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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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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 80º 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)

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

Method

This study was part of a multidisciplinary project (ATOS: POL2006-00550/CTM), and took place on board the research vessel BIO Hespérides from July 1 to 24, 2007. The cruise departed Reykjavik (Iceland), and sampling started northward across the Greenland Sea (Table 1, Fig. 1). In Arctic waters, we sampled a series of stations in the vicinity of the ice-edge, alternating between several stations free of ice and two coastal stations near the Svalbard Islands. During the study, we reached a historical minimum of Arctic ice cover (Zhang *et al.* 2008), allowing samples to be taken from areas up to 80º 50' N. The stations lasted 24 h, starting with profiles for the

measurement of salinity, temperature, and fluorescence during the early morning using a Seabird CTD911, followed by collection of water samples for the determination of chlorophyll *a* concentration (hereafter Chl *a*) with 12 l Niskin bottles fitted to a rosette during the ascending CTD casts.

At each station we conducted standard grazing dilution experiments (Landry and Hassett, 1982) to assess the microzooplankton grazing impact on primary producers and on other components of the food web (see below). This technique consists of the sequential dilution of natural water with filtered seawater to obtain a gradient of net grazing impact on phytoplankton. The water for these experiments was collected at the fluorescence maximum (Table 1) using a rosette equipped with 12-L Niskin bottles, according to the fluorescence profile. Once on deck, the water was gravity-filtered through a Pall Acropak 0.8/0.2 500 capsule (previously flushed, including tubing, with diluted HCl and thoroughly rinsed with deionised water), and then 2 replicate bottles (2.3-L acid washed polycarbonate) for each of the dilution treatments were filled with the corresponding required amount of filtered seawater. Afterwards, we added natural non-filtered seawater from the selected depth to the bottles to generate experimental water percentages of 13, 27, 50, 73, and 100%. At some stations, the presence of a dense bloom of the haptophyte *Phaeocystis pouchetii* made it impossible to efficiently filter the water through the Acropak capsules; therefore, filtered seawater originated from below the bloom depth.

To guarantee the homogeneity of the natural water poured from different Niskin bottles filled at the same depth, we used a 20-L intermediary carboy in which the water was gently mixed by its own flow. All the process was carried out under dim light conditions to avoid cell light-damage. To promote constant and saturated phytoplankton growth in the dilution series, each bottle received added nutrients (10 μM NH_4Cl and 0.7 μM Na_2HPO_4). In addition, four 100% (i.e., not diluted) bottles were prepared without nutrients to assess the natural growth of the algae. Two of these latter bottles were sacrificed for initial samples. Because we did not screen the water used for the dilution series to avoid damaging delicate microzooplankton, the experimental suspension may have contained some mesozooplankton. We examined the bottles by eye to observe the presence of large copepods, and the very few times this occurred did not result in inconsistent results in the dilution series.

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

(Calbet and Saiz, 2005), because recent research has revealed that such corrections should apply to many other planktonic groups, not only ciliates (Zarauz and Irigoien, 2008), and universal factors have not been developed yet. Because plankton were preserved with acidic Lugol's solution, no distinction between strict heterotrophs and auto/mixotrophs was made for flagellates, ciliates, and some dinoflagellates. However, the groups identified to species level were classified trophically according to the literature.

Instantaneous growth rates in dilution grazing experiments were derived from net growth in the un-amended bottles (no nutrients added) adding the mortality by microzooplankton grazing from dilution experiments when the latter was significant. We should note that in the cases where the grazing mortality was not significant the instantaneous growth rate of prey would be equivalent to the net growth rate in the un-amended bottles. Therefore, we may be underestimating the actual instantaneous growth rate of the prey because some mortality may still occur in the bottles, even if not measured by the dilution method. All statistical tests were conducted with JMP 7.0 statistical software.

Results

Plankton biomass and distribution

We present a summary of the different fractions of Chl *a* in Table 1 and the contribution of micro- and nanoplankton to total plankton community biomass at the sampled stations in Table 2 (a detailed summary of the species composition is presented in the Supplementary Material online). The main feature of the data for the Arctic stations is the almost ubiquitous presence of the haptophyte *Phaeocystis pouchetii*, which formed dense blooms (Table 2, Fig. 2) and whose distribution was directly correlated with total Chl *a* ($r=0.76$, $p<0.01$), and inversely correlated with temperature ($r = 0.77$; $p < 0.05$). Following *P. pouchetii*, diatoms (mostly chain forming species > 20 µm in total length of the genera *Chaetoceros*, *Fragilariopsis*, *Nitzschia*, *Rhizosolenia*, *Thalassiothrix*, and *Thalassiosira*) were the second biomass grouping within the phytoplankton, being dominant in the Greenland Sea and at warmer stations. Regarding micrograzers, the important contribution of large ciliates

(mostly Tintinnida, *Strombidium* spp., and the mixotroph *Laboea* spp.) was noticeable in the Greenland Sea and Svalbard Islands coastal station (Table 2). Actually, ciliates were the overall major contributors to microzooplankton during the study and were substantial components of the total plankton biomass (ca. 25%), whereas heterotrophic dinoflagellates (considering 50% of unidentified dinoflagellates as heterotrophic; Lessard and Swift, 1985; Sherr and Sherr, 2007) represented, on average, 12% of the total carbon biomass (Table 2). In relation to the relevance of heterotrophs, it is interesting to note that the quotient between heterotrophic and autotrophic carbon (indicative of the trophic characteristics of the system) was > 1 in the Greenland Sea and Svalbard Islands coastal stations (Fig. 3).

Despite the high heterotrophic biomass, we did not find significant correlations between any of the size fractions of Chl *a*, large heterotrophic dinoflagellates (e.g., *Gyrodinium* spp., *Protoperidinium* spp., and *Katodinium* spp.), and ciliates. However, large mixotrophic dinoflagellates (*Dinophysis* spp., *Ceratium* spp., *Gonyaulax* spp., and *Amphidinium* spp.) were positively correlated with diatoms ($r = 0.85$; $p < 0.05$). Correlations between groups do not necessarily mean causality, but they can indicate that an association exists. Likewise, unidentified > 20-µm dinoflagellates were also positively correlated with *P. pouchetti* biomass ($r = 0.72$; $p < 0.05$). However, when excluded from the analysis station 43 (the station with the maximum biomass of *P. pouchetii*) this relationship is strongly affected and it becomes non-significant.

We calculated the C:Chl *a* ratios using the Chl *a* data and the autotrophic biomass (in carbon), obtained by cell counting and conversion to carbon using literature equations (Table 2). For unidentified dinoflagellates we assumed 50% of the organisms were phototrophs. Because our microscope counting technique, based on Lugol-preserved samples, does not allow for good resolution of the lower size-fractions, we can better estimate the C:Chl *a* ratio using only > 5-µm Chl *a*. The values averaged 18 for the entire data-set, and ranged from 4 to 47.

Dilution grazing experiments

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

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

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

Despite the overall low grazing rates, some associations between the distributions of several organisms seem to be evident. For instance, mixotrophic dinoflagellates were positively correlated with diatoms. Mixotrophy is widespread among dinoflagellates, and it is not uncommon that these organisms contribute significantly to the community grazing on phytoplankton (Stoecker, 1999; Stoecker *et al.*, 2009), and particularly on diatoms (Yoo *et al.*, 2009). Moreover, the role of mixotrophic dinoflagellates in the fate of primary producers is likely to have been 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-

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

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

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

- The natural mortality hypothesis

Parallel to our study, Lasternas and Agustí (Lasternas and Agustí, in press) used a membrane permeability probe (Agustí and Sánchez, 2002) to estimate the natural mortality of the *P. pouchetii* community, and they observed that higher percentages of dead *P. pouchetii* cells (up to ca. 90% at some stations) were associated to both cold and less saline waters across the area studied. The mechanisms behind this mortality, not related to grazing rates, can be several. It could well be that we were facing a

senescent community at the end of the bloom. On the other hand, we cannot disregard viruses as playing a role in controlling the population of these and other protists and responsible for the frequent negative growth rates (Baudoux *et al.*, 2006; Jacobsen *et al.*, 2007). Unfortunately, our experimental set up was not adequate for virus-related mortality quantification.

Given the contribution of *P. pouchetii* to the total phytoplankton biomass, the natural mortality of these algae could be driving the growth rates observed on the basis of Chl *a*. Corroborating this, we observed an inverse relationship between phytoplankton instantaneous growth rates and Chl *a* distribution. Although other groups of plankton could also have shown natural mortality rates, we do not have solid evidence of this.

- The Phaeocystis pouchetii hypothesis

As previously mentioned, our cruise coincided with high abundances of *Phaeocystis pouchetii*, which commonly blooms in these waters in July (Schoemann *et al.*, 2005). The peculiarities of the food web dominated by these algae make it difficult to extract general conclusions. We believe that our low grazing estimates (not significant at most of the stations) could be partially a consequence of the presence of *P. pouchetii* . We observed the presence of many colonies in the samples, although we have not been able to quantify the percentage of free cells and colonies in the Lugol preserved samples. However, it is quite likely this species either introduced variability into the samples (colonies can be heterogeneously distributed in the samples) and precluded the establishment of significant regressions based on Chl *a*, evaded grazing when in colonial form (Hansen *et al.*, 1994; Weisse *et al.*, 1994; Tang, 2003), or chemically deterred grazers (Barnard *et al.*, 1984; Nejstgaard *et al.*, 2007; van Rijssel *et al.*, 2007). Certainly, *P. pouchetii* seems the most obvious candidate when seeking chemical deterrence of grazing. Similar to macroalgae and other phytoplankters, *Phaeocystis* spp. exude chemicals that can interfere with grazing activity (see review by van Rijssel *et al.*, 2007). Although the chemicals involved in this process have not yet been identified, it has been suggested that grazing-activated DMSP cleavage by *P. pouchetii* contributes to grazing deterrence (Wolfe *et al.*, 2000). Allelopathic interactions could also be responsible for the negative growth rates of a phytoplankton (and other protists) community, which apparently was not limited by nutrients.

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 7.5ºC, 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

ecological interactions, can be easily interpreted as growth enhancement due to increased feeding in the more concentrated treatments. However, positive slopes of phytoplankton occur when the organism considered is adversely affected by the dilution treatment. The explanation for these particular responses is not easy because they can have different non-exclusive causes. For instance, they may either be the result of strong trophic cascade effects during the incubations (Calbet *et al.*, 2008), chemical grazing deterrence by the algae or other organisms, toxic effects of the filtered seawater (Landry, 1993), mixotrophs being important contributors of phytoplankton biomass (then, favoured in less diluted conditions), or of complex cycling of nutrients between internal and external pools (Landry, 1993), because nutrients would be taken up by smaller algae more efficiently and would become limiting for larger phytoplankton. We do not believe that the last three hypotheses apply to our experiments for the following reasons: i) any toxic effect would likely be persistently evident at all the groups and stations, ii) some of the groups of phytoplankton showing positive slopes, as far as we know, were not mixotrophic (e.g., diatoms), and iii) nutrients were supplied in excess. Therefore, either (or both) trophic cascades or grazing deterrence seem to be the most reasonable explanation. If *P. pouchetii* was responsible for the positive slopes found for diatoms and other groups, its effects would not be apparent at the stations where the haptophyte was not present (stations 2 and 3). While this was the case and thus supporting the feeding deterrence hypothesis, it did not fully demonstrate the hypothesis because the response was not directly related to the *P. pouchetii* concentration.

We, therefore, contemplated the trophic cascade explanation for the positive slopes found in our (and others) study. It has been argued that changes of grazer abundance during dilution grazing incubation may result in results that are artifacts (Dolan *et al.*, 2000; Agis *et al.*, 2007; Modigh and Franzè, 2009). These changes usually involve a decrease in abundance in the most diluted treatments, the result of starvation. However, our experiments showed on many occasions the opposite, the grazers diminishing in the most concentrated treatments. This can be a consequence of predation from other microzooplankers, either protozoans (intraguild predation) or metazoans (e.g., copepod nauplii; not included in our sampling), during the incubations.

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 µm 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 µm 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 µm 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 microzoplankton 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.

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 were 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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TABLE 1. Summary of the dates of sampling (July 2007), geographic position and area, depth of sampling (m) and *in situ* chlorophyll *a* concentration (µg l^{-1} ± SE) and temperature for the different stations sampled.

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

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

Table 3. Dilution grazing experiments. Phytoplankton instantaneous growth rates without nutrients (μ±SE; d^{-1}) and mortality rates (m±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 $(SE_k^2+SE_m^2)^{1/2}$ were 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±0.029	ns	–	0.10±0.035	ns	–	0.17±0.14	ns	0.0
3	0.07±0.11	ns	–	-0.05±0.019	ns	–	0.13±0.18	ns	0.0
4	-0.23±0.11	-0.31±0.11	0.50	-0.39±0.25	-0.44±0.19	0.45	-0.22±0.13	ns	23.9
5	-0.11±0.077	-0.13±0.039	0.59	0.00±0.21	-0.34±0.056	0.84	-0.15±0.069	ns	11.6
6	-0.05±0.010	ns	–	0.29±0.11	-0.30±0.086*	0.67	-0.03±0.050	ns	0.0
9	-0.11±0.046	ns	–	-0.33±0.026	ns	–	0.07±0.090	ns	0.0
12	0.15±0.113	-0.31±0.11*	0.73	-0.25±0.026	ns	–	-0.08±0.022	ns	28.6
15	0.09±0.030	ns	–	-0.16±0.24	ns	–	0.23±0.079	ns	0.0
18	-0.06±0.059	ns	–	-0.11±0.00	ns	–	0.19±0.036	ns	0.0
20	-0.24±0.015	ns	–	-0.29±0.031	ns	–	-0.19±0.060	ns	0.0
23	-0.24±0.00	ns	–	-0.48±0.15	ns	–	-0.09±0.07	ns	0.0
27	-0.04±0.043	ns	–	-0.04±0.16	ns	–	-0.04±0.007	ns	0.0
33	0.14±0.081	-0.24±0.073	0.57	0.00±0.11	-0.30±0.11	0.48	-0.06±0.041	ns	22.9
39	-0.04±0.022	ns	–	-0.05±0.002	ns	–	-0.03±0.061	ns	0.0
43	0.01±0.152	-0.35±0.15	0.41	-0.05±0.19	-0.46±0.19**	0.45	0.17±0.069	ns	29.7
46	0.01±0.030	-0.16±0.027	0.83	-0.02±0.43	-0.25±0.058	0.70	0.34±0.70	ns	14.9

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
P. pouchetii	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			

< 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 cililiates	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.

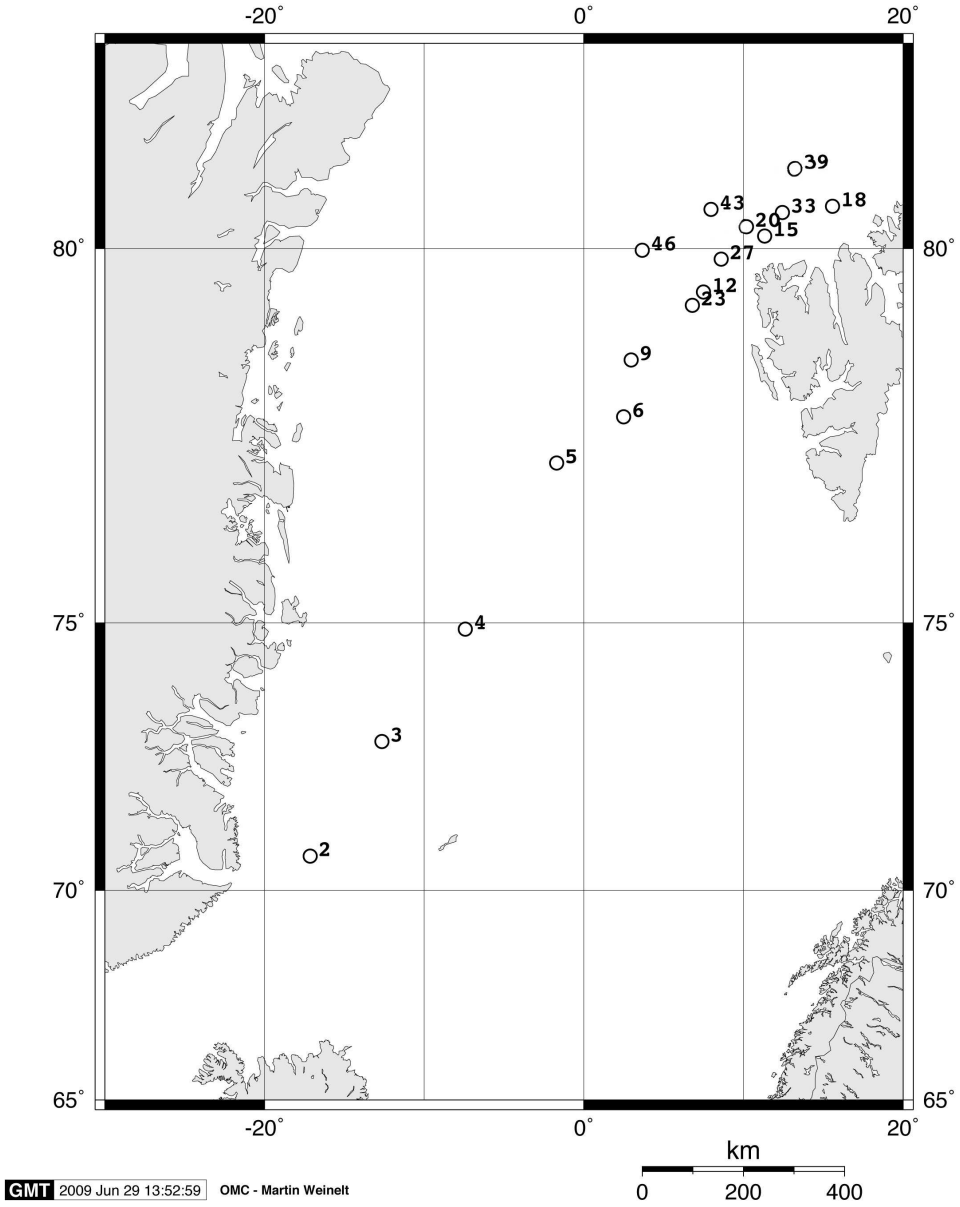
Figure 3. The quotient heterotrophic carbon / autotrophic carbon as a function of latitude. Greenland Sea and Coastal stations are indicated, the rest of stations correspond to Arctic Ocean open waters.

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 µm ciliates in St. 4 as grazers and the abundance of nanoflagellates as prey. See text for further details.



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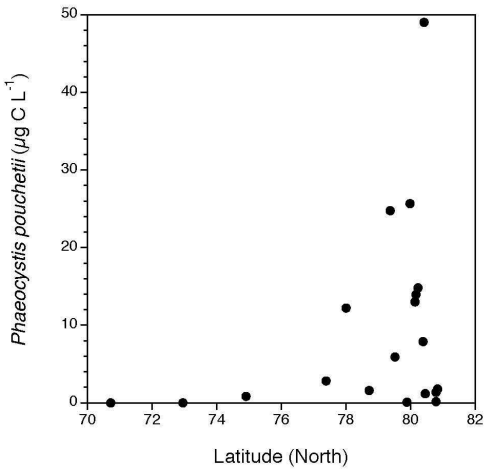

Fig. 2

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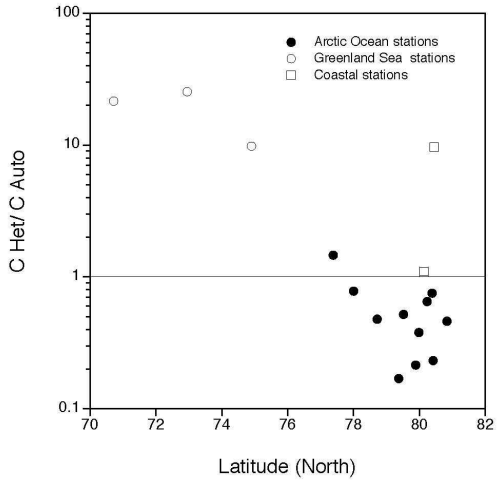

Fig. 3

209x297mm (200 x 200 DPI)

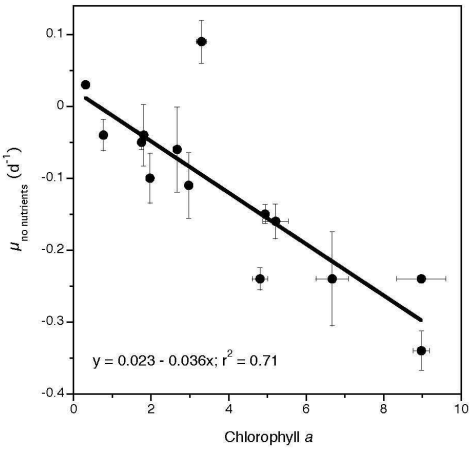

Fig. 4

209x297mm (200 x 200 DPI)

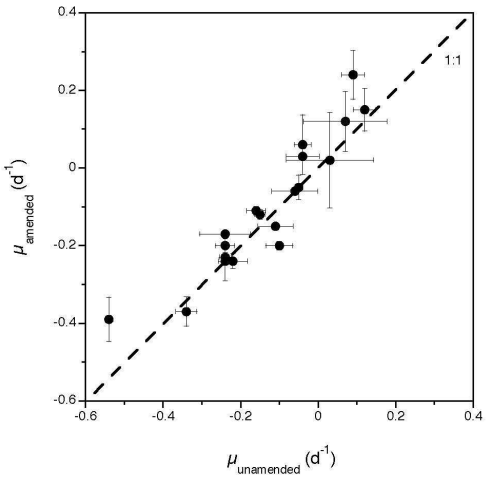

Fig. 5

209x297mm (200 x 200 DPI)

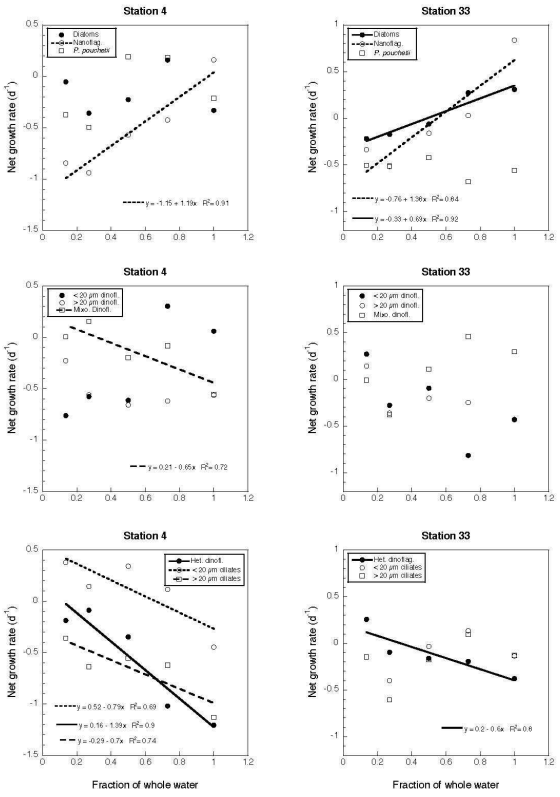

Fig. 6

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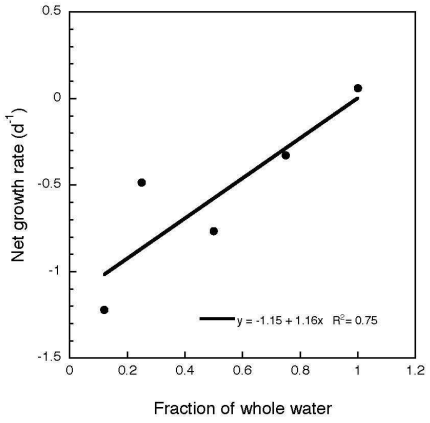
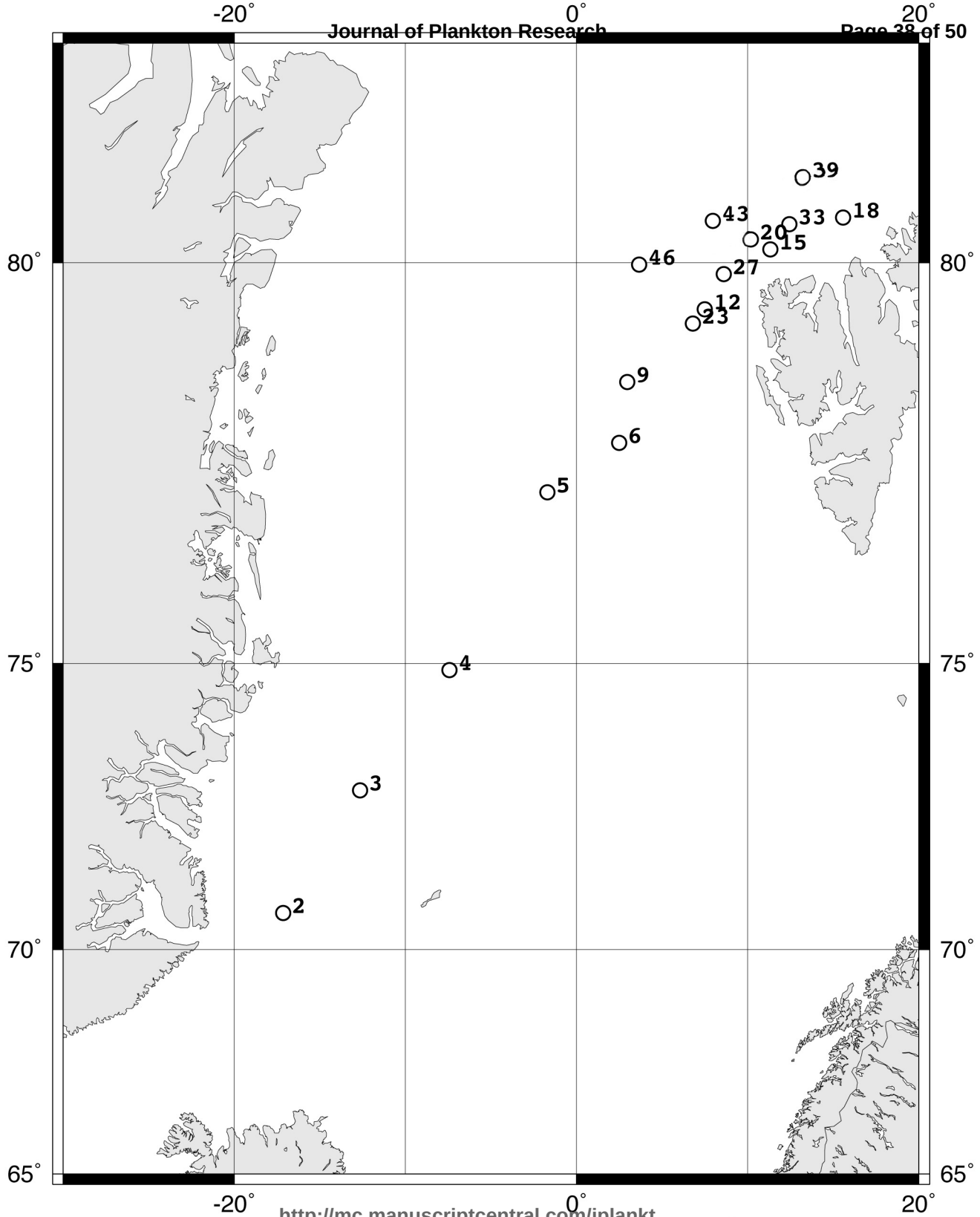


Fig. 7

209x297mm (200 x 200 DPI)



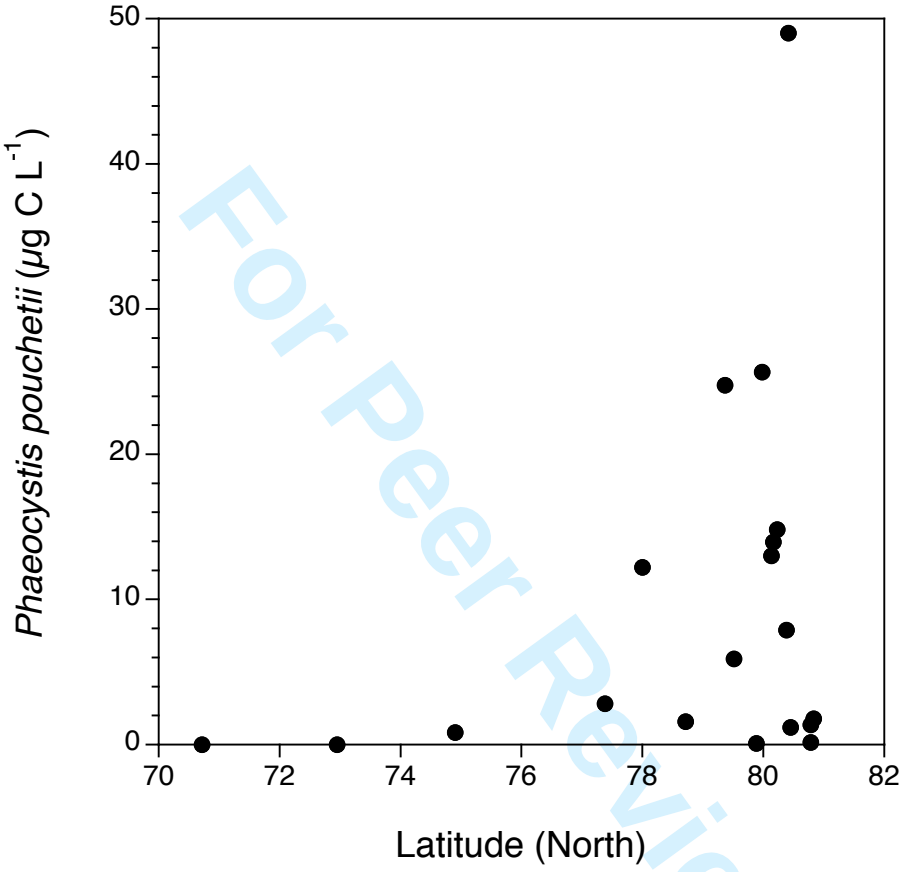


Fig. 2

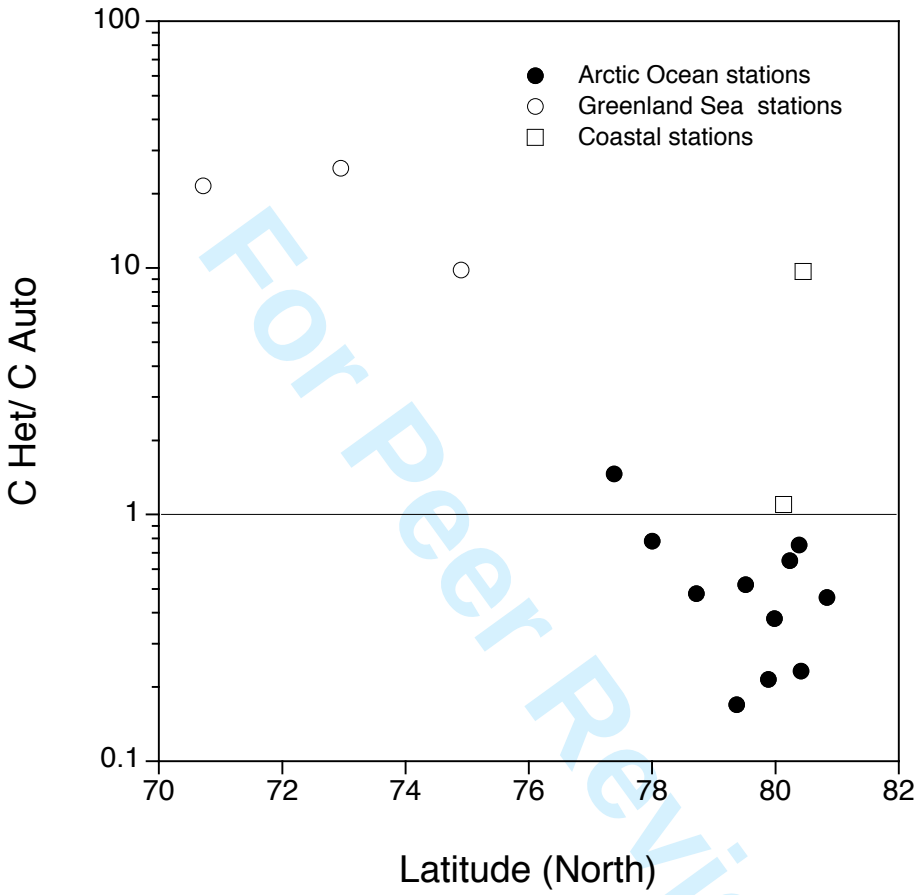


Fig. 3

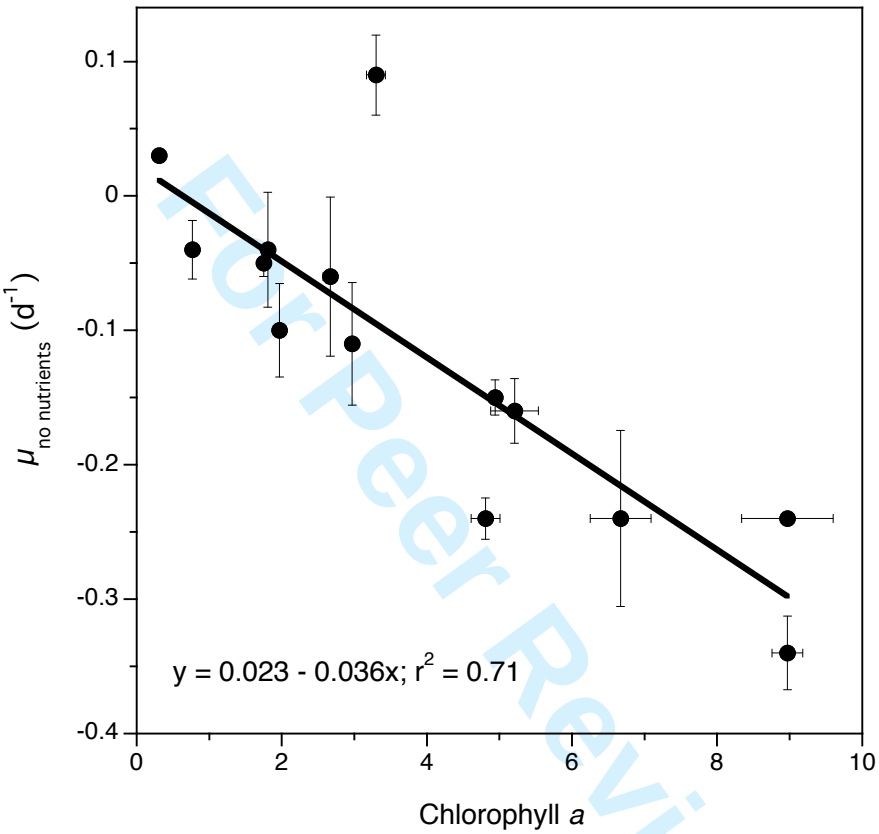


Fig. 4

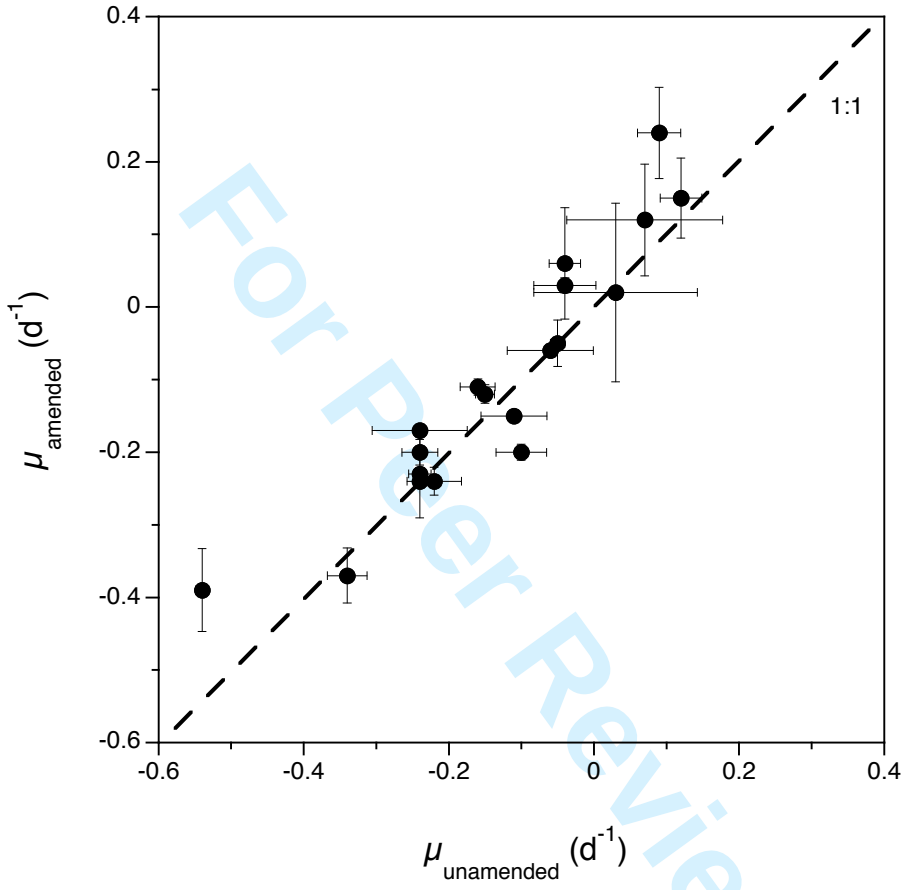


Fig. 5

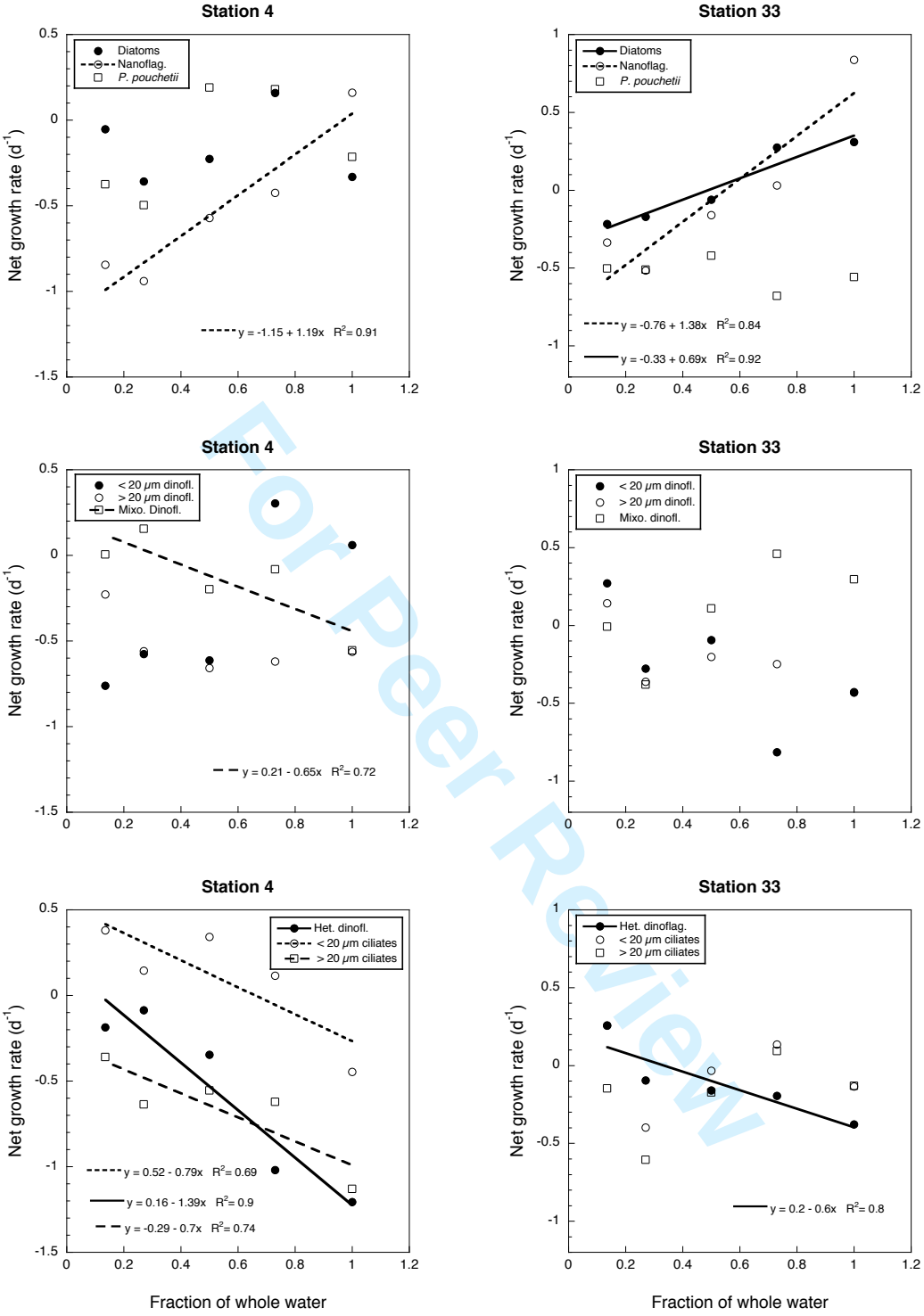


Fig. 6

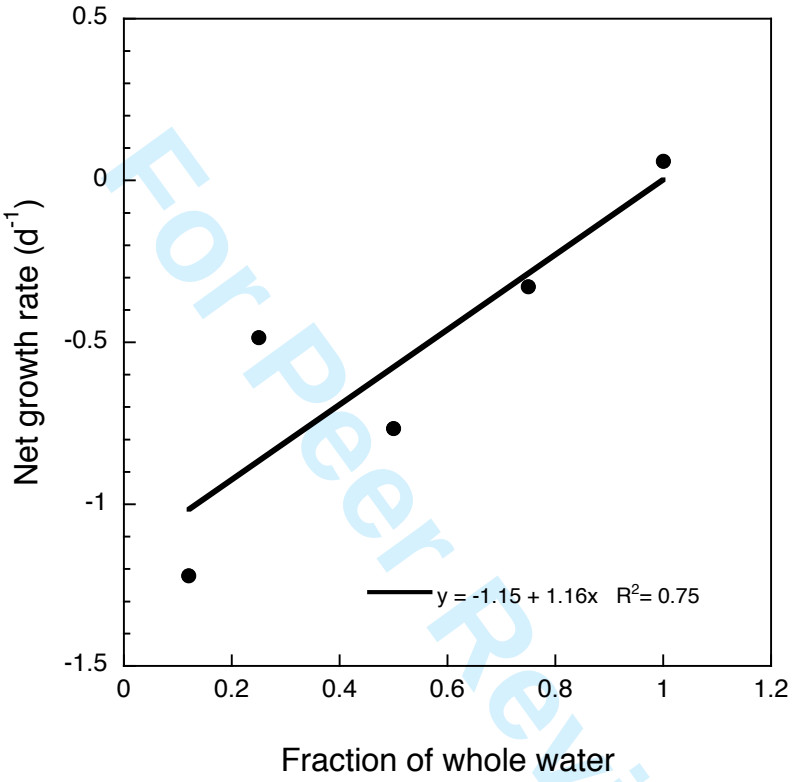


Fig. 7

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					
Chaetoceros spp.	1224	526	80959	11497	1770
Corethron hystrix	0	0	0	0	0
Eucampia zodiacus	0	0	20	20	0
Fragilariopsis spp.	280	20	60	60	130
Nitzschia spp.	0	0	20	20	2025
Rhizosolenia spp.	10	10	0	0	0
Skeletonema spp.	0	0	0	0	0
Thalassiosira spp.	955	445	290	20	750
Other centric diatoms	220	20	80	10	10
Pennated diatoms	0	0	0	0	5
Flagellates					
Phaeocystis pouchetii	0	0	0	0	212940
Choanoflagellidea	0	0	371907	8649	100905
Dictyocha sp.	35	5	10	10	50
Other nanoflagellates	29250	4500	42588	2366	227136
Dinoflagellates					
Amphidinium spp.	65	35	35	5	40
Ceratium spp.	10	0	0	0	10
Dinophysis spp.	0	0	0	0	5
Gyrodinium spp.	85	15	45	15	520
Katodinium glaucum	70	30	15	5	75
Micracanthodinium claytoi	20	20	95	15	100
Prorocentrum balticum	70	10	0	0	165
Protoperidinium bipes	85	15	20	10	25
Protoperidinium spp.	25	5	10	10	5
Torodinium robustum	50	10	10	10	265
Dinoflagellates < 20 µm	1644	274	2719.5	55.5	2497.5
Dinoflagellates > 20 µm	479.5	68.5	490	60	1270
Ciliates					
Didinium sp.	80	0	15	5	5
Laboea sp.	545	65	2220	180	310
Mesodinium spp.	55	35	5	5	0
Strombidium spp.	505	65	680	120	2004.5
Tintinnids	0	0	5	5	20
< 20 µm ciliates	3699	548	1494.5	24.5	4131
> 20 µm ciliates	420	70	240	20	1120.5

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

4	5	5	6	6	9
SE	Cells L^{-1}	SE	Cells L^{-1}	SE	Cells L^{-1}
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

tandard 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}
	5	9960		180	149858		4990	27281.5
	0	440		40	1600		280	810
	0	25		25	60		40	25
	15	1280		540	5148.5		901.5	1030
	90	57825		4275	380556		46128	533355
	0	0		0	0		0	10
	0	1575		225	2475		225	0
	315.5	10530		1310	79785		2585	12135
	5	10		10	21190		2240	8365
	68.5	4725		1125	6300		2250	450
	158565	2485146		403620	5420040		576600	20181
	0	0		0	0		0	0
	5	15		5	10		10	0
	282534	804186		50586	323880		41262	149916
	110	230		10	140		20	80
	0	0		0	0		0	0
	0	0		0	0		0	0
	167	195		65	445		35	210
	274	220		80	460		100	190
	0	10		10	10		10	30
	68.5	2475		225	14175		4725	9900
	68.5	100		40	70		30	140
	0	60		10	35		5	45
	5	120		40	120		20	60
	11532	24750		450	20025		1125	25425
	117	1065		165	990		290	950
	0	0		0	10		0	20
	130	0		0	15		5	5
	0	0		0	0		0	0
	25	180		0	120		30	65
	5	0		0	0		0	0
	132	4650		310	2636.5		72.5	400
	140	720		80	1000		120	250

	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)
	260	350	250	21.2
	0	0	0	44.2
	0	0	0	16
	430	240	240	8.6
	2250	5175	675	4.2
	0	0	0	36
	0	0	0	12.4
	1035	11475	225	18
	65	725	205	24.24
	0	450	0	9.6
	1210860	4050615	1484745	3.8
	900	1350	1350	4
	65	630	110	24.4
	34821	284559	5991	4.04
	20	300	20	13.8
	5	0	0	52.2
	5	15	5	32.8
	20	370	30	32.4
	60	370	10	15
	25	260	20	20
	225	3825	675	19.4
	20	50	20	18.8
	5	35	5	36.6
	15	40	10	27.6
	1575	12375	2925	
	680	1510	200	
	10	45	5	28.8
	20	20	0	71
	20	20	20	21
	95	440	100	33
	0	5	5	57.2
	55	2735	195	
	40	400	20	