11 At each station we conducted standard grazing dilution experiments (Landry
12 and Hassett, 1982) to assess the microzooplankton grazing impact on primary
13 producers and on other components of the food web (see below). This technique
14 consists of the sequential dilution of natural water with filtered seawater to obtain a
15 gradient of net grazing impact on phytoplankton. The water for these experiments was
16 collected at the fluorescence maximum (Table 1) using a rosette equipped with 12-L
17 Niskin bottles, according to the fluorescence profile. Once on deck, the water was
18 gravity-filtered through a Pall Acropak 0.8/0.2 500 capsule (previously flushed,
19 including tubing, with diluted HCl and thoroughly rinsed with deionised water), and
20 then 2 replicate bottles (2.3-L acid washed polycarbonate) for each of the dilution
21 treatments were filled with the corresponding required amount of filtered seawater.
22 Afterwards, we added natural non-filtered seawater from the selected depth to the
23 bottles to generate experimental water percentages of 13, 27, 50, 73, and 100%. At
24 some stations, the presence of a dense bloom of the haptophyte *Phaeocystis pouchetii*
25 made it impossible to efficiently filter the water through the Acropak capsules;
26 therefore, filtered seawater originated from below the bloom depth.
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41 To guarantee the homogeneity of the natural water poured from different Niskin
42 bottles filled at the same depth, we used a 20-L intermediary carboy in which the
43 water was gently mixed by its own flow. All the process was carried out under dim
44 light conditions to avoid cell light-damage. To promote constant and saturated
45 phytoplankton growth in the dilution series, each bottle received added nutrients (10
46 μM NH_4Cl and 0.7 μM Na_2HPO_4). In addition, four 100% (i.e., not diluted) bottles
47 were prepared without nutrients to assess the natural growth of the algae. Two of
48 these latter bottles were sacrificed for initial samples. Because we did not screen the
49 water used for the dilution series to avoid damaging delicate microzooplankton, the
50 experimental suspension may have contained some mesozooplankton. We examined
51 the bottles by eye to observe the presence of large copepods, and the very few times
52 this occurred did not result in inconsistent results in the dilution series.
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All bottles were incubated on deck in a large (600 L), dark incubator with open-circuit water running from a 5-m depth at a temperature about the same as *in situ*. The natural sunlight was dimmed with appropriate dark plastic mesh to mimic the light intensity at the fluorescence maximum. We gently mixed the bottles by repeatedly turning them upside down and moving them around the incubator at least three times per day. After 27-32 h, we finished the incubations and took samples for quantification of total and > 5- μm Chl *a* concentration. To get further insight into the actual trophic interactions during dilution experiments and to detect possible artifacts (Dolan *et al.*, 2000; Agis *et al.*, 2007; Modigh and Franzè, 2009), we additionally took samples for the determination of nano- and microplankton from the 2 initials and in one of the replicates per dilution level, and preserved them with Acidic Lugol's solution (2% final concentration). To avoid damaging the delicate cells we first added the fixative and then gently siphoned the water sample directly into the sample bottle

For total Chl *a*, we filtered 50-250 ml of water (depending on station and dilution level) under low vacuum pressure (< 100 mm Hg) through Whatmann glass fiber filters (GF/F, 25 mm diameter). For the > 5- μm fraction, we filtered 100-300-ml samples through 5- μm pore-size polycarbonate Osmonics Inc. filters (25 mm diameter). After filtration, the filters were stored frozen at -20°C until fluorometric analysis of acetone extracts, with and without acidification (Parsons *et al.*, 1984) on a Turner Designs Fluorometer.

Lugol-preserved samples were processed in the laboratory. We concentrated the most diluted treatments (13%, 25% and 50%) by first settling the entire bottle for 72 h and gently siphoning off 50-75% of the supernatant water without re-suspending the sample. Then, for all the samples, 100 ml of the concentrate was settled in Utermöhl chambers for at least 48 h prior and counted under the microscope. The whole chamber, or a fraction of it for the smallest and more abundant organisms, was counted under an inverted microscope (XSB-1A) at 100, 250, and 400X magnification, depending on the group. Fifty to one-hundred cells per group were sized, adjusted to their closest geometric shape, and converted into carbon using the equations of Menden-Deuer and Lessard (Menden-Deuer and Lessard, 2000). We did not use any correction factor to compensate for ciliate losses due to fixation, as previously suggested by Broglio *et al.* (Broglio *et al.*, 2004), and Calbet and Saiz

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3 (Calbet and Saiz, 2005), because recent research has revealed that such corrections
4 should apply to many other planktonic groups, not only ciliates (Zarauz and Irigoien,
5 2008), and universal factors have not been developed yet. Because plankton were
6 preserved with acidic Lugol's solution, no distinction between strict heterotrophs and
7 auto/mixotrophs was made for flagellates, ciliates, and some dinoflagellates.
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9 However, the groups identified to species level were classified trophically according
10 to the literature.
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17 Instantaneous growth rates in dilution grazing experiments were derived from
18 net growth in the un-amended bottles (no nutrients added) adding the mortality by
19 microzooplankton grazing from dilution experiments when the latter was significant.
20 We should note that in the cases where the grazing mortality was not significant the
21 instantaneous growth rate of prey would be equivalent to the net growth rate in the
22 un-amended bottles. Therefore, we may be underestimating the actual instantaneous
23 growth rate of the prey because some mortality may still occur in the bottles, even if
24 not measured by the dilution method. All statistical tests were conducted with JMP
25 7.0 statistical software.
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37 Results

38 *Plankton biomass and distribution*

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41 We present a summary of the different fractions of Chl *a* in Table 1 and the
42 contribution of micro- and nanoplankton to total plankton community biomass at the
43 sampled stations in Table 2 (a detailed summary of the species composition is
44 presented in the Supplementary Material online). The main feature of the data for the
45 Arctic stations is the almost ubiquitous presence of the haptophyte *Phaeocystis*
46 *pouchetii*, which formed dense blooms (Table 2, Fig. 2) and whose distribution was
47 directly correlated with total Chl *a* ($r=0.76$, $p<0.01$), and inversely correlated with
48 temperature ($r = 0.77$; $p < 0.05$). Following *P. pouchetii*, diatoms (mostly chain
49 forming species $> 20 \mu\text{m}$ in total length of the genera *Chaetoceros*, *Fragilariopsis*,
50 *Nitzschia*, *Rhizosolenia*, *Thalassiothrix*, and *Thalassiosira*) were the second biomass
51 grouping within the phytoplankton, being dominant in the Greenland Sea and at
52 warmer stations. Regarding micrograzers, the important contribution of large ciliates
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3 (mostly Tintinnida, *Strombidium* spp., and the mixotroph *Laboea* spp.) was noticeable
4 in the Greenland Sea and Svalbard Islands coastal station (Table 2). Actually, ciliates
5 were the overall major contributors to microzooplankton during the study and were
6 substantial components of the total plankton biomass (ca. 25%), whereas
7 heterotrophic dinoflagellates (considering 50% of unidentified dinoflagellates as
8 heterotrophic; Lessard and Swift, 1985; Sherr and Sherr, 2007) represented, on
9 average, 12% of the total carbon biomass (Table 2). In relation to the relevance of
10 heterotrophs, it is interesting to note that the quotient between heterotrophic and
11 autotrophic carbon (indicative of the trophic characteristics of the system) was > 1 in
12 the Greenland Sea and Svalbard Islands coastal stations (Fig. 3).
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22 Despite the high heterotrophic biomass, we did not find significant
23 correlations between any of the size fractions of Chl *a*, large heterotrophic
24 dinoflagellates (e.g., *Gyrodinium* spp., *Protoperidinium* spp., and *Katodinium* spp.),
25 and ciliates. However, large mixotrophic dinoflagellates (*Dinophysis* spp., *Ceratium*
26 spp., *Gonyaulax* spp., and *Amphidinium* spp.) were positively correlated with diatoms
27 ($r = 0.85$; $p < 0.05$). Correlations between groups do not necessarily mean causality,
28 but they can indicate that an association exists. Likewise, unidentified $> 20\text{-}\mu\text{m}$
29 dinoflagellates were also positively correlated with *P. pouchetti* biomass ($r = 0.72$; $p <$
30 0.05). However, when excluded from the analysis station 43 (the station with the
31 maximum biomass of *P. pouchetii*) this relationship is strongly affected and it
32 becomes non-significant.
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43 We calculated the C:Chl *a* ratios using the Chl *a* data and the autotrophic
44 biomass (in carbon), obtained by cell counting and conversion to carbon using
45 literature equations (Table 2). For unidentified dinoflagellates we assumed 50% of
46 the organisms were phototrophs. Because our microscope counting technique, based
47 on Lugol-preserved samples, does not allow for good resolution of the lower size-
48 fractions, we can better estimate the C:Chl *a* ratio using only $> 5\text{-}\mu\text{m}$ Chl *a*. The
49 values averaged 18 for the entire data-set, and ranged from 4 to 47.
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Dilution grazing experiments

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3 Table 3 shows the outcome of the dilution grazing experiments based on the different
4 size-fractions of Chl *a*. Unanticipated results from these experiments include the
5 following: i) the little, or even at times negative, phytoplankton instantaneous growth
6 (range: -0.24 to 0.14; average -0.04 for total Chl *a*) and ii) the low microzooplankton
7 grazing impact on primary producers. Significant microzooplankton grazing (i.e.,
8 significant negative slopes in the dilution experiments) were found only in 6 out of 16
9 experiments for total and <5- μm Chl *a*, and no significant mortality was detected in
10 the >5- μm fraction at any of the stations. Overall, microzooplankton grazing cannot
11 explain the observed phytoplankton negative net growth rates (especially in the >5-
12 μm size-fractions) and must be attributed to other causes (see discussion). However, it
13 is interesting to note the inverse relationship between the phytoplankton net growth
14 rates and Chl *a* concentration (Fig. 4) in the Arctic Ocean stations. This points
15 towards a naturally-occurring, density-dependent mortality effect, probably linked to
16 the presence of *Phaeocystis pouchetii*, although not significantly related to the
17 occurrence of either this or any other planktonic group. Likewise, and related to net
18 phytoplankton growth rates, there was no clear evidence of nutrient limitation at most
19 of the stations (Fig. 5).

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The phytoplankton and microzooplankton composition analysis in the initial and final dilution experiments revealed a very complex food web scenario, with frequent negative growth rates both for autotrophs and heterotrophs, frequent positive slopes, and very few cases of significant microzooplankton grazing impact (Table 4). Overall, it is difficult to extract any clear interpretation or global pattern out of the dilution data for the different plankton groups. Nevertheless, we decided to present these data because negative dilution grazing results in the literature have seldom been discussed (Dolan and McKeon, 2005). As example, we show in Fig. 6 the dilution experiment plots for stations 4 and 33. It is interesting to note in these plots the positive slopes for some prey and the negative slopes (theoretically indicative of grazing) for some potential grazers of phytoplankton. It should be noted that the mortality rates of top predators (large ciliates and heterotrophic dinoflagellates) are not necessarily estimated well by the dilution method. However, we think the information about the changes of these groups in the incubation bottles is relevant and should be also presented in the results.

Discussion

Community composition and microzooplankton grazing on phytoplankton

A main characteristic that distinguishes our study from previous ones in Arctic waters is the peculiarity of the composition of the heterotrophic microbial community. Past works stressed the relevance of heterotrophic dinoflagellates in Arctic waters, especially when diatoms dominated the autotrophic community (Levinsen *et al.*, 1999; Rysgaard *et al.*, 1999; Sherr *et al.*, 1997; Sherr and Sherr, 2007; Sherr *et al.*, 2009). We found a population rich in ciliates, which accounted for ca. 2/3 of the total microzooplankton biomass (assuming 50% of the unidentified dinoflagellates were heterotrophs). This high contribution was especially evident in the Greenland Sea, where they completely dominated the microplankton. We suspect that in these waters we were facing a very unusual situation, likely the result of temporal imbalances in the structure of the community (e.g., the end of a phytoplankton bloom), as the biomass of producers seems unable to maintain such a high abundance of grazers.

Despite the relatively high biomass of microzooplankton, the grazing rates on phytoplankton (Chl *a*) were low. Certainly, trophic relationships between predators and prey do not necessarily have to be directly related to biomass, especially in areas where annual blooms are intense. If a phytoplankton bloom is senescent, grazers, even if abundant, may not graze phytoplankton cells in poor health (the end of the bloom situation). Similar inconsistencies between grazing impacts and grazer biomass have also been reported for Antarctic waters (Caron *et al.*, 2000). Regarding the Arctic Ocean, we find contrasting results for microzooplankton grazing impact in different areas. For instance, during the summer along the western coast of Greenland, Levinsen *et al.* (Levinsen *et al.*, 1999) studied the microzooplankton grazing impact by indirect methods and concluded that if this group had a purely autotrophic diet they could remove 362% of primary daily production. Nevertheless, the authors pointed out that cannibalism could likely reduce the actual impact of this group on phytoplankton. Also by indirect measurements, Rysgaard *et al.* (Rysgaard *et al.*, 1999) estimated that the combined grazing activity of ciliates and dinoflagellates in Young Sound (NE Greenland) would potentially remove only 14% of the annual

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3 primary production, a value that contrasts strongly with the previous estimate.
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5 Levinsen and Nielsen (Levinsen and Nielsen, 2002) found that potential
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7 microzooplankton grazing could account for 32-55% of the primary production in
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9 Disko Bay. These data, as well as the 40-114% primary production daily grazed in
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11 Jones Sound (Paranjape, 1987), the 37-88% in Baffin Bay (Paranjape, 1987), the 64-
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13 97% in the Barents Sea (Verity *et al.*, 2002), and the 2-293% (average 110 and 81%
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15 of phytoplankton growth rates for > 10 μm and < 10 μm phytoplankton, respectively)
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17 grazed in the southeast Bering Sea (Olson and Strom, 2002) are much higher than the
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19 values observed in our study. Recent research in the high Western Arctic Ocean
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21 (Sherr *et al.*, 2009), however, advocates a lower control of microzooplankton grazing
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23 on primary producers (average $22 \pm 26\%$). The study by Sherr *et al.* (Sherr *et al.*,
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25 2009), even if in a different area, with lower average temperatures, and with a
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27 community of phytoplankton not dominated by *Phaeocystis*, but by diatoms, is the
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29 study that *a priori* seems more appropriate to contrast with ours; both were located in
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31 open waters near the ice-edge zone, and both were conducted at high latitudes using
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33 the same methodology. Our data agree with the results of the Sherr *et al.* (Sherr *et al.*,
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35 2009) study, which does not show significant grazing in about half of the experiments
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37 at the fluorescence maximum and contains total average grazing rates of $< 0.1 \text{ d}^{-1}$.
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39 Similarly, in the Sherr *et al.* (Sherr *et al.*, 2009) study they found low and even
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41 negative values for phytoplankton growth rates during summer. They attributed this
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43 natural mortality to low light levels (samples were collected at the base of the
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45 euphotic zone) and to post-bloom conditions (protist grazing rates were low because
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47 the diatom blooms were senescent).

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46 Despite the overall low grazing rates, some associations between the
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48 distributions of several organisms seem to be evident. For instance, mixotrophic
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50 dinoflagellates were positively correlated with diatoms. Mixotrophy is widespread
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52 among dinoflagellates, and it is not uncommon that these organisms contribute
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54 significantly to the community grazing on phytoplankton (Stoecker, 1999; Stoecker *et*
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56 *al.*, 2009), and particularly on diatoms (Yoo *et al.*, 2009). Moreover, the role of
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58 mixotrophic dinoflagellates in the fate of primary producers is likely to have been
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60 underestimated for several reasons. First, the presence of their own chloroplasts may
mask the detection of prey inside the organisms, and second, the different feeding
mechanisms displayed by this group (direct engulfment, tube-feeding, and pallium-

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3 feeding; Hansen and Calado, 1999) make it quite difficult to correctly assess their
4 contribution to total community grazing. Because *pallium*-feeding and tube-feeding
5 do not leave evident remains of the preyed cell inside the predator and because the
6 *pallium* and peduncles are not persistent structures, they are not easily quantified
7 when microscopically observing preserved samples. Therefore, we are of the opinion
8 that a predator-prey association between armoured mixotrophic dinoflagellates and
9 diatoms is meaningful. Certainly, other microbial grazers, such as ciliates among
10 others, can impact on diatoms (Aberle *et al.*, 2007), but we did not obtain proof of this
11 behaviour in our study. On the other hand, the diatom-dinoflagellate relationship has
12 been widely suggested in the literature (e.g., Sherr and Sherr, 2007; Saito *et al.*, 2006;
13 Calbet, 2008), although seldom quantified in natural communities (Archer *et al.*,
14 1996).

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28 *Finding explanations for the low microzooplankton grazing impact, but the high net*
29 *mortality rates of phytoplankton:*

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33 It is difficult to ascertain whether the low grazing found in our study is a general
34 characteristic describing the system or if it was the result of some particular
35 conditions. It is surprising, however, that we found many negative net phytoplankton
36 growth rates (based on Chl *a* changes) not associated with microzooplankton grazing.
37 The simplest explanation for this would be that the incubation light-level was not
38 appropriate, the cells adjusting their Chl *a* contents to the new conditions. This could
39 actually be the cause at some stations; however, the same pattern persists in cell
40 counts in many others. Therefore, we have to find alternative hypotheses, which may
41 be nonexclusive:

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50 - *The natural mortality hypothesis*

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53 Parallel to our study, Lasternas and Agustí (Lasternas and Agustí, in press) used a
54 membrane permeability probe (Agustí and Sánchez, 2002) to estimate the natural
55 mortality of the *P. pouchetii* community, and they observed that higher percentages of
56 dead *P. pouchetii* cells (up to ca. 90% at some stations) were associated to both cold
57 and less saline waters across the area studied. The mechanisms behind this mortality,
58 not related to grazing rates, can be several. It could well be that we were facing a
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3 senescent community at the end of the bloom. On the other hand, we cannot disregard
4 viruses as playing a role in controlling the population of these and other protists and
5 responsible for the frequent negative growth rates (Baudoux *et al.*, 2006; Jacobsen *et*
6 *al.*, 2007). Unfortunately, our experimental set up was not adequate for virus-related
7 mortality quantification.
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13 Given the contribution of *P. pouchetii* to the total phytoplankton biomass, the
14 natural mortality of these algae could be driving the growth rates observed on the
15 basis of Chl *a*. Corroborating this, we observed an inverse relationship between
16 phytoplankton instantaneous growth rates and Chl *a* distribution. Although other
17 groups of plankton could also have shown natural mortality rates, we do not have
18 solid evidence of this.
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24 25 - *The Phaeocystis pouchetii hypothesis*

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27 As previously mentioned, our cruise coincided with high abundances of *Phaeocystis*
28 *pouchetii*, which commonly blooms in these waters in July (Schoemann *et al.*, 2005).
29 The peculiarities of the food web dominated by these algae make it difficult to extract
30 general conclusions. We believe that our low grazing estimates (not significant at
31 most of the stations) could be partially a consequence of the presence of *P. pouchetii*.
32 We observed the presence of many colonies in the samples, although we have not
33 been able to quantify the percentage of free cells and colonies in the Lugol preserved
34 samples. However, it is quite likely this species either introduced variability into the
35 samples (colonies can be heterogeneously distributed in the samples) and precluded
36 the establishment of significant regressions based on Chl *a*, evaded grazing when in
37 colonial form (Hansen *et al.*, 1994; Weisse *et al.*, 1994; Tang, 2003), or chemically
38 deterred grazers (Barnard *et al.*, 1984; Nejstgaard *et al.*, 2007; van Rijssel *et al.*,
39 2007). Certainly, *P. pouchetii* seems the most obvious candidate when seeking
40 chemical deterrence of grazing. Similar to macroalgae and other phytoplankters,
41 *Phaeocystis* spp. exude chemicals that can interfere with grazing activity (see review
42 by van Rijssel *et al.*, 2007). Although the chemicals involved in this process have not
43 yet been identified, it has been suggested that grazing-activated DMSP cleavage by *P.*
44 *pouchetii* contributes to grazing deterrence (Wolfe *et al.*, 2000). Allelopathic
45 interactions could also be responsible for the negative growth rates of a phytoplankton
46 (and other protists) community, which apparently was not limited by nutrients.
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Overall, we believe that the net transfer of energy to higher trophic levels in this ecosystem would be greatly diminished if the autotrophic community was dominated by *Phaeocystis*, provided that other groups not considered here (e.g., copepods) do not exert a strong impact on this alga. However, the low tolerance of *P. pouchetii* to relatively high temperatures (Schoemann *et al.*, 2005), as shown by the reduced presence at St. 27 where temperatures reached 7.5°C, indicates a limited relevance of this species under global warming scenarios. Certainly, this does not mean that other *Phaeocystis* species, such as *P. globosa*, which is adapted to warmer waters, cannot replace *P. pouchetii*, further diminishing the trophic efficiency of the system because *P. globosa* is seldom consumed by zooplankton (see review by Nejstgaard *et al.*, 2007).

-The dilution grazing artifact hypothesis

Chl *a* is a rough proxy for phytoplankton because it does not capture the complexity of this group, and its use in dilution experiments has been questioned because chlorophyll content per cell may change during the incubation (McManus, 1995). Moreover, the need for a close examination of the microzooplankton community during dilution experiments to detect and correct possible artifacts has also been discussed (Dolan *et al.*, 2000; Agis *et al.*, 2007; Modigh and Franzè, 2009). To shed light on this point and to deepen our understanding of the food web interactions in Arctic waters, we further examined changes in the entire microbial community during the dilution experiments. We did not make any attempt to compare the rates obtained based on Chl *a*, with those derived from cell counts and their corresponding conversion to carbon because the uncertainties associated when depicting a trophic role (autotrophy vs heterotrophy) to unidentified dinoflagellates and nanoflagellates. Besides, the contribution of mixotrophic species at some stations (e.g. the mixotrophic ciliate *Laboea* sp. represented most of the planktonic biomass at station St. 3; Supplementary Material online) precluded any comparison.

When opening the planktonic black box in the dilution experimental bottles we faced unanticipated results suggesting a complex and intricate food web, in which choosing the major microbial grazers of phytoplankton was not so straightforward. A clear example of a puzzling response was the occurrence of positive regression slopes between the net growth rates of certain groups against the dilution factor. Positive slopes for heterotrophs and mixotrophs, even if sometimes the result of complicated

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3 ecological interactions, can be easily interpreted as growth enhancement due to
4 increased feeding in the more concentrated treatments. However, positive slopes of
5 phytoplankton occur when the organism considered is adversely affected by the
6 dilution treatment. The explanation for these particular responses is not easy because
7 they can have different non-exclusive causes. For instance, they may either be the
8 result of strong trophic cascade effects during the incubations (Calbet *et al.*, 2008),
9 chemical grazing deterrence by the algae or other organisms, toxic effects of the
10 filtered seawater (Landry, 1993), mixotrophs being important contributors of
11 phytoplankton biomass (then, favoured in less diluted conditions), or of complex
12 cycling of nutrients between internal and external pools (Landry, 1993), because
13 nutrients would be taken up by smaller algae more efficiently and would become
14 limiting for larger phytoplankton. We do not believe that the last three hypotheses
15 apply to our experiments for the following reasons: i) any toxic effect would likely be
16 persistently evident at all the groups and stations, ii) some of the groups of
17 phytoplankton showing positive slopes, as far as we know, were not mixotrophic
18 (e.g., diatoms), and iii) nutrients were supplied in excess. Therefore, either (or both)
19 trophic cascades or grazing deterrence seem to be the most reasonable explanation. If
20 *P. pouchetii* was responsible for the positive slopes found for diatoms and other
21 groups, its effects would not be apparent at the stations where the haptophyte was not
22 present (stations 2 and 3). While this was the case and thus supporting the feeding
23 deterrence hypothesis, it did not fully demonstrate the hypothesis because the
24 response was not directly related to the *P. pouchetii* concentration.
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43 We, therefore, contemplated the trophic cascade explanation for the positive
44 slopes found in our (and others) study. It has been argued that changes of grazer
45 abundance during dilution grazing incubation may result in results that are artifacts
46 (Dolan *et al.*, 2000; Agis *et al.*, 2007; Modigh and Franzè, 2009). These changes
47 usually involve a decrease in abundance in the most diluted treatments, the result of
48 starvation. However, our experiments showed on many occasions the opposite, the
49 grazers diminishing in the most concentrated treatments. This can be a consequence
50 of predation from other microzooplankers, either protozoans (intraguild predation) or
51 metazoans (e.g., copepod nauplii; not included in our sampling), during the
52 incubations.
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It is relatively easy to mathematically simulate a dilution grazing experiment involving a grazer that reduces their abundance inversely related to the dilution level during the incubation. We can actually base our example on data from one of our experiments. For instance, we can use as an example the response of $< 20 \mu\text{m}$ ciliates at Station 4, and the positive slope for nanoflagellates, one of their likely prey (Fig. 6). We assume nanoflagellates doubled their abundance in 24h, and that $< 20 \mu\text{m}$ ciliates were the only group grazing on them. If ciliate feeding rates were linearly related to food concentration we will most likely obtain, after the incubation period, a negative slope for nanoflagellates when plotting net growth rates as function of dilution level, as predicted by dilutions (although it would not be a true estimate of the natural grazing rate on this group because grazers varied their concentrations in the experimental bottles). However, if feeding was saturated, we could easily mimic the results found in the experiments using a constant feeding rate of only 76 nanoflagellates consumed per ciliate per day (Fig. 7). This happens because the grazing pressure is in this case only proportional to the abundance of grazers, and we have a higher net growth rate of grazers ($< 10 \mu\text{m}$ ciliates) in the more concentrated treatments. Moreover, varying the concentration of grazers, prey and the growth and grazing rates we can also obtain non significant from zero grazing estimates, which are not true rates, but artifacts of the method in very special situations. This mathematical exercise was not intended to correct our grazing rates, as suggested by Modigh and Franzè (Modigh and Franzè, 2009), because given the complexity of the food web we cannot anticipate the microzooplankton group responsible for most of the phytoplankton grazing impact. However, we can use our reasoning to prove that positive slopes (and non-significant slopes) are easily the result of a combination of trophic cascades during the incubations (the main grazers decreasing their abundance in the most concentrated treatments) with a saturated feeding responses. The picture complicates further if microzooplankton feeding behaviour changes with food concentration, as described by Teixeira and Figueras (Teixeira and Figueras, 2009), and if there is nutrient limitation during the incubations. Actually, and regarding this latter artifact, severe nutrient limitation during the dilution experiments inversely proportional to the dilution level will most likely favour fake negative slopes, exaggerating the grazing activity of microzooplankton because phytoplankton instantaneous growth rates will be higher in the most diluted treatments, where more nutrients per cell are available.

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In summary, the data presented here depict a planktonic Arctic community dominated by *P. pouchetii* and rich in microzooplankton, which at first sight did not seem to be exerting a strong control on a phytoplankton community in decline. However, several natural and causes as well as artifacts may have been playing important roles in some of our experiments, precluding clear grazing estimates in this very complex food web. Even though, it may seem disappointing to conclude that our rates might not be actual estimates, but bound for the lower grazing impact of the microzooplankton in the area, they point out the need for presenting negative results, when these are not consequence of evident mistakes or artifacts (Dolan and McKeon, 2005). Only with a whole picture of the existing data we will be able to extract solid conclusions on the dynamics of marine systems. Maybe in the future someone will find the way of extracting information from such results. Specifically regarding dilution grazing experiments, this study calls for special caution when applying the technique, originally developed for oligotrophic areas, to rich environments where saturated feeding responses may be common. In any case, as previously suggested (Dolan *et al.*, 2000; Agis *et al.*, 2007; Modigh and Franzè, 2009), it is evident we need a detailed examination of the grazer and prey dynamics during the incubations if we want to present trustable microzooplankton grazing estimates. By presenting data on counts-based rates we will enhance our resolution and avoid artifacts associated with chlorophyll analysis. However, these sorts of data involve a considerable amount of time and are highly dependent on the taxonomic skills of the researcher.

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43 44 **Acknowledgements**

45
46 This research was supported by the Spanish grants POL2006-0550/CTM to C. Duarte,
47 CTM2009-08783 to A.C., CTM2006-12344-C02-01 to M. A, CTM2007-60052 to E.S., and
48 grant 2005SGR-00219 from the Generalitat de Catalunya to the research group, and a PhD
49 fellowship, BES-2005-7491 to RA. The authors wished to thank the crew of the BIO
50 Hesperides and the special help of the technical staff of the UTM (D. Afonso, R. Ametller, J.
51 Llinàs J. Pozo, J. Sanz, and X. Vidal) for their invaluable logistic help. Plankton
52 composition was analyzed by Dr. M. Delgado. Five anonymous reviewers highly contributed
53 to improve earlier versions of the ms.
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TABLE 1. Summary of the dates of sampling (July 2007), geographic position and area, depth of sampling (m) and *in situ* chlorophyll *a* concentration ($\mu\text{g l}^{-1} \pm \text{SE}$) and temperature for the different stations sampled.

| Station | Date | Latitude | Longitude | Area | Sampling depth | Total Chl <i>a</i> | > 5 μm Chl <i>a</i> | Temp. ($^{\circ}\text{C}$) |
|---------|------|-------------|-------------|----------------|----------------|--------------------|--------------------------------|------------------------------|
| 2 | 2 | 70° 43.19 N | 17° 07.70 W | Greenland Sea | 20 | 0.48 \pm 0.02 | 0.15 \pm 0.00 | -1.2 |
| 3 | 3 | 72° 57.21 N | 12° 39.19 W | Greenland Sea | 25 | 1.31 \pm 0.21 | 0.79 \pm 0.00 | 0 |
| 4 | 4 | 74° 53.89 N | 7° 24.50 W | Greenland Sea | 32 | 1.41 \pm 0.02 | 0.49 \pm 0.01 | 0.8 |
| 5 | 5 | 77° 23.29 N | 1° 40.57 W | Arctic Ocean | 15 | 6.67 \pm 0.42 | 2.90 \pm 0.13 | 4 |
| 6 | 6 | 78° 00.44 N | 2° 29.94 E | Arctic Ocean | 23 | 1.75 \pm 0.03 | 1.09 \pm 0.03 | 4 |
| 9 | 7 | 78° 43.72 N | 2° 58.51 E | Arctic Ocean | 15 | 2.97 \pm 0.07 | 1.48 \pm 0.03 | 5 |
| 12 | 8 | 79° 30.83 N | 7° 29.74 E | Arctic Ocean | 20 | 5.21 \pm 0.33 | 2.57 \pm 0.07 | 5 |
| 15 | 9 | 80° 08.39 N | 11° 19.54 E | Svalbard Coast | 20 | 3.30 \pm 0.13 | 1.81 \pm 0.36 | 5 |
| 18 | 10 | 80° 26.90 N | 15° 35.38 E | Svalbard Coast | 35 | 2.67 \pm 0.10 | 0.81 \pm 0.08 | 5 |
| 20 | 12 | 80° 13.98 N | 10° 10.97 E | Arctic Ocean | 24 | 4.81 \pm 0.20 | 2.31 \pm 0.07 | 5 |
| 23 | 13 | 79° 22.16 N | 6° 49.39 E | Arctic Ocean | 17 | 8.97 \pm 0.63 | 5.01 \pm 0.08 | 5 |
| 27 | 15 | 79° 52.71 N | 8° 36.44 E | Arctic Ocean | 30 | 1.81 \pm 0.09 | 1.38 \pm 0.07 | 7.5 |
| 33 | 17 | 80° 23.46 N | 12° 25.98 E | Arctic Ocean | 25 | 1.97 \pm 0.01 | 0.66 \pm 0.00 | 5 |
| 39 | 19 | 80° 49.96 N | 13° 12.82 E | Arctic Ocean | 39 | 0.77 \pm 0.02 | 0.28 \pm 0.03 | 5 |
| 43 | 22 | 80° 25.29 N | 7° 57.57 E | Arctic Ocean | 20 | 8.97 \pm 0.21 | 2.28 \pm 0.12 | -1 |
| 46 | 23 | 79° 59.15 N | 3° 39.63 E | Arctic Ocean | 16 | 4.94 \pm 0.07 | 0.73 \pm 0.06 | -1.5 |

Table 2. In situ biomass in $\mu\text{gC L}^{-1}$ of the different protist groups considered at the stations sampled. Numbers between parentheses are SE of 2 replicates. The quotient “total carbon / $> 5 \mu\text{m Chl } a$ ” are also shown.

| Station | Diatoms | Nanofl | P. pouchetii | < 20 μm dinofl. | > 20 μm dinofl. | Mixo. dinofl. | Het. dinofl. | < 20 μm ciliat | > 20 μm ciliat | Total biomass | C / > 5 μm Chl a |
|---------|----------------|----------------|----------------|----------------------------|----------------------------|----------------|----------------|---------------------------|---------------------------|---------------|-----------------------------|
| 2 | 1.25 (0.07) | 0.36 (0.06) | 0.00 (0.00) | 0.51 (0.08) | 0.24 (0.03) | 0.24 (0.02) | 0.28 (0.07) | 4.48 (0.46) | 34.91 (4.88) | 42.26 | 14.03 |
| 3 | 1.55 (0.21) | 3.12 (0.05) | 0.00 (0.00) | 0.84 (0.02) | 0.22 (0.03) | 0.12 (0.01) | 0.08 (0.00) | 4.74 (0.33) | 50.53 (4.00) | 61.18 | 4.75 |
| 4 | 1.26 (0.15) | 0.60 (0.01) | 0.83 (0.12) | 0.77 (0.12) | 0.57 (0.01) | 0.72 (0.08) | 0.43 (0.08) | 12.2 (0.00) | 20.90 (2.31) | 38.31 | 7.73 |
| 5 | 1.77 (0.47) | 0.51 (0.12) | 2.81 (0.32) | 14.1 (3.40) | 0.26 (0.06) | 1.76 (0.28) | 0.47 (0.13) | 0.66 (0.19) | 11.49 (3.03) | 33.83 | 4.75 |
| 6 | 1.53 (0.05) | 3.78 (0.81) | 12.2 (2.30) | 10.3 (4.38) | 0.87 (0.23) | 1.20 (0.27) | 0.69 (0.13) | 3.70 (0.11) | 6.08 (1.66) | 40.42 | 20.64 |
| 9 | 1.79 (0.11) | 1.02 (0.09) | 1.59 (0.10) | 1.93 (0.32) | 0.34 (0.03) | 0.72 (0.06) | 0.15 (0.02) | 0.40 (0.11) | 0.83 (0.06) | 8.77 | 3.88 |
| 12 | 0.81 (0.03) | 1.81 (0.05) | 5.90 (0.82) | 1.49 (0.04) | 0.75 (0.11) | 0.11 (0.04) | 0.17 (0.01) | 1.91 (0.15) | 0.94 (0.27) | 13.88 | 3.44 |
| 15 | 2.97 (0.48) | 3.82 (0.12) | 13.0 (1.27) | 5.56 (0.14) | 3.09 (0.74) | 0.99 (0.05) | 1.53 (0.24) | 2.45 (0.33) | 15.09 (6.67) | 48.50 | 12.80 |
| 18 | 0.18 (0.02) | 4.01 (1.87) | 1.19 (0.71) | 6.14 (0.72) | 5.24 (0.28) | 0.27 (0.05) | 3.33 (0.67) | 2.12 (0.15) | 59.9 (7.61) | 82.38 | 11.46 |
| 20 | 4.24 (0.36) | 3.43 (0.22) | 14.8 (2.40) | 3.99 (0.07) | 3.89 (0.73) | 0.98 (0.08) | 0.57 (0.17) | 6.59 (0.42) | 4.52 (0.50) | 43.02 | 10.39 |
| 23 | 67.8 (5.19) | 3.10 (0.39) | 24.8 (2.63) | 5.00 (0.28) | 1.79 (0.53) | 3.65 (1.20) | 0.65 (0.11) | 5.73 (0.21) | 7.12 (0.81) | 119.58 | 20.18 |
| 27 | 25.1 (1.21) | 1.36 (0.10) | 0.09 (0.01) | 5.49 (0.34) | 1.91 (0.10) | 2.55 (0.23) | 0.51 (0.04) | 0.81 (0.24) | 1.74 (0.38) | 39.59 | 23.22 |
| 33 | 6.47 (0.38) | 2.33 (0.28) | 7.89 (4.22) | 3.36 (0.47) | 2.70 (0.53) | 1.05 (0.17) | 0.70 (0.05) | 4.56 (0.13) | 5.60 (0.63) | 34.67 | 29.68 |
| 39 | 6.73 | 1.61 | 1.79 | 2.10 | 2.50 | 0.25 | 0.34 | 1.45 | 1.02 | 17.78 | 42.16 |

| | | | | | | | | | | | |
|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|-------|
| | (1.05) | (0.15) | (0.04) | (0.08) | (0.39) | (0.03) | (0.04) | (0.25) | (0.20) | | |
| 43 | 6.09 | 1.15 | 49.0 | 1.96 | 9.73 | 1.37 | 1.08 | 2.77 | 4.77 | 77.93 | 27.63 |
| | (0.78) | (0.20) | (7.40) | (0.20) | (2.02) | (0.06) | (0.16) | (0.27) | (0.73) | | |
| 46 | 3.67 | 2.52 | 25.7 | 1.65 | 2.33 | 1.39 | 1.02 | 5.94 | 3.43 | 47.61 | 46.53 |
| | (0.18) | (0.06) | (9.41) | (0.39) | (0.34) | (0.21) | (0.02) | (0.57) | (0.12) | | |

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Table 3. Dilution grazing experiments. Phytoplankton instantaneous growth rates without nutrients ($\mu \pm \text{SE}$; d^{-1}) and mortality rates ($m \pm \text{SE}$; d^{-1}) for total and the 2 fractions of chlorophyll *a* at the different stations. %SS correspond to the calculated impact on the phytoplankton standing stock. Determination coefficient for the regression analysis is also provided. SE error for μ was obtained using the equation $(\text{SE}_k^2 + \text{SE}_m^2)^{1/2}$ where SE_k is the SE of the average of the 100% un-amended bottles and SE_m is the standard error associated to the slope of the regression equation (when significant).

| Station | μ (TOTAL) | m (TOTAL) | r^2 (TOTAL) | μ (< 5 μm) | m (< 5 μm) | r^2 (< 5 μm) | μ (> 5 μm) | m (> 5 μm) | %SS |
|---------|-------------------|-------------------|---------------|----------------------------|--------------------------|----------------------------|----------------------------|--------------------------|------|
| 2 | 0.12 \pm 0.029 | ns | – | 0.10 \pm 0.035 | ns | – | 0.17 \pm 0.14 | ns | 0.0 |
| 3 | 0.07 \pm 0.11 | ns | – | -0.05 \pm 0.019 | ns | – | 0.13 \pm 0.18 | ns | 0.0 |
| 4 | -0.23 \pm 0.11 | -0.31 \pm 0.11 | 0.50 | -0.39 \pm 0.25 | -0.44 \pm 0.19 | 0.45 | -0.22 \pm 0.13 | ns | 23.9 |
| 5 | -0.11 \pm 0.077 | -0.13 \pm 0.039 | 0.59 | 0.00 \pm 0.21 | -0.34 \pm 0.056 | 0.84 | -0.15 \pm 0.069 | ns | 11.6 |
| 6 | -0.05 \pm 0.010 | ns | – | 0.29 \pm 0.11 | -0.30 \pm 0.086* | 0.67 | -0.03 \pm 0.050 | ns | 0.0 |
| 9 | -0.11 \pm 0.046 | ns | – | -0.33 \pm 0.026 | ns | – | 0.07 \pm 0.090 | ns | 0.0 |
| 12 | 0.15 \pm 0.113 | -0.31 \pm 0.11* | 0.73 | -0.25 \pm 0.026 | ns | – | -0.08 \pm 0.022 | ns | 28.6 |
| 15 | 0.09 \pm 0.030 | ns | – | -0.16 \pm 0.24 | ns | – | 0.23 \pm 0.079 | ns | 0.0 |
| 18 | -0.06 \pm 0.059 | ns | – | -0.11 \pm 0.00 | ns | – | 0.19 \pm 0.036 | ns | 0.0 |
| 20 | -0.24 \pm 0.015 | ns | – | -0.29 \pm 0.031 | ns | – | -0.19 \pm 0.060 | ns | 0.0 |
| 23 | -0.24 \pm 0.00 | ns | – | -0.48 \pm 0.15 | ns | – | -0.09 \pm 0.07 | ns | 0.0 |
| 27 | -0.04 \pm 0.043 | ns | – | -0.04 \pm 0.16 | ns | – | -0.04 \pm 0.007 | ns | 0.0 |
| 33 | 0.14 \pm 0.081 | -0.24 \pm 0.073 | 0.57 | 0.00 \pm 0.11 | -0.30 \pm 0.11 | 0.48 | -0.06 \pm 0.041 | ns | 22.9 |
| 39 | -0.04 \pm 0.022 | ns | – | -0.05 \pm 0.002 | ns | – | -0.03 \pm 0.061 | ns | 0.0 |
| 43 | 0.01 \pm 0.152 | -0.35 \pm 0.15 | 0.41 | -0.05 \pm 0.19 | -0.46 \pm 0.19** | 0.45 | 0.17 \pm 0.069 | ns | 29.7 |
| 46 | 0.01 \pm 0.030 | -0.16 \pm 0.027 | 0.83 | -0.02 \pm 0.43 | -0.25 \pm 0.058 | 0.70 | 0.34 \pm 0.70 | ns | 14.9 |

1 ns: not significant ($p > 0.05$); * Grazing saturation: 3-point method used (Gallegos 1989); ** One outlier removed

Table 4. Dilution grazing experiments. Instantaneous growth rates without nutrients (μ ; d^{-1}) and mortality rates (m ; d^{-1}) for the different planktonic groups considered. Determination coefficient for the regression analysis is also provided. Positive m values indicate positive slope.

| Plank group | | St 2 | St 3 | St 4 | St 5 | St 6 | St 9 | St 12 | St 15 | St 18 | St 20 | St 23 | St 27 | St 33 | St 39 | St 43 | St 46 |
|----------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Diatoms | μ | -0.10 | 0.83 | -0.21 | -0.41 | 0.02 | 0.82 | 1.49 | 0.24 | -0.38 | 0.21 | -0.03 | -0.36 | -0.20 | -0.08 | 0.05 | 0.17 |
| | m | ns | ns | ns | ns | ns | 1.16 | ns | 1.08 | ns | ns | ns | ns | 0.68 | ns | ns | 0.72 |
| | r^2 | | | | | | 0.91 | | 0.86 | | | | | 0.92 | 0.66 | | 0.93 |
| Nanoflagellates | μ | -0.08 | 0.69 | -0.22 | 0.58 | 0.08 | 0.99 | 0.87 | 0.62 | -0.06 | -0.42 | -0.06 | -0.62 | 0.62 | 0.12 | -0.18 | -0.61 |
| | m | ns | ns | 1.19 | ns | -0.33 | ns | ns | 1.59 | ns | ns | ns | ns | 1.38 | 0.95 | 0.66 | 1.05 |
| | r^2 | | | 0.91 | | 0.96 | | | 0.96 | | | | | 0.94 | 0.81 | 0.77 | 0.79 |
| <i>P. pouchetii</i> | μ | nd | nd | -0.47 | 0.95 | -3.97 | 1.20 | 0.87 | -0.02 | 0.44 | -0.37 | 0.04 | 0.11 | -0.62 | -0.21 | -0.25 | 0.19 |
| | m | | | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | 1.19 |
| | r^2 | | | | | | | | | | | | | | | | 0.93 |
| < 20 μm dinofl. | μ | -0.29 | -0.17 | -0.48 | -1.39 | -0.77 | 0.83 | 0.32 | -0.49 | -0.19 | -0.52 | 0.01 | -0.78 | -0.23 | -0.55 | -0.35 | -0.29 |
| | m | ns | ns | 1.14 | | 0.94 | 0.48 | 1.28 | ns | ns | ns | ns | ns | ns | ns | 0.93 | ns |
| | r^2 | | | 0.72 | | 0.86 | 0.79 | 0.96 | | | | | | | | | 0.77 |
| > 20 μm dinofl. | μ | 0.96 | 1.53 | -0.79 | -0.37 | -0.30 | 0.31 | 1.19 | -0.48 | 0.02 | 0.26 | 0.52 | 0.32 | -0.23 | -0.02 | -0.38 | -0.50 |
| | m | -0.53 | -0.71 | ns | ns | ns | ns | ns | ns | ns | ns | 2.08 | ns | ns | ns | ns | ns |
| | r^2 | 0.78 | 0.88 | | | | | | | | | 0.88 | | | | | |
| Mixo. dinofl. | μ | 0.26 | 0.10 | 0.32 | -0.14 | -0.61 | -0.04 | 0.63 | -0.17 | 0.06 | 1.30 | 0.12 | -0.75 | 0.21 | 0.24 | -0.03 | 0.03 |
| | m | ns | ns | -0.65 | ns | ns | ns | ns | ns | ns | -0.97 | ns | ns | ns | ns | 0.47 | ns |
| | r^2 | | | 0.72 | | | | | | | 0.90 | | | | | 0.87 | |
| Het. dinofl. | μ | 1.17 | 0.28 | 0.29 | -1.04 | -0.22 | 1.88 | 1.02 | -0.34 | -0.01 | 0.19 | 0.47 | 0.05 | 0.12 | 0.23 | 0.54 | -0.06 |
| | m | -0.58 | ns | -1.39 | ns | ns | ns | ns | ns | ns | ns | 1.00 | ns | -0.60 | ns | ns | ns |
| | r^2 | 0.79 | | 0.90 | | | | | | | | 0.76 | | 0.80 | | | |

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|-----------------------------|-------|-------|-------|-------|-------|-------|-------|------|-------|-------|-------|-------|-------|-------|------|-------|-------|
| < 20 μm ciliates | μ | -0.12 | 0.25 | 0.70 | 1.21 | -0.61 | 1.03 | 0.95 | -0.54 | 0.15 | -0.30 | 0.50 | 0.10 | -0.16 | 0.13 | 0.12 | -0.16 |
| | m | ns | ns | -0.79 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | r^2 | | | 0.69 | | | | | | | | | | | | | |
| > 20 μm ciliates | μ | -0.08 | -0.17 | 0.03 | -0.71 | 0.26 | -1.05 | 0.17 | -1.71 | -0.45 | -0.44 | -0.73 | -1.26 | -0.14 | 0.06 | -0.20 | -1.07 |
| | m | ns | ns | -0.70 | ns | ns | ns | ns | 0.55 | ns | ns | ns | ns | ns | ns | ns | ns |
| | r^2 | | | 0.74 | | | | | 0.84 | | | | | | | | |

ns: not significant regression equation ($p > 0.05$); nd: not determined because not enough cells;

Figure legends

Figure 1. Map of the surveyed area indicating the sampling stations.

Figure 2. *Phaeocystis pouchetii* biomass as related to latitude.

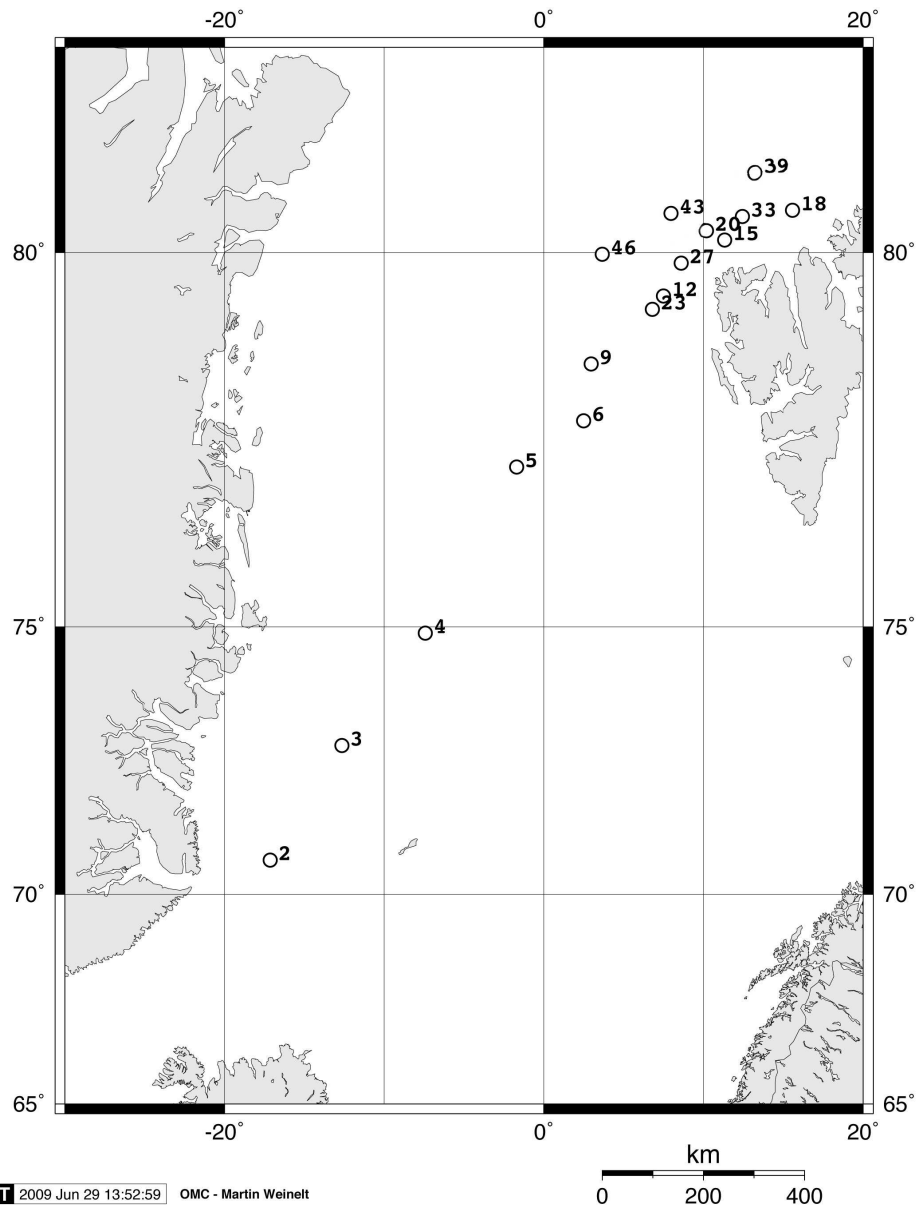
Figure 3. The quotient heterotrophic carbon / autotrophic carbon as a function of latitude. Greenland Sea and Coastal stations are indicated, the rest of stations correspond to Arctic Ocean open waters.

Figure 4. Relationship between instantaneous phytoplankton growth rates in the un-amended (without added nutrients) bottles (d^{-1}) and chlorophyll *a* concentration (μg Chl *a* l^{-1}).

Figure 5. Comparison of instantaneous phytoplankton growth rates (from total Chl *a* analysis) in bottles amended (with added nutrients) and un-amended. The discontinuous line represents the 1:1.

Figure 6. Example of grazing dilution plots for the microbial components of the planktonic community of stations 4 (left) and 33 (right).

Figure 7. Simulated outcome of a dilution grazing experiment using the abundance and growth rates of $< 20 \mu m$ ciliates in St. 4 as grazers and the abundance of nanoflagellates as prey. See text for further details.



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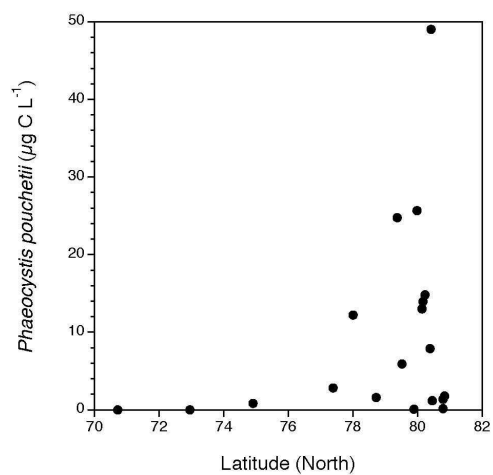


Fig. 2

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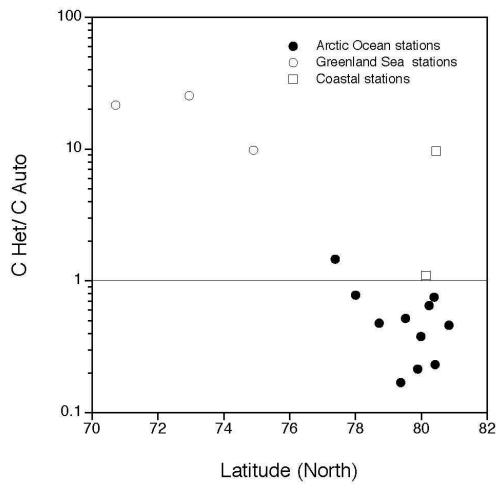


Fig. 3

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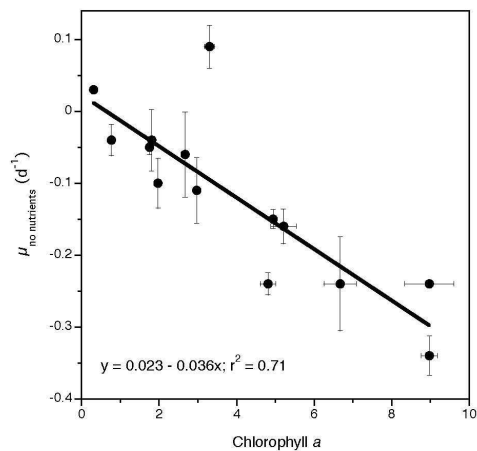


Fig. 4

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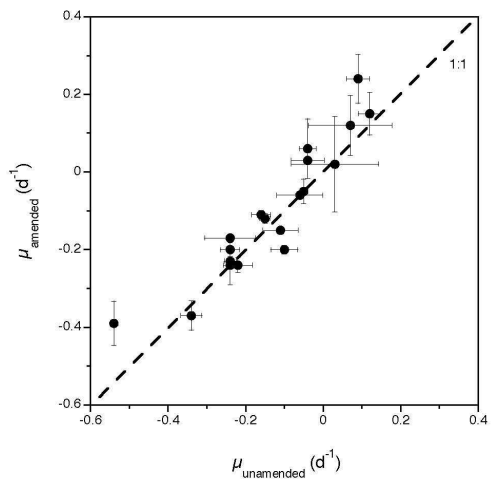


Fig. 5

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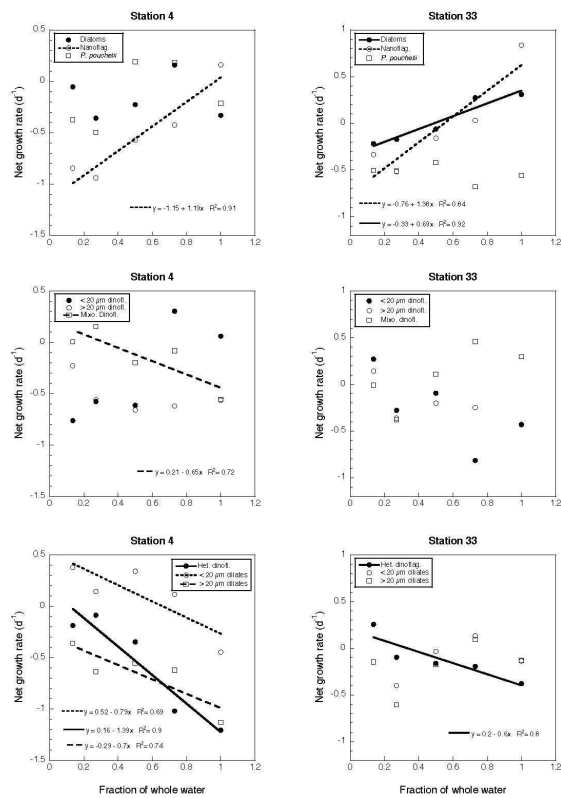


Fig. 6

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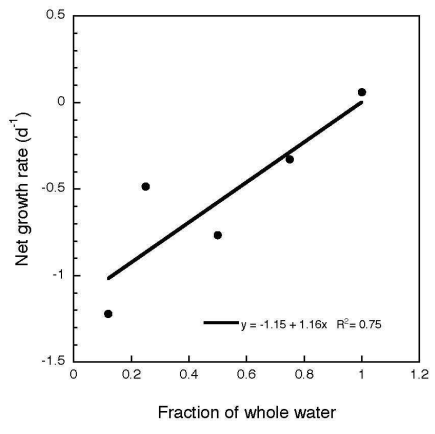
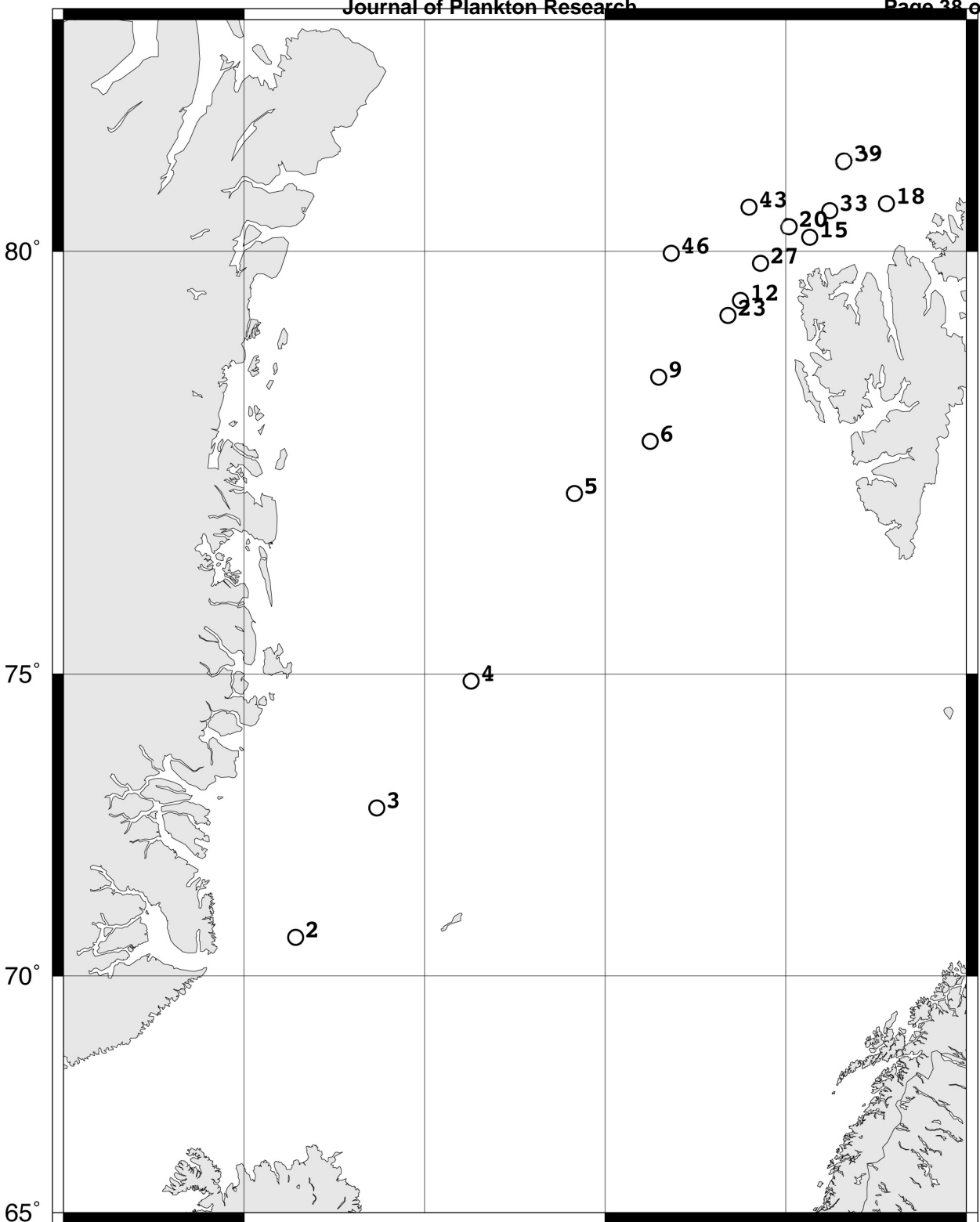


Fig. 7

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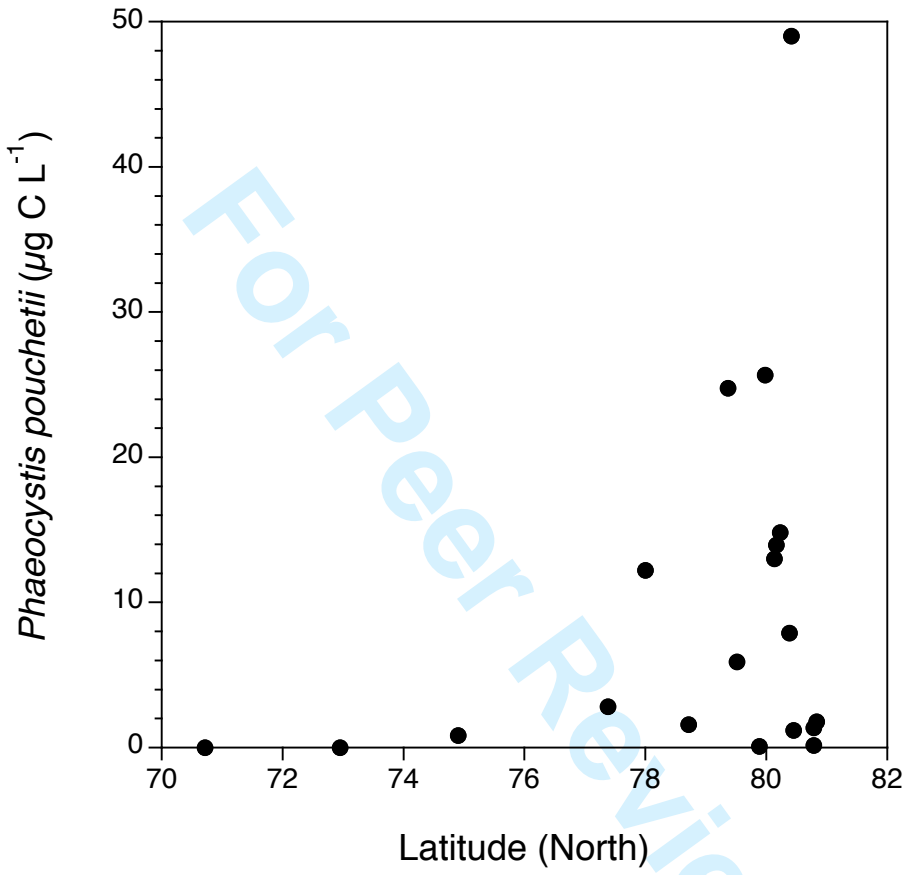


Fig. 2

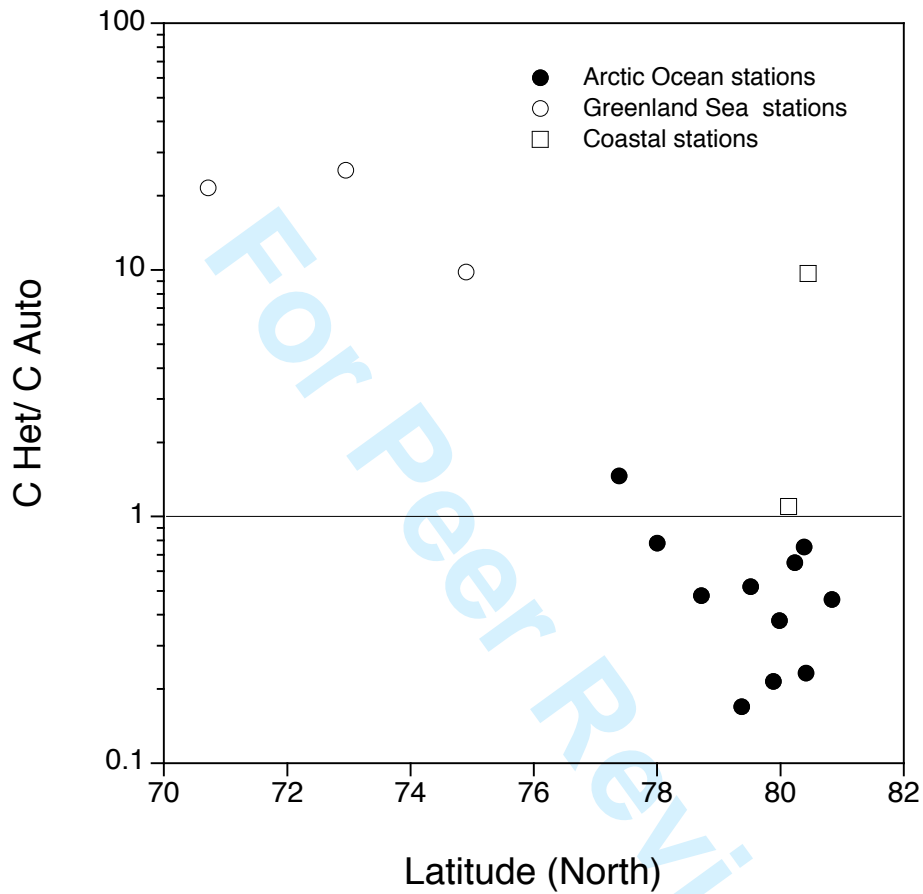


Fig. 3

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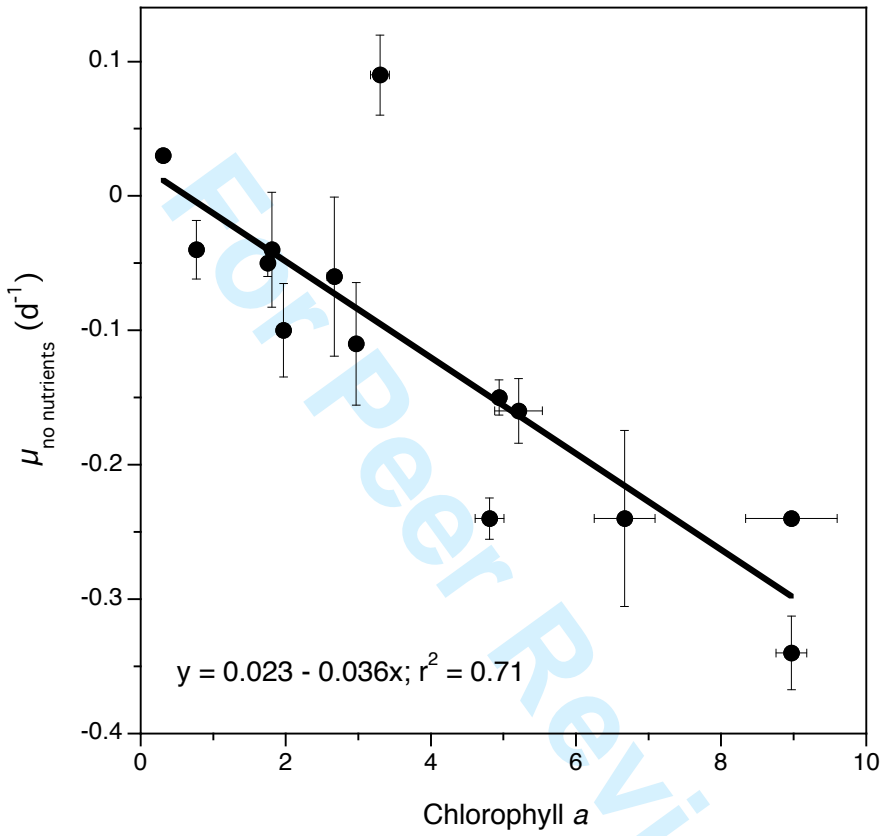


Fig. 4

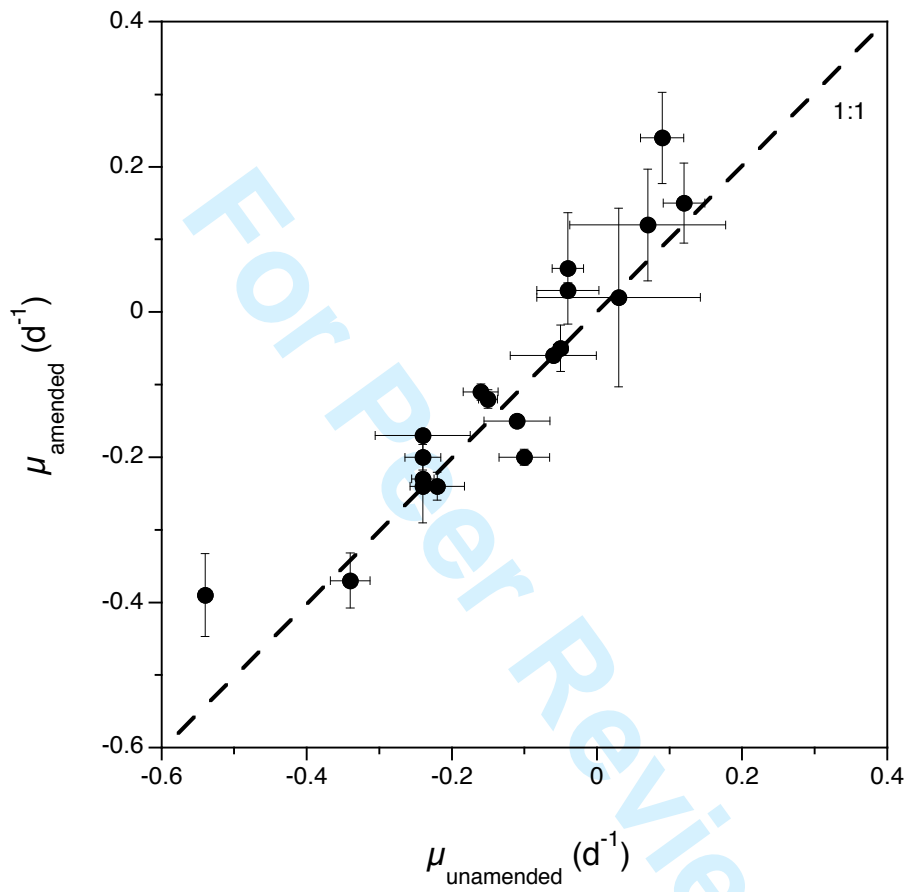


Fig. 5

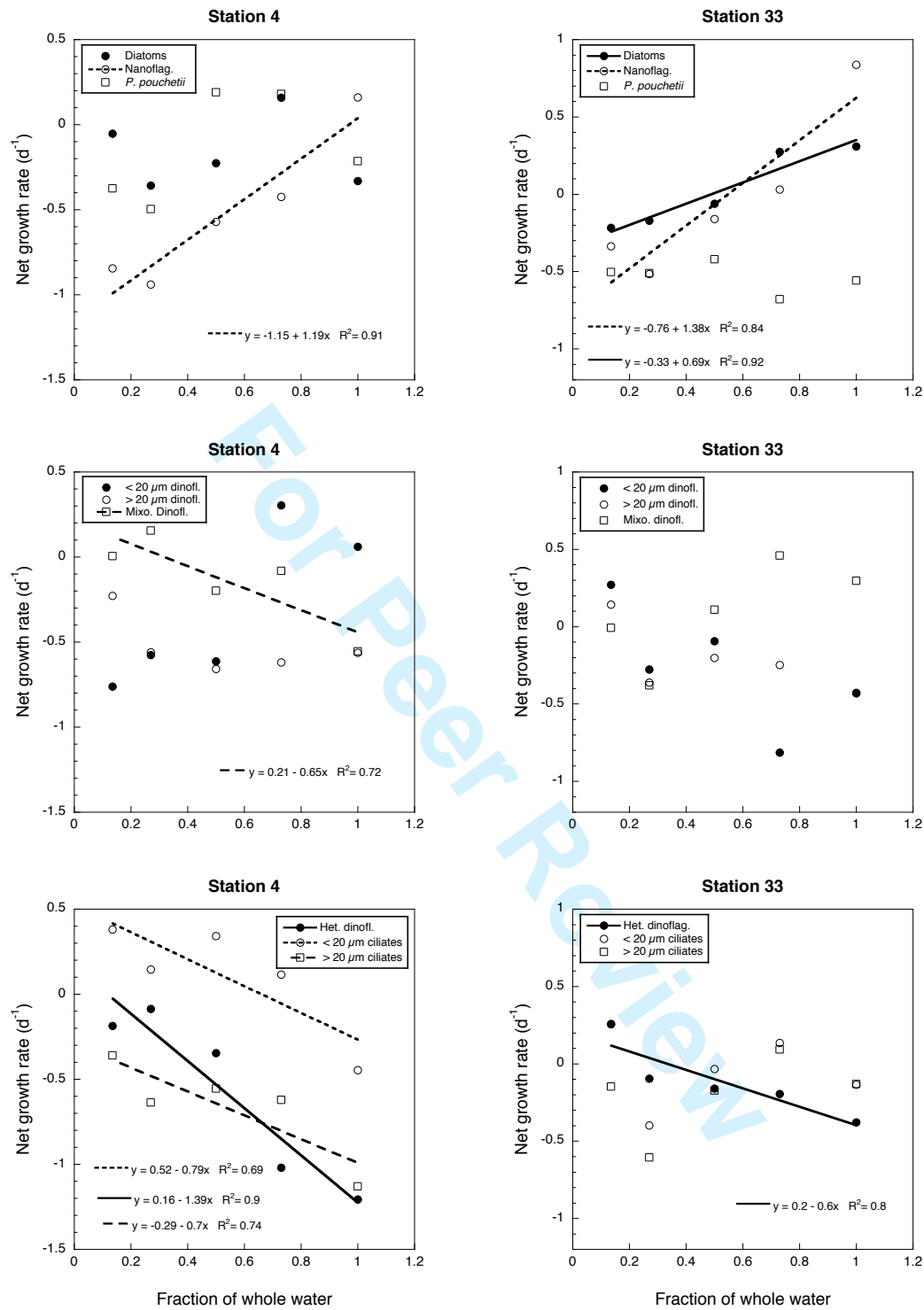


Fig. 6

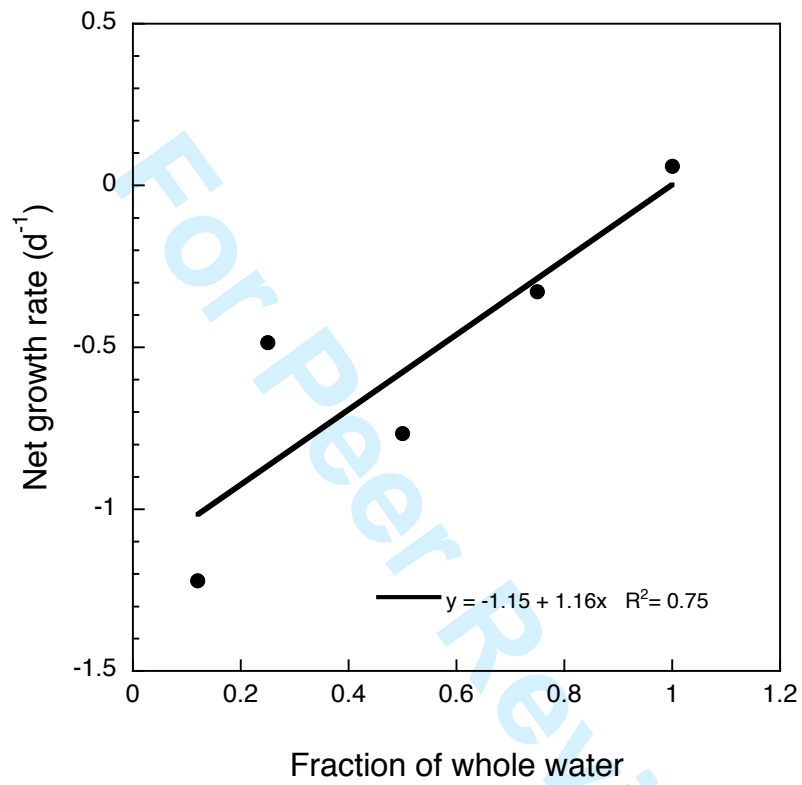


Fig. 7

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60**Web Annex 1.** Species composition and abundance of the plankton at the different stations sa

| Station | 2 | 2 | 3 | 3 | 4 |
|-----------------------------------|-----------------------|----|-----------------------|----|-----------------------|
| | Cells L ⁻¹ | SE | Cells L ⁻¹ | SE | Cells L ⁻¹ |
| Diatoms | | | | | |
| <i>Chaetoceros spp.</i> | 1224 | | 526 | | 80959 |
| <i>Corethron hystrix</i> | 0 | | 0 | | 11497 |
| <i>Eucampia zodiacus</i> | 0 | | 0 | | 0 |
| <i>Fragilariopsis spp.</i> | 280 | | 20 | | 20 |
| <i>Nitzschia spp.</i> | 0 | | 0 | | 60 |
| <i>Rhizosolenia spp.</i> | 10 | | 10 | | 60 |
| <i>Skeletonema spp.</i> | 0 | | 0 | | 130 |
| <i>Thalassiosira spp.</i> | 955 | | 445 | | 20 |
| Other centric diatoms | 220 | | 20 | | 10 |
| Pennated diatoms | 0 | | 0 | | 0 |
| Flagellates | | | | | |
| <i>Phaeocystis pouchetii</i> | 0 | | 0 | | 0 |
| <i>Choanoflagellidea</i> | 0 | | 0 | | 212940 |
| <i>Dictyocha sp.</i> | 35 | | 5 | | 371907 |
| Other nanoflagellates | 29250 | | 4500 | | 8649 |
| Dinoflagellates | | | | | |
| <i>Amphidinium spp.</i> | 65 | | 35 | | 35 |
| <i>Ceratium spp.</i> | 10 | | 0 | | 5 |
| <i>Dinophysis spp.</i> | 0 | | 0 | | 0 |
| <i>Gyrodinium spp.</i> | 85 | | 15 | | 45 |
| <i>Katodinium glaucum</i> | 70 | | 30 | | 15 |
| <i>Micracanthodinium claytoni</i> | 20 | | 20 | | 95 |
| <i>Prorocentrum balticum</i> | 70 | | 10 | | 0 |
| <i>Protoperidinium bipes</i> | 85 | | 15 | | 20 |
| <i>Protoperidinium spp.</i> | 25 | | 5 | | 10 |
| <i>Torodinium robustum</i> | 50 | | 10 | | 10 |
| Dinoflagellates < 20 µm | 1644 | | 274 | | 2719.5 |
| Dinoflagellates > 20 µm | 479.5 | | 68.5 | | 55.5 |
| Ciliates | | | | | |
| <i>Didinium sp.</i> | 80 | | 0 | | 15 |
| <i>Laboea sp.</i> | 545 | | 65 | | 2220 |
| <i>Mesodinium spp.</i> | 55 | | 35 | | 5 |
| <i>Strombidium spp.</i> | 505 | | 65 | | 680 |
| Tintinnids | 0 | | 0 | | 5 |
| < 20 µm ciliates | 3699 | | 548 | | 1494.5 |
| > 20 µm ciliates | 420 | | 70 | | 240 |

mpled. SE corresponde to the Standard Error of 2 replicate samples. Sizes correspo

| SE | 4 Cells L ⁻¹ | 5 SE | 5 Cells L ⁻¹ | 6 SE | 6 Cells L ⁻¹ | 9 |
|----|----------------------------|---------|----------------------------|---------|----------------------------|--------|
| | 180 | 1630 | 530 | 2875 | 465 | 50 |
| | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0 | 35 | 15 | 0 | 0 | 3600 |
| | 130 | 10545 | 1110 | 1330 | 10 | 1820 |
| | 225 | 41850 | 15750 | 15300 | 900 | 28575 |
| | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 | 0 | 0 |
| | 90 | 10405 | 1325 | 6930 | 550 | 10195 |
| | 0 | 30 | 10 | 125 | 5 | 5 |
| | 5 | 12375 | 3825 | 2025 | 1125 | 9450 |
| | | | | | | |
| | 30758 | 1138785 | 129735 | 4947228 | 934092 | 357492 |
| | 8649 | 0 | 0 | 0 | 0 | 0 |
| | 20 | 75 | 25 | 35 | 5 | 35 |
| | 2366 | 262353 | 60543 | 227757 | 49011 | 170097 |
| | | | | | | |
| | 10 | 15 | 5 | 240 | 80 | 10 |
| | 10 | 0 | 0 | 5 | 5 | 0 |
| | 5 | 10 | 10 | 5 | 5 | 5 |
| | 130 | 155 | 55 | 705 | 95 | 50 |
| | 5 | 20 | 10 | 370 | 130 | 0 |
| | 10 | 30 | 20 | 20 | 0 | 15 |
| | 35 | 7425 | 1125 | 4050 | 900 | 2925 |
| | 5 | 888 | 222 | 20 | 10 | 230 |
| | 5 | 25 | 5 | 40 | 10 | 5 |
| | 25 | 35 | 15 | 55 | 5 | 5 |
| | 388.5 | 45675 | 11025 | 33525 | 14175 | 10800 |
| | 20 | 550 | 110 | 1835 | 455 | 335 |
| | | | | | | |
| | 5 | 20 | 0 | 15 | 5 | 5 |
| | 50 | 0 | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 | 0 | 0 |
| | 304.5 | 65 | 15 | 95 | 5 | 0 |
| | 10 | 0 | 0 | 0 | 0 | 0 |
| | 301 | 686.5 | 203.5 | 6555 | 215 | 205 |
| | 100.5 | 530 | 140 | 330 | 90 | 130 |

standard Error of 2 replicate samples. Sizes correspond to equivalent spherical diam

| | 9 | 12 | 12 | 15 | 15 | 18 |
|----|-----------------------|-----------|-----------------------|-----------|-----------------------|-----------|
| SE | Cells L ⁻¹ | SE | Cells L ⁻¹ | SE | Cells L ⁻¹ | |
| | 30 | 3115 | 805 | 1845 | 325 | 5 |
| | 0 | 55 | 5 | 110 | 60 | 0 |
| | 450 | 10 | 10 | 30 | 30 | 0 |
| | 760 | 370 | 50 | 1080 | 260 | 135 |
| | 2475 | 9450 | 450 | 27000 | 5850 | 170 |
| | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 1575 | 675 | 0 |
| | 195 | 1770 | 410 | 15935 | 675 | 849.5 |
| | 5 | 10 | 10 | 242 | 232 | 5 |
| | 1350 | 900 | 450 | 3150 | 900 | 479.5 |
| | | | | | | |
| | 23064 | 1326180 | 184512 | 3992955 | 389205 | 268119 |
| | 0 | 0 | 0 | 49011 | 14415 | 0 |
| | 25 | 10 | 0 | 5 | 5 | 35 |
| | 14415 | 194553 | 4641 | 224874 | 23064 | 605430 |
| | | | | | | |
| | 10 | 60 | 20 | 120 | 20 | 390 |
| | 0 | 0 | 0 | 0 | 0 | 0 |
| | 5 | 0 | 0 | 15 | 5 | 0 |
| | 10 | 145 | 15 | 790 | 170 | 935 |
| | 0 | 120 | 0 | 120 | 20 | 685 |
| | 5 | 20 | 10 | 80 | 20 | 10 |
| | 225 | 110 | 50 | 3825 | 225 | 479.5 |
| | 30 | 40 | 10 | 105 | 25 | 890.5 |
| | 5 | 5 | 5 | 70 | 40 | 10 |
| | 5 | 15 | 5 | 10 | 10 | 5 |
| | 1800 | 8325 | 225 | 18000 | 450 | 98022 |
| | 45 | 435 | 75 | 1695 | 405 | 2429 |
| | | | | | | |
| | 5 | 5 | 5 | 10 | 0 | 10 |
| | 0 | 0 | 0 | 45 | 5 | 1400 |
| | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0 | 65 | 15 | 145 | 35 | 35 |
| | 0 | 0 | 0 | 5 | 5 | 5 |
| | 55 | 1040 | 70 | 2555 | 325 | 1465 |
| | 10 | 140 | 40 | 810 | 370 | 760 |

| | 18 | 20 | 20 | 23 | 23 | 27 |
|----|-----------------------|-----------|-----------------------|-----------|-----------------------|-----------|
| SE | Cells L ⁻¹ | SE | Cells L ⁻¹ | SE | Cells L ⁻¹ | |
| 9 | 5 | 9960 | 180 | 149858 | 4990 | 27281.5 |
| 10 | 0 | 440 | 40 | 1600 | 280 | 810 |
| 11 | 0 | 25 | 25 | 60 | 40 | 25 |
| 12 | 15 | 1280 | 540 | 5148.5 | 901.5 | 1030 |
| 13 | 90 | 57825 | 4275 | 380556 | 46128 | 533355 |
| 14 | 0 | 0 | 0 | 0 | 0 | 10 |
| 15 | 0 | 1575 | 225 | 2475 | 225 | 0 |
| 16 | 315.5 | 10530 | 1310 | 79785 | 2585 | 12135 |
| 17 | 5 | 10 | 10 | 21190 | 2240 | 8365 |
| 18 | 68.5 | 4725 | 1125 | 6300 | 2250 | 450 |
| 19 | | | | | | |
| 20 | | | | | | |
| 21 | | | | | | |
| 22 | | | | | | |
| 23 | 158565 | 2485146 | 403620 | 5420040 | 576600 | 20181 |
| 24 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 5 | 15 | 5 | 10 | 10 | 0 |
| 26 | 282534 | 804186 | 50586 | 323880 | 41262 | 149916 |
| 27 | | | | | | |
| 28 | | | | | | |
| 29 | | | | | | |
| 30 | 110 | 230 | 10 | 140 | 20 | 80 |
| 31 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33 | 167 | 195 | 65 | 445 | 35 | 210 |
| 34 | 274 | 220 | 80 | 460 | 100 | 190 |
| 35 | 0 | 10 | 10 | 10 | 10 | 30 |
| 36 | 68.5 | 2475 | 225 | 14175 | 4725 | 9900 |
| 37 | 68.5 | 100 | 40 | 70 | 30 | 140 |
| 38 | 0 | 60 | 10 | 35 | 5 | 45 |
| 39 | 5 | 120 | 40 | 120 | 20 | 60 |
| 40 | 11532 | 24750 | 450 | 20025 | 1125 | 25425 |
| 41 | 117 | 1065 | 165 | 990 | 290 | 950 |
| 42 | | | | | | |
| 43 | | | | | | |
| 44 | | | | | | |
| 45 | 0 | 0 | 0 | 10 | 0 | 20 |
| 46 | 130 | 0 | 0 | 15 | 5 | 5 |
| 47 | 0 | 0 | 0 | 0 | 0 | 0 |
| 48 | 25 | 180 | 0 | 120 | 30 | 65 |
| 49 | 5 | 0 | 0 | 0 | 0 | 0 |
| 50 | 132 | 4650 | 310 | 2636.5 | 72.5 | 400 |
| 51 | 140 | 720 | 80 | 1000 | 120 | 250 |
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| | 27 | 33 | 33 | 39 | 39 | 43 |
|----|-----------------------|-----------|-----------------------|-----------|-----------------------|-----------|
| SE | Cells L ⁻¹ | SE | Cells L ⁻¹ | SE | Cells L ⁻¹ | |
| | 7434.5 | 2610 | 190 | 2345 | 675 | 1350 |
| | 150 | 220 | 40 | 75 | 15 | 0 |
| | 5 | 5 | 5 | 15 | 5 | 0 |
| | 50 | 450 | 110 | 300 | 20 | 890 |
| | 72075 | 25650 | 2250 | 9675 | 225 | 9000 |
| | 10 | 25 | 15 | 10 | 10 | 0 |
| | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1195 | 11000 | 1750 | 4760 | 340 | 15885 |
| | 225 | 1660 | 250 | 2410 | 470 | 255 |
| | 0 | 4275 | 225 | 2475 | 225 | 0 |
| | | | | | | |
| | 2883 | 1588533 | 850485 | 360375 | 8649 | 8014740 |
| | 0 | 0 | 0 | 0 | 0 | 900 |
| | 0 | 60 | 20 | 20 | 10 | 285 |
| | 11532 | 194553 | 24189 | 154599 | 13965 | 207801 |
| | | | | | | |
| | 20 | 140 | 20 | 65 | 15 | 90 |
| | 0 | 0 | 0 | 0 | 0 | 5 |
| | 0 | 10 | 10 | 0 | 0 | 35 |
| | 50 | 215 | 25 | 180 | 30 | 150 |
| | 10 | 410 | 30 | 110 | 10 | 320 |
| | 20 | 25 | 15 | 20 | 0 | 75 |
| | 900 | 2250 | 450 | 45 | 5 | 3825 |
| | 20 | 70 | 50 | 10 | 0 | 120 |
| | 5 | 65 | 5 | 25 | 15 | 55 |
| | 0 | 160 | 20 | 15 | 5 | 45 |
| | 1575 | 16200 | 2250 | 16875 | 675 | 15075 |
| | 20 | 1745 | 345 | 1040 | 150 | 3340 |
| | | | | | | |
| | 0 | 0 | 0 | 0 | 0 | 30 |
| | 5 | 40 | 0 | 0 | 0 | 110 |
| | 0 | 0 | 0 | 0 | 0 | 120 |
| | 25 | 310 | 80 | 155 | 35 | 225 |
| | 0 | 15 | 15 | 0 | 0 | 0 |
| | 120 | 1905 | 145 | 725 | 115 | 1055 |
| | 50 | 520 | 80 | 150 | 30 | 280 |

| | 43 | 46 | 46 | |
|----|-----------------------|-----------|----------------------------|-------|
| SE | Cells L ⁻¹ | SE | verage cell size (ESD; μm) | |
| 9 | 260 | 350 | 250 | 21.2 |
| 10 | 0 | 0 | 0 | 44.2 |
| 11 | 0 | 0 | 0 | 16 |
| 12 | 430 | 240 | 240 | 8.6 |
| 13 | 2250 | 5175 | 675 | 4.2 |
| 14 | 0 | 0 | 0 | 36 |
| 15 | 0 | 0 | 0 | 12.4 |
| 16 | 1035 | 11475 | 225 | 18 |
| 17 | 65 | 725 | 205 | 24.24 |
| 18 | 0 | 450 | 0 | 9.6 |
| 19 | | | | |
| 20 | | | | |
| 21 | | | | |
| 22 | | | | |
| 23 | 1210860 | 4050615 | 1484745 | 3.8 |
| 24 | 900 | 1350 | 1350 | 4 |
| 25 | 65 | 630 | 110 | 24.4 |
| 26 | 34821 | 284559 | 5991 | 4.04 |
| 27 | | | | |
| 28 | | | | |
| 29 | | | | |
| 30 | 20 | 300 | 20 | 13.8 |
| 31 | 5 | 0 | 0 | 52.2 |
| 32 | 5 | 15 | 5 | 32.8 |
| 33 | 20 | 370 | 30 | 32.4 |
| 34 | 60 | 370 | 10 | 15 |
| 35 | 25 | 260 | 20 | 20 |
| 36 | 225 | 3825 | 675 | 19.4 |
| 37 | 20 | 50 | 20 | 18.8 |
| 38 | 5 | 35 | 5 | 36.6 |
| 39 | 15 | 40 | 10 | 27.6 |
| 40 | | | | |
| 41 | 1575 | 12375 | 2925 | |
| 42 | 680 | 1510 | 200 | |
| 43 | | | | |
| 44 | | | | |
| 45 | | | | |
| 46 | 10 | 45 | 5 | 28.8 |
| 47 | 20 | 20 | 0 | 71 |
| 48 | 20 | 20 | 20 | 21 |
| 49 | 95 | 440 | 100 | 33 |
| 50 | 0 | 5 | 5 | 57.2 |
| 51 | 55 | 2735 | 195 | |
| 52 | 40 | 400 | 20 | |
| 53 | | | | |
| 54 | | | | |
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