Plankton development and trophic transfer in seawater enclosures with nutrients and *Phaeocystis pouchetii* added

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ABSTRACT: In high latitude planktonic ecosystems where the prymnesiophyte alga *Phaeocystis pouchetii* is often the dominant primary producer, its importance in structuring planktonic food webs is well known. In this study we investigated how the base of the planktonic food web responds to a *P. pouchetii* colony bloom in controlled mesocosm systems with natural water enclosed *in situ* in a West Norwegian fjord. Similar large (11 m^3) mesocosm studies were conducted in 2 successive years and the dynamics of various components of the planktonic food web from viruses to mesozooplankton investigated. In 2002 (4 to 24 March), 3 mesocosms comprising a control containing only fjord water; another with added nitrate (N) and phosphate (P) in Redfield ratios; and a third with added N, P, and cultured solitary cells of *P. pouchetii*, were monitored through a spring bloom cycle. In 2003 (27 February to 2 April) a similar set of mesocosms were established, but cultured *P. pouchetii* was not added. As expected, during both years, addition of N and P without addition of silicate resulted in an initial small diatom bloom followed by a colonial bloom of *P. pouchetii* (600 to 800 µg C l^{-1}). However, the hypothesis that addition of solitary cells of *P. pouchetii* would enhance subsequent colony blooms was not supported. Interestingly, despite the large production of *Phaeocystis* colonial material, little if any was transferred to the grazing food web, as evidenced by non-significant effects on the biomass of micro- and mesozooplankton in fertilized mesocoms. Separate experiments utilizing material from the mesocosms showed that colonies formed from solitary cells at rates that required only ca. 1 % conversion efficiencies. The results are discussed from the perspective of future research still required to understand the impact of life cycle changes of this enigmatic phytoplankter on surrounding ecosystems.

KEY WORDS: *Phaeocystis pouchetii* · Mesocosms · Nutrients · Fjord · Biocomplexity

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INTRODUCTION

The phytoplankton genus *Phaeocystis* is a cosmopolitan group that typically produces prodigious blooms of gelatinous colonies in high latitude marine environments. A particularly salient aspect of this genus is its ability to change between the well-known colonial stage and the less studied motile solitary stage. Colonies may be mm to cm in diameter and contain more than hundreds of cells in the periphery of a

gelatinous polysaccharide 'skin' (Chen et al. 2002). Solitary cells may be either motile or non-motile, and are typically 3 to 9 µm in diameter (Rousseau et al. 1994). This unusually large range of sizes between colonies and solitary cells (ca. 6 to 11 orders of magnitude in biovolume) can significantly alter material flow among trophic levels and export from the upper ocean (Wassmann et al. 1990, Lancelot et al. 1998). Furthermore, each stage is thought to function in different ways in order to reduce losses to either small or large zooplankton and viruses, and thus *Phaeocystis* spp. effectively function as dual species (Weisse et al. 1994, Smaal & Twisk 1997, Hamm et al. 1999, Jacobsen 2000, Verity 2000, Jakobsen & Tang 2002, Tang 2003).

The dual life history of colonial and solitary cell stages was described over 50 yr ago (Kornmann 1955), and the dominant morphology appears to alter the ecosystem function from a regenerative system (solitary cells) to one associated with the classical fisheries food chain (colonies, Fernandez et al. 1992). Transitions between colonial and solitary cell stages are probably under ecological control, but the mechanisms and ecological benefits are poorly known. Several investigators have proposed that such a life cycle is a form of 'bet-hedging', whereby one morphotype confers protection against predation or viral attack while the other enables higher growth efficiency. However, this is currently under debate (e.g. review by Schoemann et al. 2005, Nejstgaard et al. (2006). Interestingly, in at least 1 species, *Phaeocystis globosa*, a complex sexual life cycle involving haploid and diploid solitary and diploid colonial cells has been observed (Valero et al. 1992, Rousseau et al. 1994, Peperzak et al. 2000), but this transition has not been reported in all *Phaeocystis* species, including *P. pouchetii* (Jacobsen 2002). In our laboratory we have observed differential gene expression in *P. globosa* between solitary cells and colonies, but most of the detected genes have not been identified (Frischer unpubl. data).

The environmental stimuli that trigger transitions between solitary and colonial life stages are also poorly understood. Some investigators have reported that the transformation from solitary cells to colonies of *Phaeocystis globosa* can be induced by chemical cues from grazers (Jakobsen & Tang 2002, Long 2005), as well as various environmental stimuli including light, nutrients and the presence of diatoms as attachment sites (Boalch 1987, Verity et al. 1988b, Rousseau et al. 1994, Escaravage et al. 1995, Peperzak et al. 1998). However, to our knowledge there are no reports in the literature of the induction of *P. pouchetii* single cells to colonies under laboratory conditions.

Phaeocystis spp. blooms generally occur seasonally either prior to, during, or immediately following a bloom of diatoms. However, the absolute and relative abundance of *Phaeocystis* spp. colonies during blooms varies among locations and among years at a given location. In some cases, *Phaeocystis* spp. may be a minor component or may develop almost monospecific blooms (Lancelot et al. 1998). Even the solitary cell stage can dominate (Wassmann et al. 2005). While silicate availability can be a strong predictor of diatom occurence (see Egge & Aksnes 1992), silicate availability is often not a predictor of whether *Phaeocystis* spp. will occur (Verity et al. 1988a, Wassmann et al. 2000, Reigstad et al. 2002, Larsen et al. 2004), nor do diatoms or *Phaeocystis* spp., in either life cycle stage have clear physiological advantages over the other, although colonies may be preferred when nitrogen is present as nitrate, whereas solitary cells better assimilate ammonium (Riegman & van Boekel 1996, Hamm et al. 1999).

Thus, there remains a considerable amount of debate concerning the quantitative response of high latitude planktonic communities to colonial blooms of *Phaeocystis* spp. and what, if any, adaptive advantage is provided to *Phaeocystis* spp. by colony formation (Verity & Medlin 2003). Since colony formation may defend the algae against predation, bacterial and viral attacks, we hypothesize that its results in trophic sequestration of nutrients and energy into forms not easily accessible to planktonic grazing and regenerating communities. In this study we describe experimental manipulations in 11 m³ mesocosms of natural plankton communities in a west Norwegian fjord designed to stimulate colonial blooms of *P. pouchetii* to test this hypothesis and to quantify the response of the planktonic community (from viruses to mesozooplankton) to such a bloom.

MATERIALS AND METHODS

Mesocosm initiation. We conducted 2 sets of experiments, each consisting of 3 transparent polyethylene enclosures (4.5 m deep, 2 m diameter, ca. 11 m³, 90% transmission of photosynthetically available radiation, PAR, made by ANI-TEX, Notodden). The experiments were conducted in the Raunefjord at the Norwegian National Mesocosm Center located at the Marine Biological field station of the University of Bergen in western Norway (60° 16′ N, 05° 14′ E). The design of the mesocosms is illustrated in Fig. 1. Studies were conducted between 4 March and 24 March 2002 and 27 February and 2 April 2003. Additional details of the location and the mesocosm facility can be found at http://www.bio.uib.no/lsf/inst2.html.

The mesocosms were filled *in situ* 1 d prior to the initiation of each experiment by pumping unfiltered fjord water from 5 m depth using a large submersible

centrifugal pump, specially designed to minimally damage live plankton, with a flow rate of ca. 1.5 m^3 min^{-1} (ITT Flycht A/S, Model 3085-182). To ensure that each mesocosm was as similar as possible, the individual mesocosms were filled sequentially, a third at a time, such that the process was staggered. All mesocosms were filled within 1 h. The mesocosms were well mixed by an airlift system that recirculated the entire volume ca. 5 times d^{-1} (ca. 40 l min^{-1}) for the duration of each experiment (Jacobsen et al. 1995). To allow introduction of new species, avoid substantial pH changes, and replace sampled water over the course of the experiment, 10% of the water in each mesocosm was renewed daily with fjord water (ca. 2.5 m) using small submersible aquarium pumps (Fig. 1). During the 2002 experiment, water renewal was maintained from 4 to 18 March and during the 2003 experiment from 27 February to 20 March. For additional discussion concerning the importance of water renewal see Egge (1993) and Williams & Egge (1998).

Treatments. During the 2 experiments, each with 3 mesocosms, 2 mesocosms were amended with nitrate (NaNO$_3$) and phosphate (KH$_2$PO$_4$) ('NP') corresponding to an initial enrichment of 16 µM nitrate and 1 µM phosphate by the addition of 100 ml each of stock solutions of NaNO$_3$ (1.76 M) and KH$_2$PO$_4$ (0.11 M). Nutrients removed by the 10% water renewal were replaced daily by the addition of 10 ml each of the

Fig. 1. Mesocosm design used in this study

nutrient stock solutions equivalent to daily additions of 0.1 and 1.6 µM, respectively. In the 2002 experiment, based on the low nutrient concentrations measured on March 12, both nutrient enriched mesocosms were augmented with additional nutrients corresponding to 8 µM nitrate and 0.5 µM phosphate during the evening of 12 March. The third mesocosm in each experiment was left unamended and served as a control treatment. During the 2002 experiment, 40 l of a late exponential growth stage (8.25×10^4 cells ml^{-1}) culture of *Phaeocystis pouchetii* solitary cells were added to 1 of the nutrient amended mesocosms ('NPF') corresponding to an initial addition of ca. 300 cells ml^{-1}, in order to investigate if higher initial abundance of flagellated cells would induce more or earlier colony formation. The culture of *P. pouchetii* was originally isolated from Raunefjorden in 2001 and grown in *f*/2-Si media (Guillard 1975).

Sampling procedures and analyses. Salinity and temperature profiles in each mesocosm were determined using a SD204 CTD (SAIV). Surface irradiance (PAR) was recorded continuously with a LI-COR 190 quantum sensor (LI-COR) mounted horizontally ca. 4 m above the sea surface. Irradiance was averaged every 15 min and stored using a LI-COR 1400 data logger. *In situ* light profiles from surface to the bottom of the mesocosms were occasionally obtained using a horizontally mounted LI-192 underwater quantum sensor. Surface water samples for analysis of nutrient concentration, viral and bacterial abundance, chlorophyll *a* (chl *a*), phytoplankton and microzooplankton were collected in 30 l carboys 12 h after initiation of the experiment and approximately every third day at 08:00 h afterward until the termination of the experiments. Because the mesocosms were fully mixed, it was not necessary to analyze depth-profiled samples for these parameters. Chl *a* was determined in triplicate water samples (20 to 100 ml) according to Parsons et al. (1984). Water was filtered onto 25 mm 0.45 µm cellulose-acetate filters (Sartorius), immediately extracted in 90% acetone overnight at 4°C, and analyzed using a Turner Designs 10-AU fluorometer. Concentrations of ammonium, phosphate, and silicate were determined on fresh samples at each sampling event. Ammonium concentrations were determined fluorometrically using a Turner Designs 10-AU fluorometer as described by Holms et al. (1999). Phosphate and silicate concentrations were determined colorimetrically as described by Valderrama (1995) using a Shimadzu UV-160 spectrophotometer. Nitrate concentrations were determined as described by Hagebø & Rey (1984) on chloroform fixed samples (1% vol:vol) using a nutrient autoanalyzer (Skalar). Chloroform fixed samples were stored at 4°C and analyzed within 1 mo of collection.
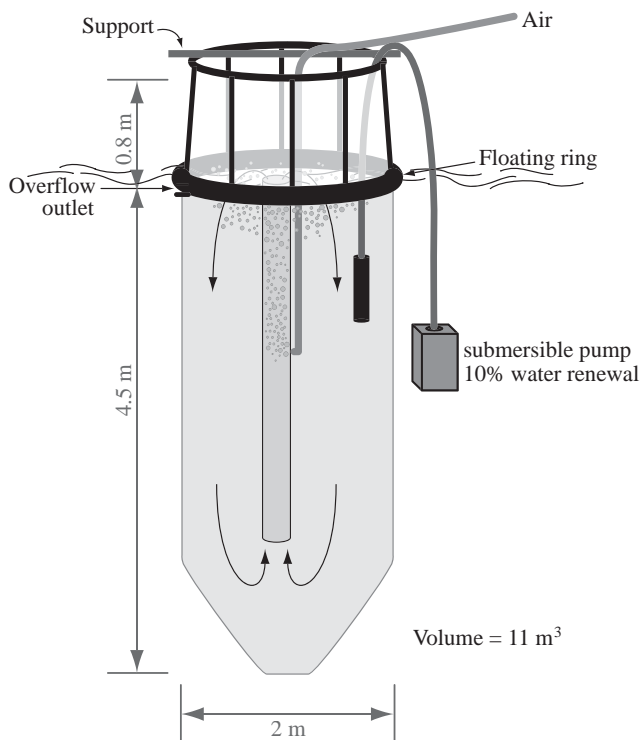
To enumerate phytoplankton and microzooplankton >10 µm, EDTA (10 mM final concentration) was added to 60 ml of whole water samples, mixed gently by inversion, fixed with HPLC grade glutaraldehyde (0.5% final concentration), and stored at 4°C. In trial experiments, 10 mM EDTA effectively dispersed *Phaeocystis pouchetii* colonies without seemingly affecting cell morphology or total cell abundance (data not shown).

Phytoplankton and microzooplankton <10 µm were identified and enumerated by epifluoresence microscopy. Unfiltered freshwater samples (60 ml) were gently passed through a 10 µm nylon mesh and fixed with HPLC grade glutaraldehyde (0.5% final concentration) and stored at 4°C. Each sample was stained with proflavin (100 ng ml^{-1} final concentration stock = 5.5 µg ml^{-1}) and DAPI (25 ng ml^{-1} final concentration stock = 500 ng ml^{-1}) as described by Porter & Feig (1980) and Haas (1982), except that higher concentrations of stain were required to effectively stain *Phaeocystis pouchetii* cells. We made 3 epifluorescent slides by gently filtering 15 ml of the fixed and stained samples onto black 0.2 µm (25 mm diameter) polycarbonate filters using a hand-pump with <0.5 mm Hg vacuum pressure. Filters were mounted onto glass slides with a cover slip and stored at –20°C until analysis. Cells were visualized by epifluoresence microscopy using an Olympus BX-60 fluorescence microscope equipped with a 60× Planapo NA 1.40 oil objective. For each slide, a minimum of 20 fields or 300 cells were examined either manually or using a semi-automated custom image analysis system 'Skipper' (www.skipperimaging.com) after digitally capturing images using a Retiga 1300 Cooled Color 12-bit digital camera (image resolution 1200 × 1024 pixels).

Flagellated *Phaeocystis pouchetii* solitary cells and other phytoplankton and microzooplankton were identified based on their fluorescence, shape and size, and were enumerated in water samples that passed through the 10 µm nylon mesh. Microflagellates (autotrophic, heterotrophic, and mixotrophic flagellates), diatoms, and total *P. pouchetii* cells (solitary and colonial) were identified and enumerated from the EDTA treated samples. Ciliates and large dinoflagellates (<50 µm diameter) were enumerated in the EDTA treated samples by screening and counting all cells present on the whole slide (15 ml sample). In 2003, cell volumes were calculated by approximation of simple geometrical 3D shapes and converted into cell carbon as described in Menden-Deuer & Lessard (2000).

The concentration and size of *Phaeocystis pouchetii* colonies were determined within hours of water collection. Water samples (50 to 100 ml) were gently concentrated 5 to 10 fold by reverse filtration though a 10 µm mesh. The entire concentrated sample was placed into 3 ml well plates (3 ml per well) and allowed to settle for 1 h at 4°C. Colony concentrations and size were determined by averaging counts from at least 4 wells using a Nikon Diaphot inverted microscope equipped with a 40× objective. All sample processing was conducted at *in situ* temperature (5°C).

Mesozooplankton were sampled at the start of each experiment by filtering 4 m^3 of water though a 90 µm WP plankton net in triplicate before, between, and after filling of the meoscosms, and at the end of the experiment by emptying each mesocosm through the same net. In addition to sampling at the start and end of each experiment, during each experiment mesozooplankton were sampled periodically by net hauls (30 cm diameter, 90 µm mesh) from the bottom to the surface of the mesocosms. During the first experiment (2002), mesocosms were sampled on 9, 15, and 24 March; during the second experiment (2003), mesozooplankton were sampled on 24 March. Mesozooplankton were fixed immediately in 4% buffered formaldehyde and identified, enumerated and sized using a Wild M10 dissecting microscope. Mesozooplankton abundance was converted into carbon biomass by applying accepted size-specific carbon conversion factors (Conover & Lalli 1972, Theilacker & Kimball 1984, Båmstedt 1986, Daan 1986, Whyte et al. 1987, Blom et al. 1989, Båmstedt et al. 1990, Falkenhaug 1991, Widdows 1991, Karlson & Båmstedt 1994, Gorsky & Fenaux 1998).

Bacterial abundance was determined in each mesocosm ca. every third day during each experiment. Total cell abundance was determined by direct counting of DAPI stained cells in combination with the estimation of the physiological status of individual cells using the VSP (vital stain probe) technique as described by Howard-Jones et al. (2001). Upon collection, whole water samples (15 ml) were equilibrated in sterile glycerol (25% final concentration) and stored at –20°C. Sample analysis was completed within 3 mo of sample collection. Cells were visualized using an Olympus BX 60 fluorescence microscope equipped with a 100× UPLANFL NA 1.35 oil objective. Enumeration was facilitated using 'Skipper' after capturing digital micrographs using a Photonic Science Color Cool View cooled, 3-chip, color integrating charged-coupled device camera system (image resolution 768 × 576 pixels or 640 × 480 pixels), as previously described by Williams et al. (1998).

Total viral abundance was estimated by flow cytometry (FCM) as previously described (Marie et al. 1999a,b). All FCM analyses were performed using a FACSCalibur Flow Cytometer (Becton Dickinson) equipped with an air-cooled laser providing 15 mW at 488 nm using a standard filter set. Water samples were fixed with EM grade glutaraldehyde (0.5% final concentration) for 30 min at 4°C, flash frozen in liquid nitrogen, and stored at –70°C until analysis. Prior to counting, samples were thawed, diluted 10- to 200-fold

in sterile filtered (0.02 µm) TE buffer (Tris 10 mM, EDTA 1 mM, pH 8), and stained for 10 min at 80°C in the dark with SYBR Green I solution (5 µl per 500 µl sample; Molecular Probes). Fluorescent microspheres (Molecular Probes) with a diameter of 0.95 µm were added to all samples as an internal counting standard. The discriminator was set on green fluorescence and the samples were analyzed for 1 min at a count event rate between 100 and 1000 s^{-1}.

Colony formation rates. During the first mesocosm experiment (2002), colony formation rates were estimated. Water from the NPF mesocosm was gravity-filtered through 47 mm 8 µm pore size polycarbonate filters in order to remove all *Phaeocystis pouchetii* colonies present in the water while allowing *P. pouchetii* solitary cells to pass. Aliquots of 2.5 ml of colony-free water were gently pipetted into each of 24 wells in sterile acid-cleaned well plates (Nalge Nunc International). In order to test the hypothesis that surfaces for settlement stimulate colony formation (e.g. Rosseau et al. 1994), half of the wells received 50 µl of a 1 mg ml^{-1} solution of diatomaceous earth (Fisher Scientific, equivalent to ca. 10^2 to 10^3 diatomaceous particles ml^{-1}). The diatomaceous earth was equilibrated in filtered NPF-mesocosm water for 24 h prior to the experiment. Well plates were incubated at *in situ* temperature (5°C) under dim light with a 14:10 light/dark photoperiod. Light levels during the light and dark periods averaged 62 and 8 µmol photons m^{-2} s^{-1}, respectively. Light levels were estimated using a hand-held QSL 100 light meter (Biospherical Instruments). We initiated 3 sets of experiments beginning on 10, 13 and 16 March, respectively, and colonies were counted in each well using a Leica zoom stereomicroscope daily for 6 d. All process manipulation and analysis were conducted at 5°C in a walk-in cold room.

Statistical analyses. The statistical packages SAS (v8.0; SAS Institute) and SigmaStat (SPSS, v3.00) were used for all statistical analyses. Net *Phaeocystis pouchetii* growth rates and production were calculated from liner regressions of log concentrations. Comparisons of absolute cell concentration between and within mesocosms and comparisons of growth rates were conducted by ANOVA and Student's *t*-tests. Significance was reported for all correlations at the 95% confidence level (p < 0.05).

RESULTS

General overview

The addition of nitrate (N) and phosphate (P) to the mesocosms significantly increased the concentration of these nutrients above those of the unamended control mesocosms (Figs. 2 & 3) and the outside fjord water (Fig. 4), and resulted in large blooms of *Phaeocystis pouchetii* colonies (max. 100 to 300 colonies ml^{-1}; 600 to 800 µg C l^{-1}) in each fertilized mesocosm (Figs. 5d–f, 6d–f, 7a–c). However, despite the large production of *P. pouchetii*, little if any of this production was transferred to the grazing food web. The initial addition of cultured *P. pouchetii* solitary cells did not result in early initiation of colony formation or eventual higher concentrations of *P. pouchetii* colonies compared to other fertilized mesocosms, as was initially hypothesized (Fig. 5d-f). Instead, higher diatom, microflagellate and dinoflagellate concentrations occurred in the NPF treatment, possibly suggesting that, unlike native *P. pouchetii* solitary flagellated cells, cultured cells were more susceptible to zooplankton grazing (Figs. 5g–i & 8). These major trends are described in further detail below.

Irradiance and hydrography

Irradiance patterns were similar during both experimental studies. In both experimental years the maximum daily irradiance varied from 100 to 1300 µmol photons m^{-2} s^{-1} at the surface and from ca. 1 to 4 orders of magnitude lower at the bottom of the mesocosms. During the 2002 experiment there were 16 mostly sunny days and 5 mostly cloudy days, and during the 2003 experimental period there were 19 mostly sunny days and 17 mostly cloudy days. Because both experimental periods bracketed the spring equinox, there were ca. 12 h of light d^{-1} in both years. The temperature and salinity of the mesocosms were also similar during both years and changed little during the experimental periods. Temperatures in the mesocosms closely followed those in the surrounding fjord (<0.2°C difference) and increased gradually from the start of each experiment to its conclusion. In 2002 water temperatures increased from 4.9 to 5.6°C over the course of the study period, and in 2003 from 3.5 to 4.5°C over the course of the experiment. During 2002 the salinity in each mesocosm was between 31.0 and 31.7 PSU, and in 2003 between 31.5 and 31.7 PSU in each mesocosm over the duration of the experiment. These temperature and salinity ranges are typical for Bergen-area fjords in March (Erga & Heimdal 1984). Based on daily depth profiles of temperature, salinity and *in situ* fluorescence in each mesocosm, there was no evidence of stratification in the mesocosms, indicating that the systems were well mixed.

Nutrients and chlorophyll

Nutrient (N, P, and Si) and pigment (chl *a* and phaeophytin) concentrations in both mesocosms and the
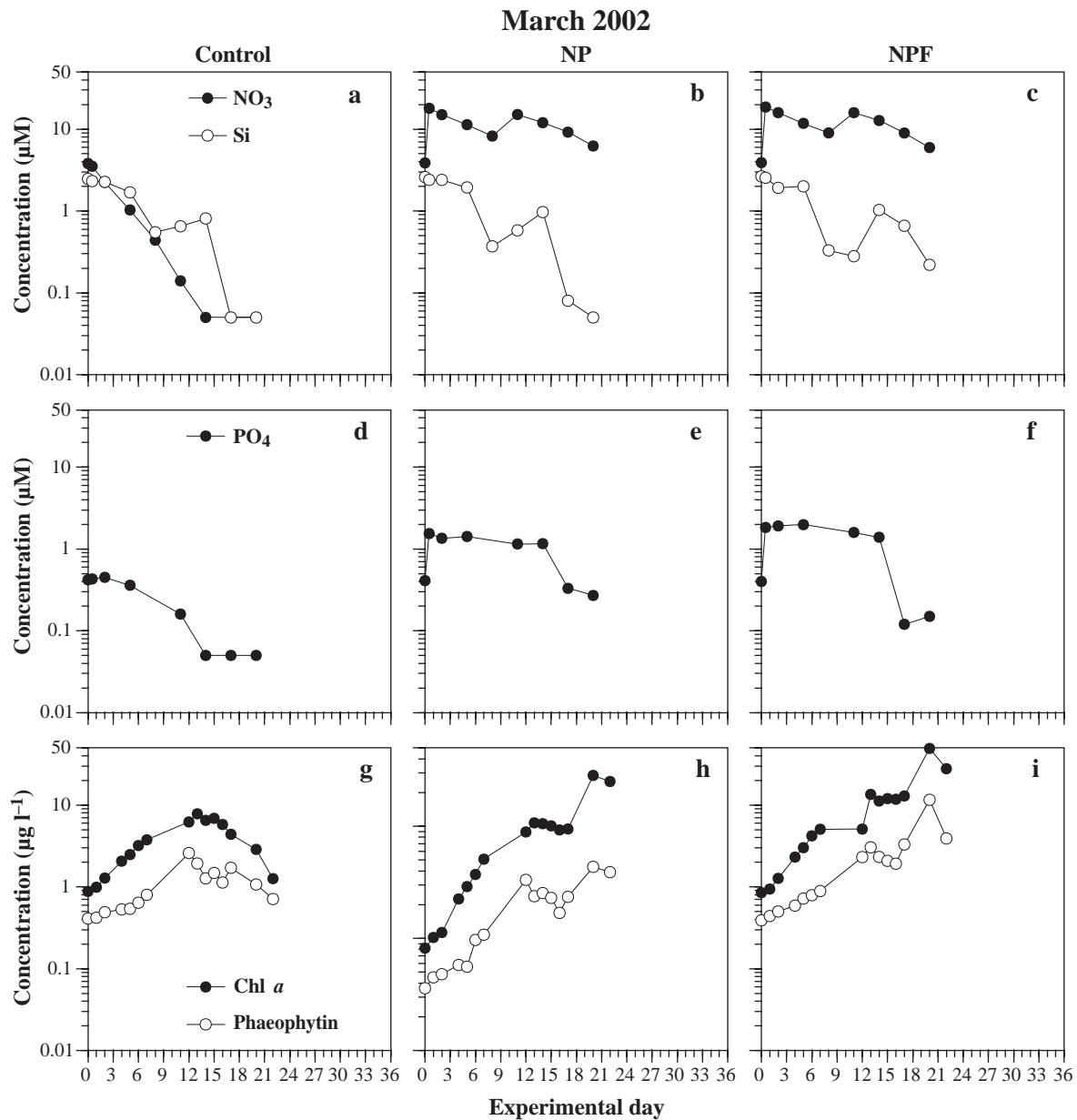
March 2002

Fig. 2. Nutrients, chl *a* and phaeophytin pigments in 2002 in (a,d,g) unamended control (b,c,h) fertilized (NP; b,e,h) and (c,f,i) NP fertilized plus cultured *Phaeocystis pouchetii* solitary cells. (a–c) Concentrations of NO_3 and Si; (d–f) concentration of PO_4; (g–i) concentrations of chl *a* and phaeophytin

fjord are shown in Figs. 2 to 4. Initial nutrient concentrations (N, P, Si) prior to fertilization in 2002 and 2003 were similar, but N concentrations (3.9 and 5.5 µM) and Si concentrations (2.6 and 5.1 µM), respectively, were slightly lower in 2002 than in 2003 (N, p = 0.005; Si, p = 0.001). Initial phosphorus concentrations in 2002 (0.41 µM) and 2003 (0.37 µM) were not significantly different (p = 0.11) from each other. During both years, nutrient concentrations after fertilization were not significantly different in any of the fertilized mesocosms (Figs. 2 & 3). The addition of 40 l of expended *f*/2 algal

growth media along with the cultured *Phaeocystis pouchetii* solitary cells in the 2002 NPF mesocosm did not significantly elevate initial nutrient concentrations in the NPF versus NP treatments (Fig. 2).

In the unamended mesocosms, N and P concentrations decreased consistently for 15 to 20 d, after which concentrations went below analytical detection limits (0.05 µM). In 2003, Si in the unfertilized mesocosm declined somewhat more rapidly than in 2002, and fell below the detection limit after 10 d. After the addition of nutrients to the fertilized mesocosms, nutrient con-
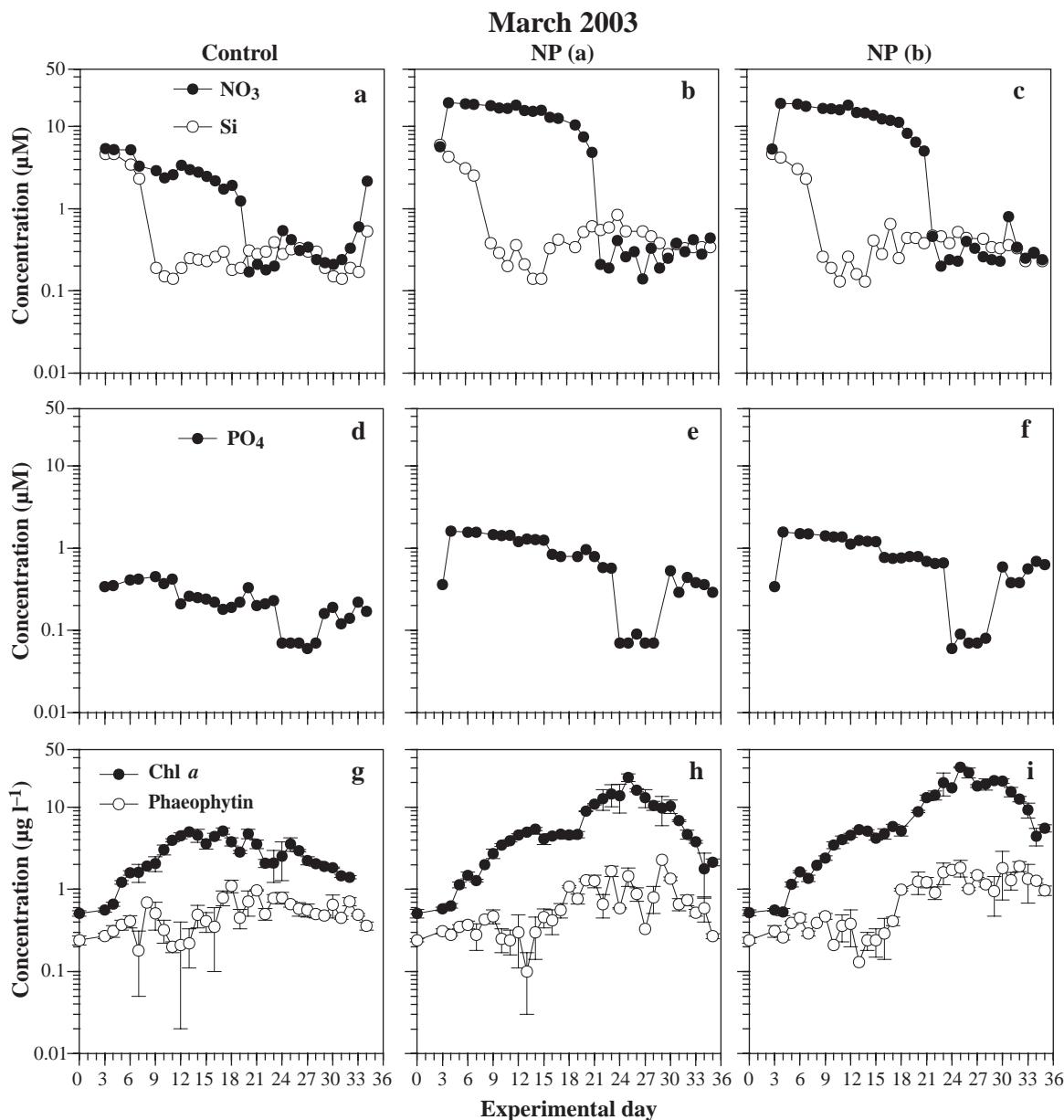
March 2003

Fig. 3. Nutrients, chl *a* and phaeophytin pigments in 2003 in (a,d,g) unamended control and replicate (b,c,h) NP (a) fertilized and NP (b) fertilized mesocosms. (a–c) Concentrations of NO_3 and Si; (d–f) concentration of PO_4; (g–i) mean ± SD concentrations of chl *a* and phaeophytin

centrations decreased over the course of the experiment, reaching detection limits after ca. 10 to 15 d, similar to the unamended mesocosms (Figs. 2a,b & 3a,b). The decrease in N and P concentrations in the 2002 mesocosms was slowed by an additional spike of nutrients (8 µM NO_3 and 0.5 µM PO_4) on 12 March 2002.

Initial chl *a* concentrations were slightly higher in 2002 (0.85 µg l^{-1}) than in 2003 (0.51 µg l^{-1}), but were not significantly different from each other (p = 0.06). Likewise, initial phaeophytin concentrations were

slightly higher in 2002 (0.39 µg l^{-1}) compared to 2003 values (0.24 µg l^{-1}), but were not significantly different from each other (p = 0.06). In all mesocosms (unfertilized and fertilized), there was an initial small increase in chl *a* concentrations ranging from 1 to 5 µg l^{-1} which diminished in the unfertilized mesocosms and was succeeded by a dramatic increase in chl *a* concentrations to 25–30 µg l^{-1} in the fertilized mesocosms. In the NPF mesocosm (2002), Chl *a* concentrations reached a maximum of 49.1 µg l^{-1} on 24 March. Chl *a* concentrations peaked after 20 d in both fertilized meso-
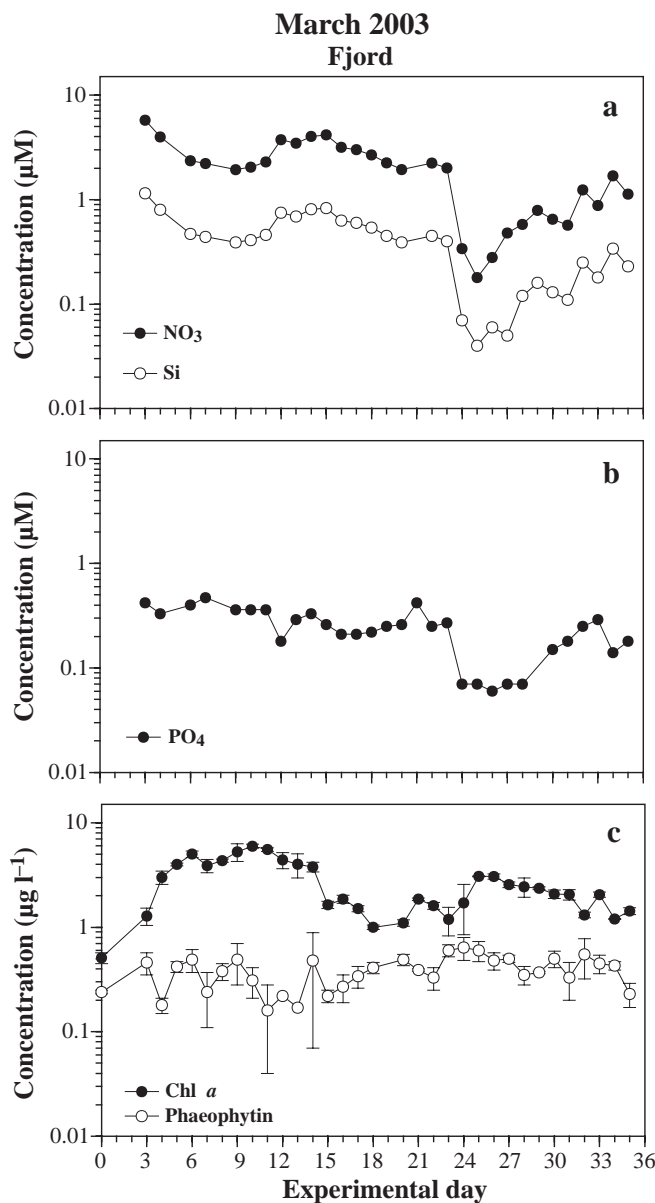
March 2003
Fjord

Fig. 4. Nutrients, chl *a* and phaeophytin pigment concentrations in Raunefjorden during 2003 study. (a) Concentrations of NO_3 and Si; (b) concentration of PO_4; (c) mean ± SD concentrations of chl *a* and phaeophytin

cosms in 2002 (24 March 2002). In 2003, chl *a* concentrations peaked 25 d after the experiment began, also on 24 March. During the longer experiment in 2003, a decline in chl *a* concentrations was observed in both fertilized mesocosms beginning 25 d after the start of the experiment on 24 March until the end of the experiment (3 April) when chl *a* concentrations were only slightly higher then initial concentrations, 1.4 and 2.1 µg l^{-1} in mesocosms NP(a) and NP(b), respectively (Fig. 3g–i).

Bacteria and viruses

Both bacteria and viruses increased similarly in 2002 and 2003 studies, although slightly greater (but not significantly so, p = 0.064), net bacterial growth was observed in 2002 than in 2003 (Fig. 5a–c and 6a–c). Overall there were no differences in bacterial abundance or net production between the control and fertilized mesocosms, suggesting that bacterial populations did not respond directly to fertilization with inorganic nutrients (N and P) or the *Phaeocystis pouchetii* bloom by increasing in abundance. Bacterial abundance increased in the 2002 experiments from an average of $6.3 ± 2.3 × 10^5$ cells ml^{-1} at the start of the experiment to $3.95 ± 1.2 × 10^6$ cells ml^{-1} after 21 d (6.3-fold). In 2003 bacterial abundance increased from an average of $1.2 ± 0.3 × 10^6$ cells ml^{-1} at the start of the experiment to $4.9 ± 1.2 × 10^6$ cells ml^{-1} after 35 d (4.1-fold). Apparent bacterial growth rates estimated from regression analysis of bacterial abundance were $1.9 × 10^5$ cells d^{-1} in 2002 ($r^2 = 0.6$) and $6.0 × 10^4$ cells d^{-1} in 2003 ($r^2 = 0.2$).

Similar to bacterial abundance, the total abundance of viral particles did not differ between unfertilized and fertilized mesocosms. Viral particle abundance increased from an average of $1.5 ± 0.25 × 10^7$ particles ml^{-1} at the start of the experiment to $6.4 ± 6.0 × 10^7$ particles ml^{-1} after 21 d (4.3-fold). In 2003 viral abundance increased from an average of $1.4 ± 0.7 × 10^7$ particles ml^{-1} at the start of the experiment to $3.95 ± 0.1 × 10^7$ particles ml^{-1} after 35 d (2.8-fold). The ratio of virus particles to bacterial cells (VBR) in all 2002 mesocosms varied between 6 and 39 and averaged 14 ± 8. In 2003 the VBR varied from 4.5 to 24.5 with an average of 9.9 ± 5.

Phaeocystis

Figs. 5d–f & 6d–f show 3 forms of *Phaeocystis pouchetii* including solitary flagellated cells, colonies and total cells associated with colonies (non-motile single cells). Single non-motile cells associated with colonies were not determined in the 2002 experiment.

The initial abundance of flagellated cells was similar in 2002 (207 ± 12 ml^{-1}) and 2003 (130 ± 96 ml^{-1}) and increased after an initial lag period of ca. 8 d in 2002 and 15 d in 2003. In both sets of experiments, the abundance of solitary flagellated cells increased in each mesocosm and was within the same order of magnitude in fertilized and unfertilized treatments. During the lag phase of the 2002 experiment (Days 0 to 5), daily growth rates averaged 0.007 d^{-1}, while average negative net growth rates of –0.04 d^{-1} were calculated during the early portion (Days 0 to 15) of the 2003 study (Table 1). Maximum growth rates, which oc-
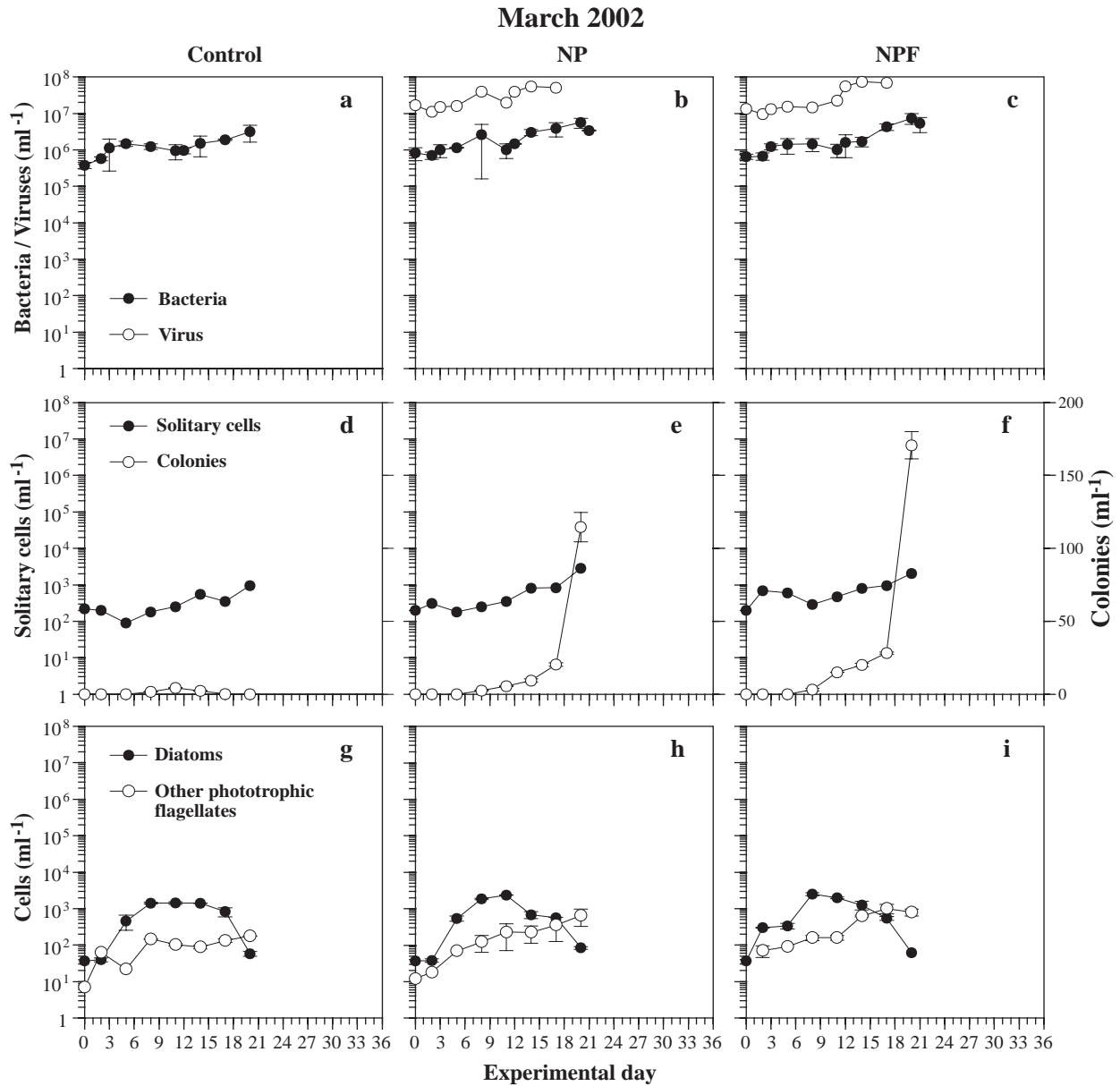
March 2002

Fig. 5. Bacterioplankton, virioplankton, *Phaeocystis pouchetii* flagellated cells and colonies, other phototrophic flagellate cells, and diatoms during the 2002 study. (a–c) Abundance of bacterioplankton and virioplankton (virioplankton was not sampled in control); (d–f) abundance of *P. pouchetii* flagellated cells and colonies; (g–i) mean ± SD abundance of other phototrophic flagellate cells and diatoms

curred after the lag phase, varied from 0.33 to 1.22 d^{-1} with an overall average maximum growth rate of 0.21 ± 0.10 d^{-1} (Table 1). There was no significant difference in the average or maximum growth rates of solitary flagellated cells in fertilized versus unfertilized treatments (p = 0.51) or between years (p = 0.09). In the 2003 study, when biomass was estimated, *Phaeocystis pouchetii* single flagellated cells accounted for only 6.3 ± 1.2 µC l^{-1} at their maximum densities, and this was similar between the mesocosms (Fig. 7a–c).

Phaeocystis pouchetii colonies were not detected in any of the 2002 mesocosms at the initiation of the experiments and were present in very low abundance (ca. 6 colonies ml^{-1}) at the start of the experiments in 2003. Colonies developed in each year and in all mesocosms, although colony abundance in the unfertilized mesocosms was very low. Colonies first appeared in the 2002 mesocosms in each treatment 8 d from the initiation of the experiment on 12 March. In 2003, the concentration of colonies began to increase in the fer-

Table 1. Average and (maximum) instantaneous net daily growth rates (d^{-1}) for chl *a*, bacteria and *Phaeocystis pouchetii* flagellated cells and colonies in 2002 and 2003 studies. NP: nitrate and phosphate; NPF: nitrate + phosphate + *P. pouchetii*. Initiation: no colonies present or net increase in abundance; during 2002 this phase was from Days 0 to 5, during 2003 from Days 0 to 15. Exponential: colonies present and colonies exhibiting net increase in abundance; during 2002, this phase was from Days 6 to 22, during 2003 from Days 7 to 29. Decay: colonies present and colonies exhibiting net decrease in abundance; during 2002 no decay phase was observed, during 2003 this phase was from Days 30 to 35. na: data not available

BloomPhase	Series 1 (2002)			Series 2 (2003)			
	Control	NP	NPF	Fjord	Control	NP(a)	NP(b)
Chl *a* (µg l^{-1})							
Initiation	0.21 (0.25)	0.25 (0.35)	0.25 (0.30)	0.08 (0.85)	−0.05 (0.61)	0.06 (0.60)	0.12 (0.78)
Exponential	−0.04 (0.25)	0.13 (0.56)	0.13 (0.97)	0.03 (0.59)	−0.10 (0.51)	−0.25 (0.65)	0.12 (0.58)
Decay	na	na	na	−0.08 (0.45)	−0.10 (−0.03)	−0.25 (0.18)	0.12 (0.22)
P. pouchetii flagellated cells (cells ml^{-1} d^{-1})							
Initiation	−0.18 (−0.05)	−0.02 (0.22)	0.22 (0.62)	na	−0.05 (0.10)	0.00 (0.00)	−0.07 (0.46)
Exponential	0.16 (0.33)	0.18 (0.41)	0.08 (0.26)	na	0.17 (0.69)	0.32 (0.62)	0.34 (1.22)
Decay	na	na	na	na	0.18	−0.20	−0.05
Phaeocystis colonies (col ml^{-1} d^{-1})							
Initiation	0.00	0.00	0.00	0.17 (0.77)	−0.09 (0.17)	−0.07 (0.034)	−0.06 (0.22)
Exponential	0.00 (0.32)	0.32 (0.58)	0.33 (0.60)	0.06 (0.10)	0.17 (0.48)	0.27 (1.02)	0.32 (0.42)
Decay	na	na	na	−0.14 (0.23)	−0.23 (−0.12)	0.15 (−0.06)	−0.02 (−0.02)
Bacteria ×10^5 (cells ml^{-1} d^{-1})							
Initiation	2.34 (2.84)	0.71 (1.34)	1.67 (2.24)	na	0.74 (3.01)	1.11 (4.91)	0.95 (3.07)
Exponential	1.69 (2.60)	2.34 (5.03)	4.56 (6.96)	na	1.43 (1.70)	1.66 (2.49)	2.25 (3.03)
Decay	na	na	na	na	na	0.94 (na)	1.19 (na)

tilized treatments on 17 March, ca. 18 d after the start of the experiment. Maximum colony concentrations were similar in all the fertilized treatments (145 ± 25 colonies ml^{-1}) and was significantly higher (p ≤ 0.001) than maximum colony concentrations in the unfertilized mesocosms (11 ± 3 colonies ml^{-1}). The rate at which colonies increased in the fertilized treatments is shown in Table 1. Net colony increase in the lag phases were zero or slightly negative during both years and in all treatments. During the exponential phase, the rate of net colony increase averaged 0.31 d^{-1} in the fertilized treatments and 0.085 d^{-1} in the unamended mesocosms (Table 1). In the 2003 study, when estimates of biomass were calculated for colonial *P. pouchetii* cells, these cells accounted for 648 ± 167 µ C l^{-1} at their maximum densities in the fertilized mesocosms (Fig. 7b,c). This biomass represents over 100 times the biomass of single cells in these same mesocosms. Even in the unfertilized mesocosm, where the abundance of *Phaeocystis* colonies was low, the estimated biomass of colonial cells was ca. 14 times higher then the average biomass of single cells in these mesocosms (Fig. 7a).

Colony dispersion was not observed during 2002, when the experiment was terminated after 21 d (13 d after colonies were first detected), but was seen during the 2003 study beginning 24 to 27 d after initiation of the experiment. By the time the experiment was terminated on 3 April, colony concentrations averaged 66 ± 38 colonies ml^{-1} in the fertilized mesocosms. Negative net colony increase rates were calculated during this period (Table 1).

Microplankton

All phototrophic microplankton groups in addition to *Phaeocystis pouchetii* are shown in Figs. 5g–i, 6g–i & 7. The diatom community, including chain-forming and solitary species, showed similar patterns in all mesocosms, reaching similar abundances in fertilized (4.8 ± 2.8 × 10^3 cells ml^{-1}) and unfertilized (4.3 ± 4.1 × 10^3 cells ml^{-1}) treatments and during both years. In general, diatom communities were low at the start of both experiments, reached a maximum early in the experiments, and declined when Si was depleted. In 2002 the diatom community was dominated by a mixture of chain-forming taxa including *Skeletonema costatum*, *Leptocylindrus* sp., *Chaetoceros socialis* and *Thalassiosira* sp. Other diatoms that occurred to a lesser extent included *Nitzschia* sp. and *Coscinodiscus* sp. Virtually all the same species that were present in 2002 were also present in 2003, but in 2003 the diatom community was largely dominated by *Chaetoceros socialis*. In 2003, when biomass estimates were calculated, maximum averaged biomass estimate on 11 March (Day 12) in the unfertilized and nutrient amended treatments was 265 ± 34 µg C l^{-1} (Fig. 7d–f).

The abundance of phototrophic flagellate species >5 µm other than *Phaeocystis pouchetii* enumerated during the 2002 experiments are shown in Fig. 5g–i. All phototrophic flagellate species between 2 and 9 µm were enumerated during the 2003 experiments and are shown in Fig. 6g–i. The abundance of the photo-
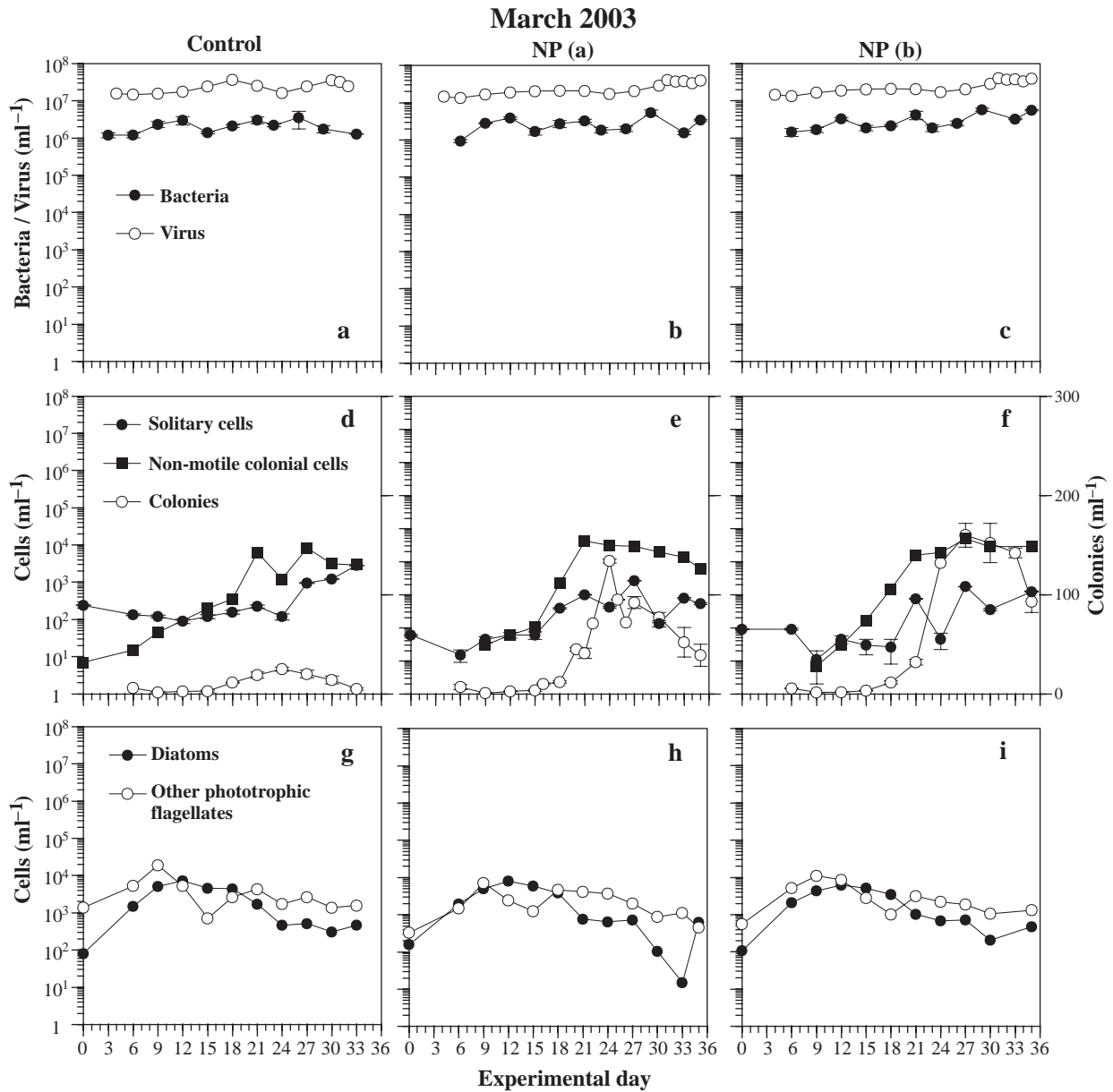
March 2003

Fig. 6. Bacterioplankton, virioplankton, *Phaeocystis pouchetii* flagellated cells, non-motile colonial cells, colonies, other photo-trophic flagellate cells and diatoms during 2003 study. (a–c) Abundance of bacterioplankton and virioplankton; (d–f) abundance of *P. pouchetii* flagellated cells, non-motile *Phaeocystis pouchetii* colonial cells and colonies; (g–i) mean ± SD abundance of other phototrophic flagellate cells and diatoms

trophic flagellates (whether only species >5 µm or those between 2 and 9 µm were counted) was not differentially affected by fertilization treatment and the subsequent colonial bloom of *P. pouchetii*. During 2002, microflagellate populations increased throughout the experiment, although absolute number of these species was relatively low (614 ± 418 ml⁻¹). The dominant larger phototrophic flagellate species included *Chrysochromulina* spp., *Apedinella* spp. and *Pyramimonas* spp. In 2003 a similar pattern to the 2002 study was observed, but in addition to a general increase in

larger phototrophic flagellate species, smaller species (2 to 3 µm) that were counted in these studies dominated the microflagellate community. These small phototrophic flagellates bloomed early in the experiment coincident with the diatom bloom, reaching maximum concentrations on the order of 10^4 cells ml⁻¹. Although these organisms dominated the abundance of this group, they were too small to be definitively identified by epifluoresence microscopy. In addition to the larger species that dominated the mesocosms during the 2002 studies, *Eutreptiella eupharyngea* and
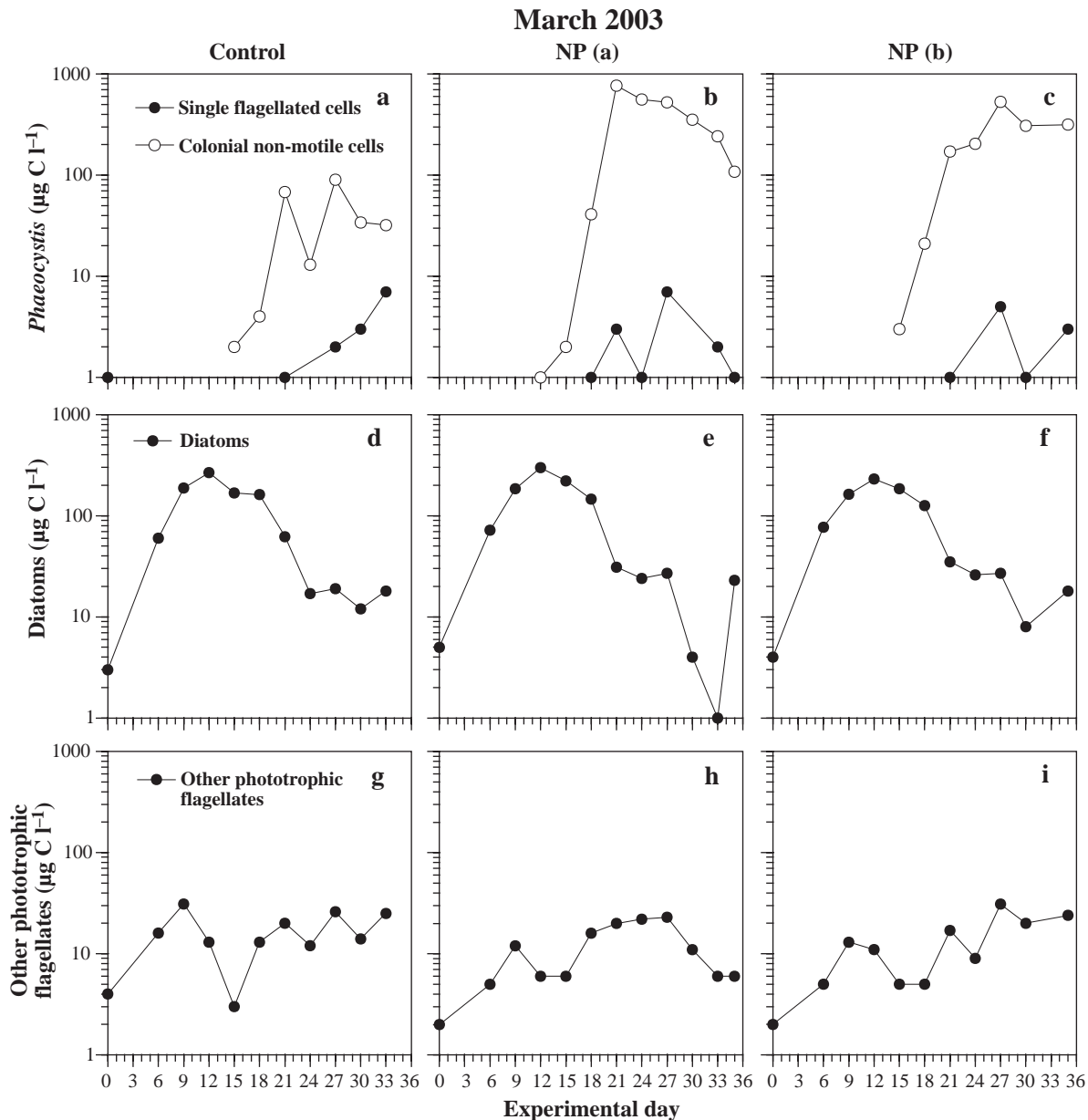
Fig. 7. Estimated biomass of *Phaeocystis pouchetii* flagellated cells, non-motile *P. pouchetii* colonial cells, other phototrophic flagellated cells and diatoms during 2003 study. (a–c) Estimated biomass of *P. pouchetii* flagellated cells and non-motile *P. pouchetii* colonial cells; (d–f) diatoms ; (g–i) other phototrophic flagellated cells

Pachysphaera pelagica were important members of the microflagellate community during the 2003 experiment. Estimates of phototrophic flagellate biomass are shown in Fig. 7g–i and reflect the relative importance of the larger flagellate species which, as was observed in 2002, generally increased throughout the study.

The abundance of heterotrophic flagellates (>5 µm) and heterotrophic nano-flagellates (<5 µm) are shown in Figs. 8a–c & 9a–c. During both years the abundance of these species generally increased over the course of the

experiments and was not differentially influenced by the nutrient fertilization and the resultant *Phaeocystis pouchetii* bloom. The average abundance of heterotrophic nanoflagellates increased from 358 ± 124 to 1736 ± 387 cells ml^{-1} in the 2002 and from 228 ± 293 to 3798 ± 1952 cells ml^{-1} in 2003. The average maximum biomass of the heterotrophic nanoflagellates and of the larger heterotrophic flagellates was roughly equivalent to each other (ca. 8.5 ± 3.8 µg C l^{-1}, Fig. 10a–c). The heterotrophic nanoflagellate community was dominated by small (2 to 3 µm) species which could not be identified
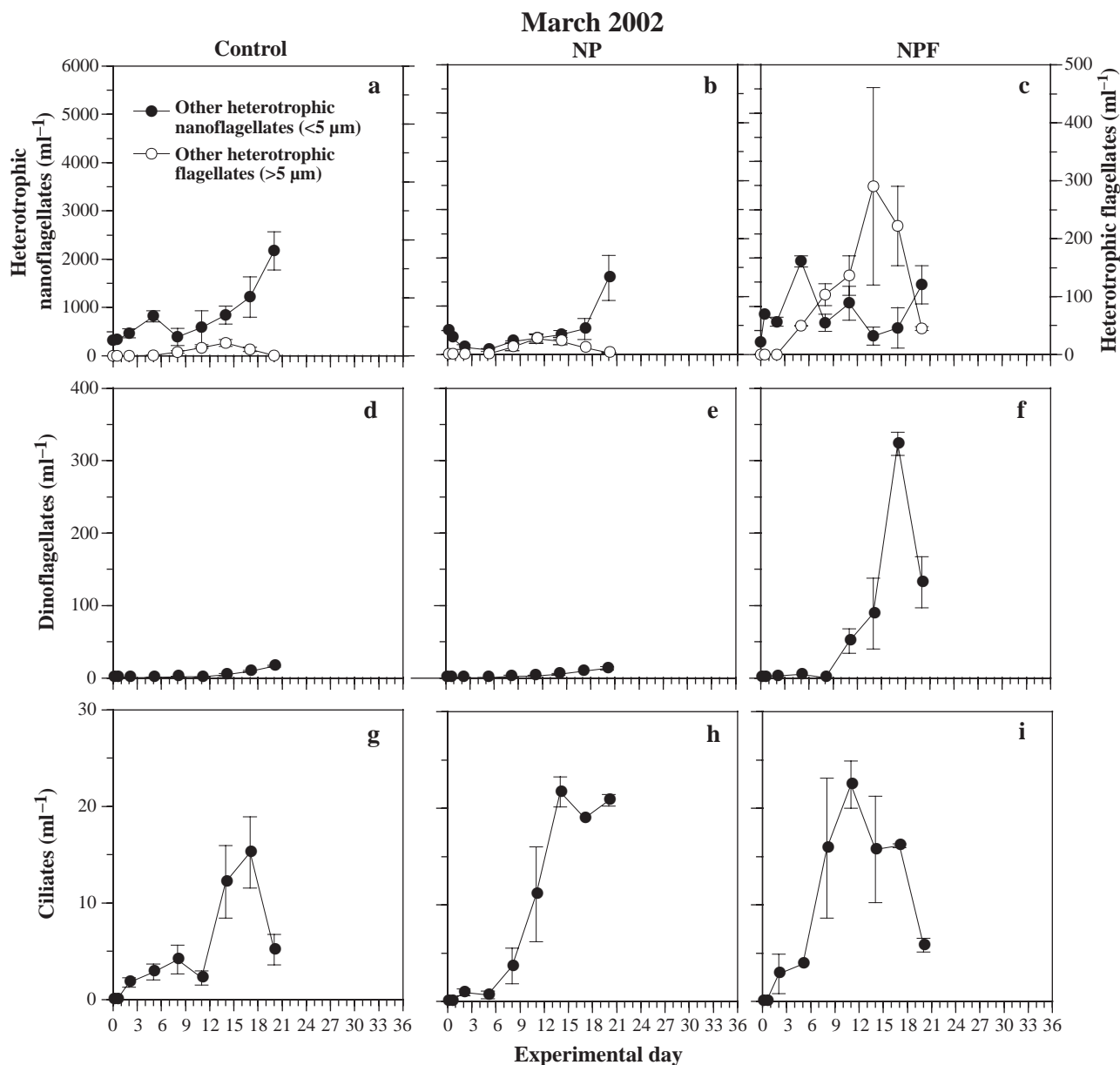
Fig. 8. Mean ± SD abundance of heterotrophic nanoflagellates (<5 µm), other heterotrophic flagellates (>5 µm, not including dinoflagellates), dinoflagellates and ciliates during 2002 study. (a–c) Abundance of heterotrophic nanoflagellates and other heterotrophic flagellates; (d–f) abundance of dinoflagellates; (g–i) abundance of ciliates

by epifluoresence microscopy. The larger heterotrophic flagellates were also largely dominated by unidentified species. During the 2002 experiment, *Rhizomonas* sp. was identified in the mesocosms and often observed associated with the chain-forming diatom *Leptocylindrus* sp., especially during the early part of the experiment when diatoms were relatively abundant.

Dinoflagellates were found at relatively low concentrations in all the mesocosms regardless of the year or fertilization treatment. Dinoflagellate abundance generally increased throughout the experiment (Figs. 8d–f

& 9d–f). The one exception to this generality was in NPF treatment during the 2002 experiment where dinoflagellate abundance reached 323 ± 16 cells ml^{-1} on 21 March near the end of the experiment (Fig. 8f). Dinoflagellate communities were dominated by *Protoperidinium bipes*, *P. pellucidum* and several species of *Gyrodinium*. The biomass of dinoflagellate communities, estimated based on the 2003 study, was relatively low and generally increased from 1 to 3 µg C l^{-1} at the start of the experiment to 10–30 µg C l^{-1} near the end of the study (Fig. 10d–f).

March 2003

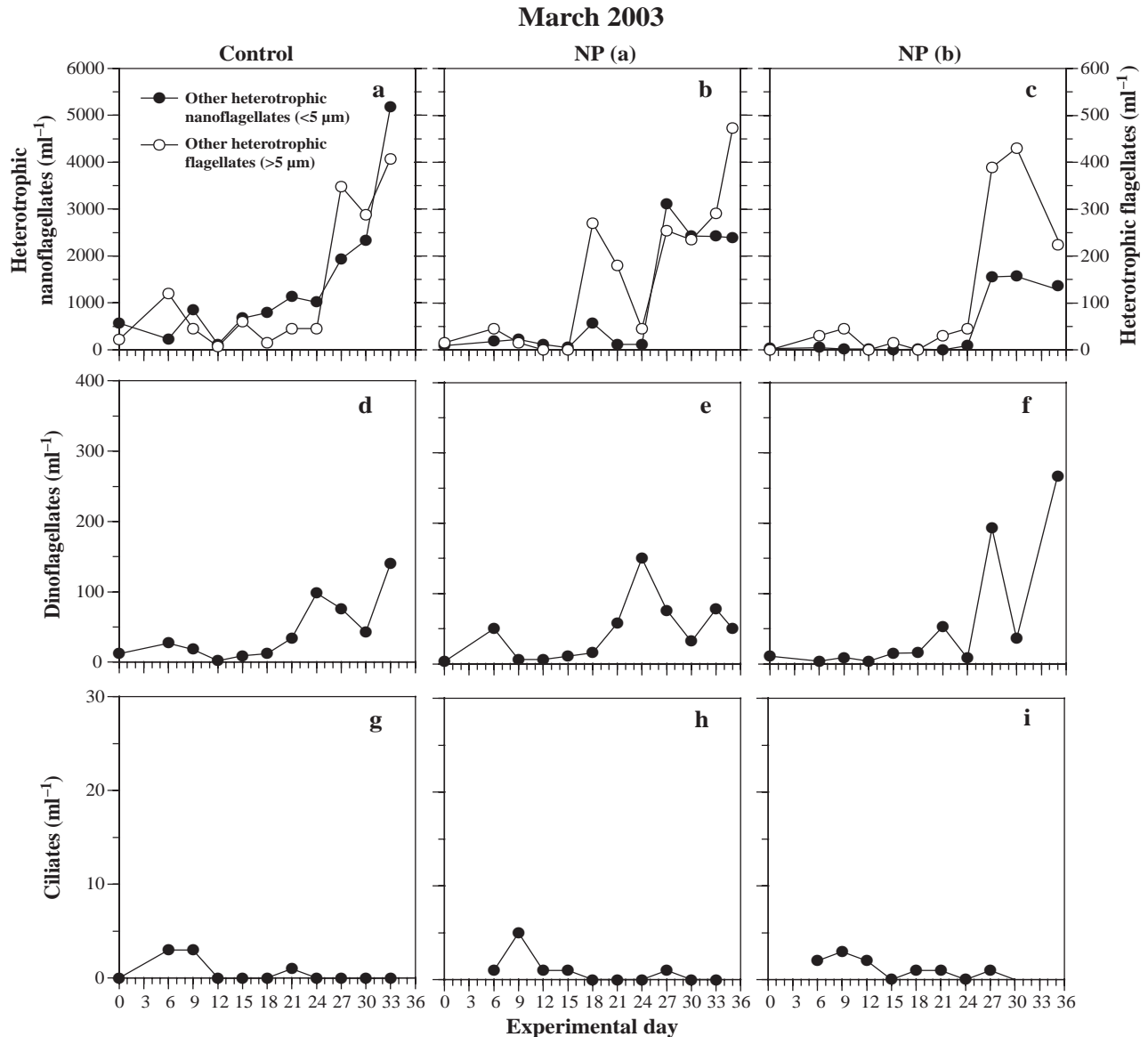Fig. 9. Abundance of heterotrophic nanoflagellates (<5 µm), other heterotrophic flagellates (>5 µm, not including dinoflagellates), dinoflagellates and ciliates during 2003 study. (a–c) Abundance of heterotrophic nanoflagellates and other heterotrophic flagellates; (d–f) abundance of dinoflagellates; (g–i) abundance of ciliates

Mesozooplankton

The overall concentrations of mesozooplankton were low, similar in both years, and were apparently not impacted by the fertilization treatment and the ensuing colonial *Phaeocystis pouchetii* bloom (Fig. 11). During the 2002 experiment the initial mesozooplankton community was dominated by barnacle nauplii (*Balanus* sp.) in all mesocosms. Some of these barnacle larvae were found in metamorphic forms during the experiment and then disappeared from the water column. At the termination of the experiment, the inside walls of the mesocosms were examined for settled barnacles,

however, none were found. During the 2003 experiment barnacle larvae were not present and copepodite stages of calanoid copepods dominated the mesozooplankton biomass (Fig. 11b). The small numbers of feeding mesozooplankton other than these 2 categories were mostly composed of molluscan meroplankton and rotifers. The copepods were dominated by CI–CIV stages of *Calanus finmarchicus*, with a small number of the calanoid copepod *Paracalanus parvus*, the cyclopoid copepod *Oithona* spp. and a small number of benthic harpacticoid copepods. The observation that the mesozooplankton did not respond by increased biomass or nauplii production in the presence
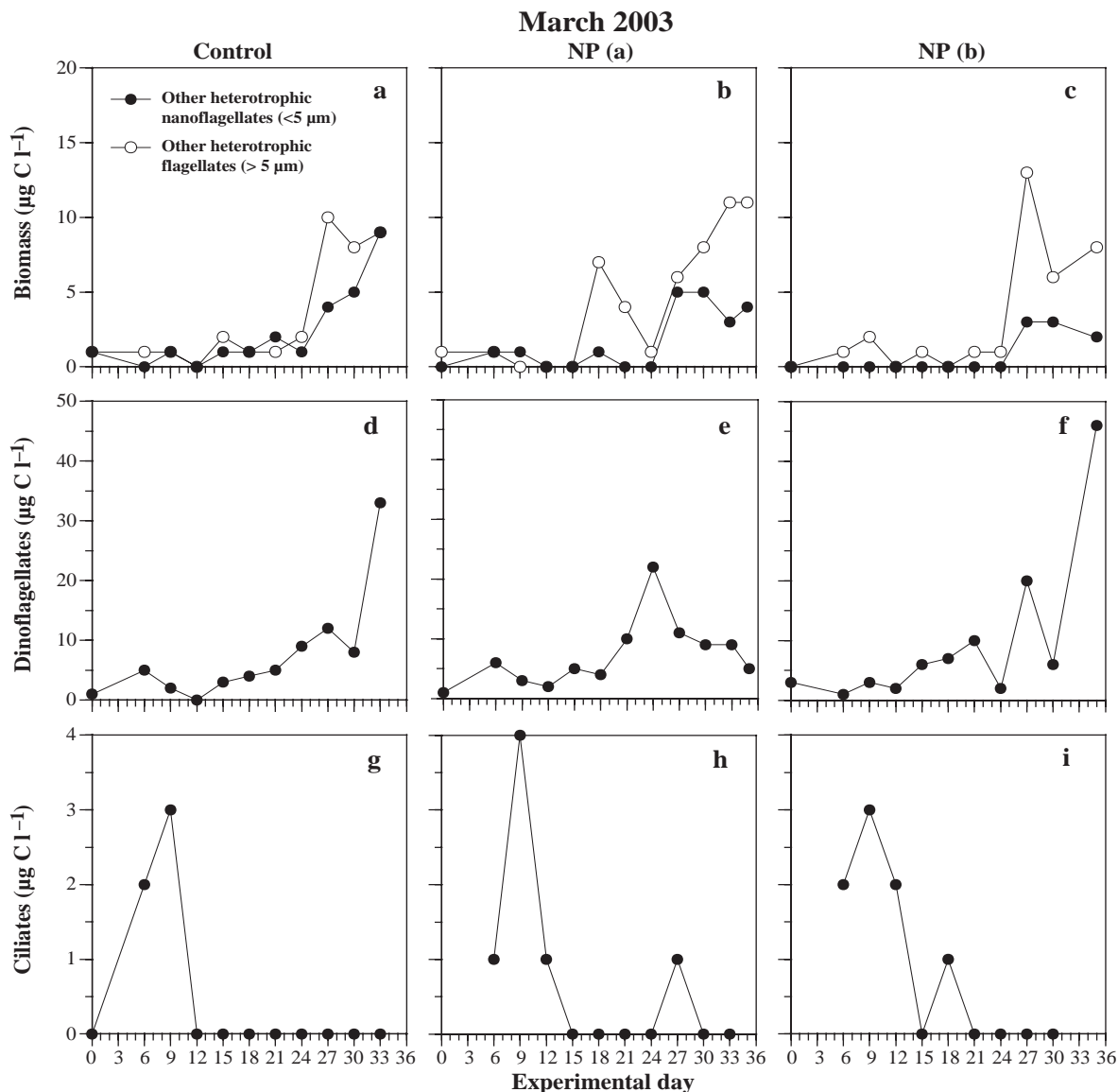
Fig. 10. Estimated biomass of heterotrophic nanoflagellates (<5 µm), heterotrophic flagellates (>5 µm, not including dino-flagellates), dinoflagellates, and ciliates during 2003 study. (a–c) Biomass of heterotrophic nanoflagellates and other hetero-trophic flagellates; (d–f) abundance of dinoflagellates; (g–i) abundance of ciliates

of a significant algal (*Phaeocystis pouchetii*) bloom supports the hypothesis that in nature *Phaeocystis* spp. (single cells or colonies) are not readily grazed and do not contribute substantially to the biomass of higher trophic levels.

Phaeocystis pouchetii colony formation studies

During the 2002 experiment, 3 incubation studies utilizing single cells from the NPF mesocosm were per-formed in order to estimate the formation rates of new small colonies from *Phaeocystis pouchetii* solitary cells (Fig. 12). The 3 studies began on 10, 13 and 16 March,

and each experiment lasted for 7 d. In all experiments new colonies increased approximately linearly over time in the well plates, with significantly ($p < 0.05$) more colonies appearing in the wells with added dia-tomaceous earth than in controls. Mean rates of new colony appearance in the 3 experiments, calculated from linear regression of the data in Fig. 12, were 1.50, 1.85 and 1.26 colonies d^{-1} without diatomaceous earth, and 2.77, 2.69 and 2.62 colonies d^{-1} in the 3 colonies with diatomaceous earth (Table 1). Correlation coeffi-cients for the six linear regressions ranged from $r^2 = 0.96$ to 0.99, and the differences between slopes of con-trols and those with added diatomaceous earth were significant ($p < 0.05$).
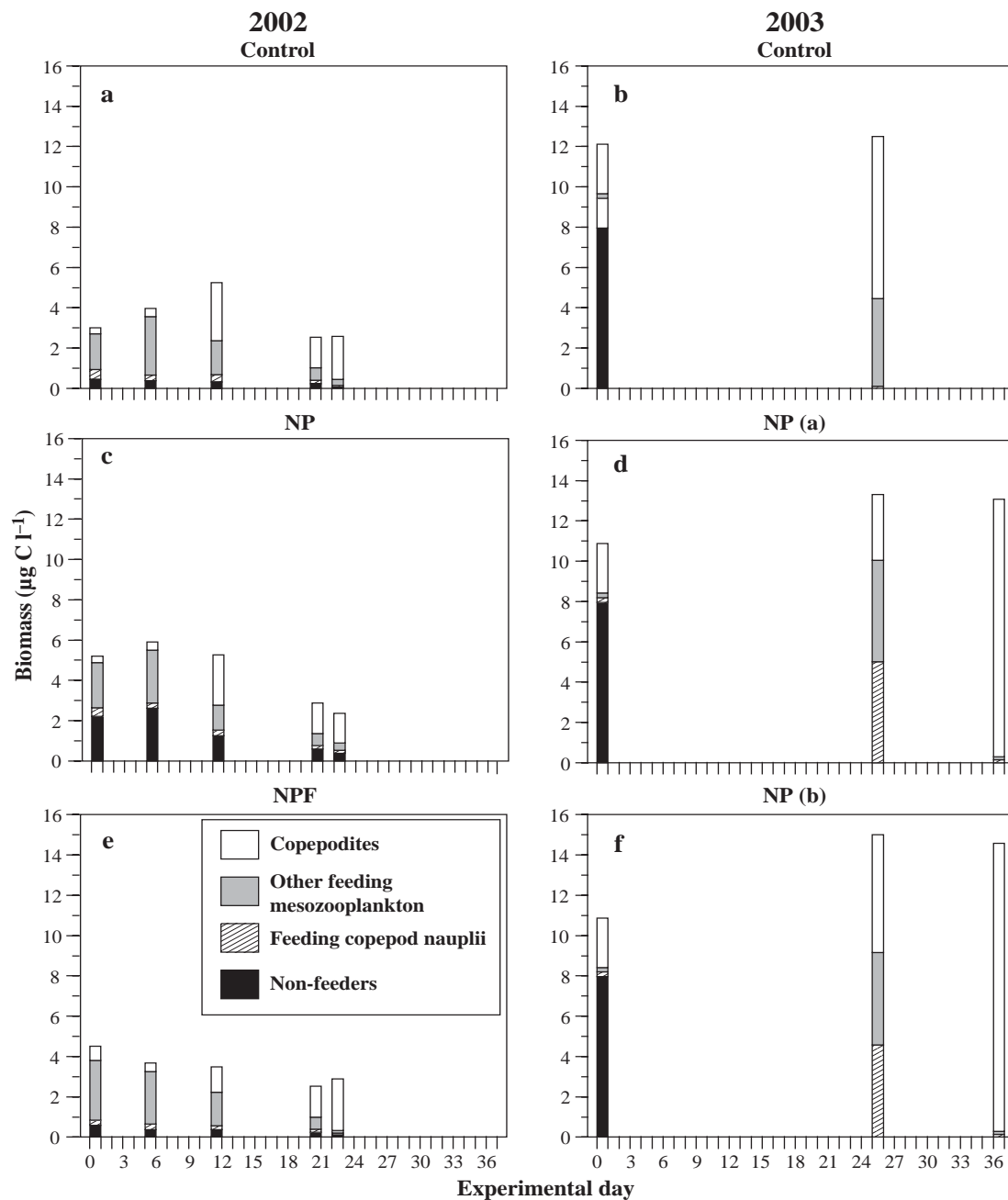
Fig. 11. Estimated biomass of mesozooplankton in (a,c,e) 2002 and (b, d, f) 2003 mesocosm studies. (a,b) Unfertilized control mesocosms; (c–f) fertilized mesocosms. In 2002, in addition to fertilization, 1 mesocosm (NPF) was further amended with *Phaeocystis pouchetii* solitary cells. Estimated biomass was comprised of copepodites (CI adults) consisting mainly of *Calanus finmarchicus*, other feeding mesozooplankton, feeding copepod nauplii consisting mainly of *C. finmarchicus* and non-feeding mesozooplankton consisting primarily of copepod eggs and *Calanus* sp. stages NI to II. During 2002 study, *Balanus* sp. larvae comprised majority of 'other feeding mesozooplankton' category

DISCUSSION

In order to investigate how the base of the planktonic food web (virus to mesozooplankton) responded to a colonial bloom of the prymnesiophyte alga *Phaeocystis pouchetii*, we compared the biomass development in fjord water enclosed *in situ* with either no further treat-

ment (controls) or added nitrate and phospate (NP) and cultured solitary cells of *P. pouchetii* (NPF). We compare results from 2 independent mesocosm studies conducted in the early spring (March), but in 2 successive years to allow for a robust interpretation of the gross effects of simple manipulated environmental variables on subsequent complex general patterns in the plankton devel-

opment. Mesocosms provide an ideal tool for studying plankton communities because they (1) simulate relatively realistic and complex communities and community dynamics (at least compared to laboratory scale systems), (2) allow manipulation of specific factors, and (3) allow the same water mass to be sampled over time (Duarte et al. 1997). Using this model system we also investigated whether the addition of *P. pouchetii* solitary cells that were in late log growth phase would accelerate the induction of colony formation, quantified *P. pouchetii* colony formation rates under realistic conditions, and explored how a typical high latitude marine food web responded to *P. pouchetii* colony domination.
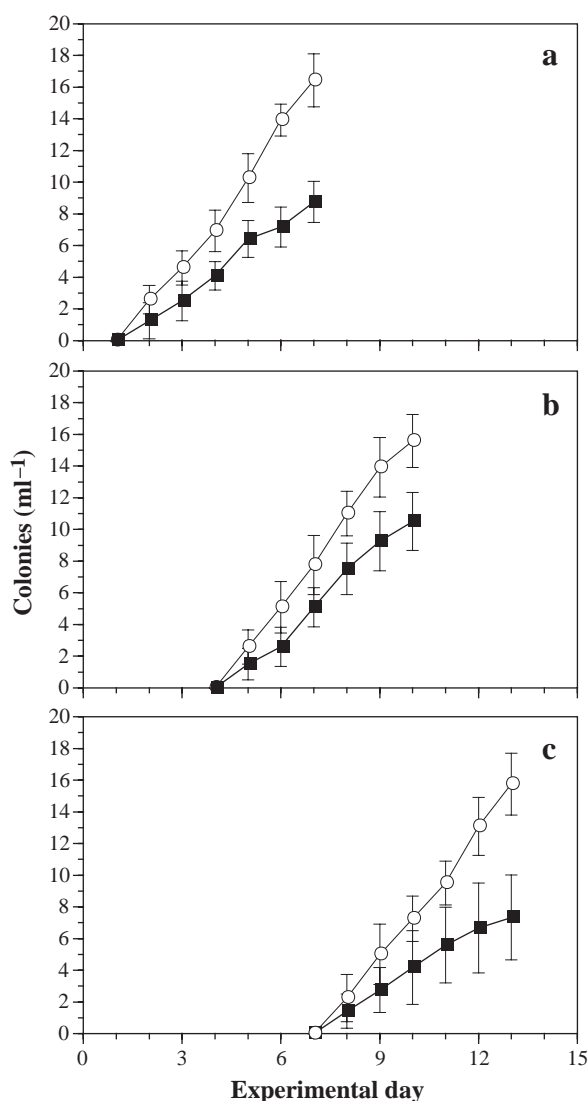

Fig. 12. Formation of *Phaeocystis pouchetti* colonies in water collected from the NPF mesocosm (2002) in (○) presence and (■) absence of diatomaceous earth. Each experiment was monitored for 7 d with water removed from mesocosm on (a) 1st day of study (10 March 2002), (b) 3rd day of study (13 March 2002) and (c) 7th day of study (16 March 2002)

Simulating colonial blooms of *Phaeocystis pouchetii*

Blooms of *Phaeocystis pouchetii* in northern latitudes have historically been reported as those of colonies, simultaneously with or just after spring blooms of chain-forming diatoms such as *S. costatum*, *Leptocylindrus* spp., and *Chaetoceros* spp. (Heimdal 1974, Eilertsen et al. 1981, Erga 1989). In Norwegian fjords, colonies of *P. pouchetii* are typically abundant between February and May, but may also occur in high numbers during the fall (Eilertsen et al. 1981). *P. pouchetii* often dominates over diatoms in waters with low silicate concentrations (<2 μM) and surplus nitrate (>5 μM) and phosphate (>0.2 μM) concentrations (Egge 1993, 1998, Egge & Jacobsen 1997). In addition, low temperature ($<10°C$) and low incident irradiance (<20 mol photons m^{-2} d^{-1}) appear to favor *P. pouchetii* over diatoms (Ploug et al 1999a,b, Jacobsen 2000). However, in nature silicate availability is not a unique predictor of *P. pouchetii* versus diatom dominance, at least not in Norwegian fjords, coastal waters and the Barents Sea. A fundamental mechanistic understanding of all the factors that contribute towards *P. pouchetii* blooms thus remains elusive (Wassmann et al. 2000, Reigstad et al. 2002, Larsen et al. 2004, Schoemann et al. 2005, Nejstgaard et al. 2006).

As expected from previous studies, the addition of nitrate and phosphate without the addition of silicate resulted in an initial small diatom bloom which deteriorated when silicate declined below 1 μM, followed by a colonial bloom of *Phaeocystis pouchetii* (600 to 800 μg C l^{-1}). This pattern was consistent in both the 2002 and 2003 experiments. Thus, although there remains a considerable amount that is not known about the factors leading to the occurrence of colonial *Phaeocystis* spp. blooms in nature, blooms of *P. pouchetii* can be reliably simulated in Western Norwegian waters using nutrient manipulated mesocosm systems.

Phaeocystis pouchetii colony formation

The concentrations of solitary *Phaeocystis pouchetii* cells in the control and nutrient-amended mesocosms were essentially the same throughout both studies. Thus, the presence of natural concentrations of solitary *P. pouchetii* cells was by itself insufficient to explain the occurrence of colonial blooms. Wassmann et al. (2005) have recently emphasized that *P. pouchetii* 'events' do not have to occur as colony blooms: in north Norwegian fjords and elsewhere in the NE North Atlantic single cells may vastly outnumber co-occurring colony cells. In the present study, solitary cells increased marginally in abundance along with colonies

in the nutrient-amended mesocosms, but the vast majority of total cells at the peak of the *P. pouchetii* bloom were contained within the colony matrix. In the absence of nutrient loading (control mesocosms), colonies were undetectable in 2002 and very low in 2003.

Adding cultured *Phaeocystis pouchetii* solitary cells, derived from the same waters and sufficient to nearly triple the natural concentration, did not result in differences in peak levels of colonies. Why? Several possible explanations exist: cultured cells did not survive, they were (selectively) eaten (further discussed in next subsection), or they could not form colonies *in situ*. An intriguing possibility derives from the colony formation incubation experiments. Initial concentrations of *P. pouchetii* solitary cells in the 3 colony initiation experiments in 2002 were 845, 750 and 657 cells ml^{-1}, respectively. Comparing these to the production rates of new colonies (Fig. 12), it is apparent that roughly only 1% of the available solitary cells are required to account for the observed appearance of new colonies. Similar conclusions were derived from cultures of a *Phaeocystis* species (then called *P. pouchetii*, but most likely *P. globosa*) from the North Sea (Veldhuis & Admiraal 1987). So, doubling or tripling the concentrations of solitary cells in the mesocosms at the time of filling might be inconsequential to subsequent colony abundance.

Mean net growth rates in numbers of colonies were 0.27 to 0.33 d^{-1} during the colony growth phase in all 4 NP-fertilized mesocosms (exponential phase, Table 1). These rates are not strictly comparable to those measured in the incubation experiments because the mesocosms include both formation of new colonies from solitary cells and division/budding of new colonies from old ones (Whipple et al. 2005), as well as potential loss factors e.g. zooplankton predation, lysis and sedimentation. There was no visual evidence in the incubation experiments of colony division/budding. However, given the similar concentrations of solitary cells in the mesocosms and in the experimental incubations, it is tempting to speculate that the formation rates of new colonies measured in the incubations may accurately represent the same processes in colonies in the mesocosms.

To our knowledge, it has previously not been possible to induce colony formation in traditional laboratory cultures of *Phaeocystis pouchetii*. Furthermore, many haptophytes do not reproduce conspicuous features such as toxicity even when cultured directly from toxic blooms *in situ*, and this may be a species-specific, or even strain-specific feature, with a complex and still largely unknown coupling to *in situ* growth conditions (e.g. Nielsen et al. 1990, Edvardsen & Paasche 1998). However, here it was possible to induce colony forma-

tion from single cells of *P. pouchetii* in the laboratory utilizing close to natural material and water from the mesocosms. This strongly supports the notion that traditional laboratory cultures may lack critical mechanisms or factors controlling growth and potential production of metabolites with key functions in natural systems. Thus, future studies should aim to unravel the controlling mechanisms, and results based on traditional laboratory cultures of *Phaeocystis* spp. and other haptophytes should be treated with caution when used for interpretation and modeling of complex natural systems.

Trophic transfer of *Phaeocystis pouchetii* biomass

Despite the large production of *Phaeocystis pouchetii* colonies, little of it appeared to be transferred to zooplankton biomass or to fuel virus production. Several components of the plankton communities did not differ saliently among mesocosms, i.e. concentrations of diatoms, bacteria and viruses, were similar between all 3 mesocosms each year, and relatively similar between years. Neither did the overall low biomass of ciliates and mesozooplankton indicate that they were significantly stimulated by the blooms of *P. pouchetii* in the fertilized mesocosms.

Only heterotrophic flagellates >5 μm and heterotrophic dinoflagellates were substantially greater in the NPF mesocosm in 2002. Perhaps the added *Phaeocystis pouchetii* solitary cells were consumed by microzooplankton? According to this notion, the initial addition of cultivated *P. pouchetii* solitary cells may have stimulated early development of several heterotrophic species, e.g. *Gyrodinium* spp., *Rhizomonas* sp. and oligotrich ciliates, that was only observed in the NPF mesocosm. In microzooplankton grazing experiments associated with these studies, heterotrophs were observed actively grazing on (also cultivated) DTAF-labeled *P. pouchetii* solitary cells (see Fu et al. 2003 for method and Anderson et al. 2002 for results). These results are also supported by 24 h dilution experiments using water from the NPF mesocosm, which showed daily community grazing rates of 33 to 95% of the standing stock of <8 μm cells early in the study (11 to 15 March), but only 4% later on 19 to 20 March (Nejstgaard et al. unpubl. data). These observations support the hypothesis that the early seeding of cultured *P. pouchetii* solitary cells did not lead to more colonies later because only a small fraction of them formed colonies and an unknown (but perhaps large) fraction was consumed by protist grazers.

The mesozooplankton biomass remained about 1 order of magnitude lower than previously recorded in comparable mesocosm experiments at this site (e.g.

Levasseur et al. 1996, Nejstgaard et al. 2001, Svensen et al. 2002). This observation was surprising given the development of strong differences in prey availability between the treatments. However, this supports the notion that *Phaeocystis pouchetii* may be a suboptimal food that does not support strong zooplankton production (Cotonnec et al. 2001, Tang et al. 2001, Klein Breteler & Koski 2003, but see Weisse et al. 1994 and Turner et al. 2002 for exceptions). The lack of trophic transfer to the zooplankton could be due to one, or several, of the defense mechanisms against predation suggested for *Phaeocystis* spp. The defense mechanisms include (1) formation of colonies to escape vigorous feeding by microzooplankton, as showed for cultured *P. globosa* (Jakobsen & Tang 2002, Tang 2003, Long 2005), (2) reduced palatability depending on the physiological state, exudates, species or even type of single cell (Estep et al. 1990, Dutz et al. 2005, Long 2005, Dutz & Koski 2006, Nejsgaard et al. 2006), (3) other factors (see review by Weisse et al. 1994).

On the other hand, due to the low abundance of mesozooplankton, they are not likely to have produced any substantial grazing pressure on the nano- or microplankton in any of the mesocosms, as was confirmed in the mesozooplankton feeding experiments coupled to the dilution experiments on 11, 15 and 19 March (Nejstgaard et al. unpubl. data). Thus, mesozooplankton could not be responsible for any of the differences in nano- and microplankton development between the mesocosms, since the composition and biomass of the mesozooplankton was almost identical between all 3 mesocosms in each year, and similarly low in both years. Moreover, even a 10-fold increase in mesozooplankton concentrations greater than those recorded here, would still show (at best) a small direct grazing pressure on the largest microzooplankton, such as ciliates larger than 20 to 30 μm (Nejstgaard et al. 1997). In addition, a large fraction of the mesozooplankton was made up of meroplankton, including barnacle larvae, which are generally less efficient grazers than copepods of the same size (Hansen et al. 1997, Desai & Anil 2004). Thus, the *P. pouchetii* blooms did not seem to be significantly grazed or support any significant production of mesozooplankton.

Bacteria and viruses

We did not observe strong variability in bacterial abundances between the fertilized and non-fertilized mesocosms, as often observed elsewhere (Cottingham et al. 1997, Joint et al. 2002, Havskum et al. 2003). One interpretation is that bacterial populations were not limited by inorganic nutrients and that increasing bacterial abundance was either the result of growth stimu-

lated by increased primary production (via release of labile DOC) or a decrease in bacterial grazing. One caveat with the former explanation is that the rate of increase in bacteria cells was similar in all 3 mesocosms, even though productivity had to be much greater in the nutrient-amended mesocosms. In other studies associated with these experiments, bacteria productivity and biomass increased in response to fertilization, although bacterial abundance did not vary among treatments (Frischer et al. 2005 and unpubl. data) Nevertheless, bacterial growth estimated from total cell abundance exceeded combined bacterial mortality, independent of external nutrient loading, such that the net increases in abundance were similar in the 3 mesocosms. In modeled systems, Thingstad and colleagues have thoroughly evaluated the balance of these factors (Thingstad & Lignell 1997, Thingstad et al. 1999). *Phaeocystis* spp. specific viruses have previously been shown to terminate blooms of *P. pouchetii* in mesocosms at this location (Jacobsen 2000), and are suspected to terminate such blooms in the nearby Raunefjord (Larsen et al. 2004). Although there was evidence for the presence of *Phaeocystis* spp.-specific viruses during these studies (Jacobsen et al. 2005 and unpubl. data), the co-variation between bacterial abundance and virus abundance observed in these studies suggest that virus communities were dominated by bacteriophages and that viral lysis did not contribute to re-proportioning biomass associated with *P. pouchetii* during the course of these experiments.

Conclusions and suggestions for future research towards understanding complexity of marine planktonic systems

The preceding discussion outlines several explanations for the observed patterns and highlights where observations revealed discrepancies with theory. Several of these are under investigation in similar mesocosm or more controlled laboratory studies including: (1) What other factors influence the transition from solitary cell to colony cell phase of the *Phaeocystis pouchetii* life cycle? Some possibilities include the role of sediments and potential stimulation by the cell surfaces of co-occurring diatoms. (2) What is the role of emigration of cells out of colonies during the demise of colony blooms. In this study, microscopy confirmed that, on the last sample days, cells within colonies had become motile and were initiating exodus, as observed in *Phaeocystis* spp. elsewhere (Parke et al. 1971, Verity et al. 1988b). (3) Does bacterial community composition change in response to *Phaeocystis* spp.? Perhaps the lack of differences in treatment effects on bacteria abundance masked activity and diversity responses.

(4) What is the role of viral lysis, in colony bloom dynamics and termination? The virus data in Figs. 5 & 6 probably reflects bacteria-specific viruses because of the similar temporal patterns of bacteria and viruses, and the numerical dominance of bacterial hosts compared to potential algal hosts. Host/virus systems are known for *P. pouchetii* (Jacobsen et al. 1996, Bratbak et al. 1998a,b, Brussaard et al. 1999), but the viruses do not seem to affect the colony life cycle phase. Incubation experiments during the present study showed no evidence of viral lysis of colonies even though *P. pouchetii*-specific viruses were present in the mesocosms (Jacobsen et al. unpubl. data). (5) Does chemical communication between life cycle stages and among life cycles stages and zooplankton influence phytoplankton species succession? (6) What are the quantitative roles of various proto- and mesozooplankton in these processes?

Complexity theory postulates that inherent in complex systems are self-organizational tendencies that structure complex systems. Entire ecosystems change in conjunction with shifts in the life cycle and dominance of *Phaeocystis* spp., and there are examples of direct biochemical communication among trophic levels in response to *Phaeocystis* spp. dynamics. From the perspective of complexity theory, the biology of *Phaeocystis* spp. involving a life-history strategy that includes small (3 to 9 µm) single cells and large (up to mm) sized colonies may be considered to represent such a self-organization property of high latitude planktonic ecosystems. The complexity apparent in the magnitude and nature of all of these interactions is large, and it remains difficult to resolve clear causality in diverse systems, even under the physically conscribed conditions created in these studies. The answers to the specific question raised here concerning the trophic effect of stimulating a colonial bloom of *P. pouchetii* by fertilization with nitrate and silicate, remain ambiguous. Certainly, the fundamental prediction that the addition of N and P to these systems would lead to significantly higher phytoplankton biomass and a colonial *P. pouchetii* bloom was supported. Furthermore, the *P. pouchetii* bloom appeared to have structured the planktonic system by limiting secondary production to levels equivalent to non-bloom conditions. However, the hypothesis that this bloom would result in a restructuring of the planktonic community was not supported. Except for the obvious differences in the abundance of *P. pouchetii*, other system components (viruses, bacteria, nano-, microplankton and mesozooplankton) appeared to be essentially unaffected by this system alternation. Apparently, *P. pouchetii* is capable of limiting system complexity by sequestering nutrients and energy into forms not easily accessible to planktonic grazing and regenerating communities that comprise northern latitude marine systems.

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