The following supplement accompanies the article

Molecular gut content analysis demonstrates that *Calanus* grazing on *Phaeocystis pouchetii* and *Skeletonema marinoi* is sensitive to bloom phase but not prey density

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SUPPLEMENTAL MATERIALS AND METHODS

Microscopic analysis of microeukaryote diversity

The seawater samples were stained with primuline (Direct Yellow 59, Sigma-Aldrich Co), fixed with a solution containing 3.6% (v/v) glutaraldehyde and 10% (v/v) glycerol (final concentrations), and gently filtered onto black-stained 25 mm diameter 0.4-µm pore-size Nucleopore filters. Filters were mounted on slides and analyzed by epifluorescence microscopy. The method is a modification from Grebecki (1962), Hobbie et al. (1977) and Caron (1983) with the glycerol added to reduce the damage of particularly small delicate protists during filtration as described in Sazhin et al. (2007).

FlowCAM analysis of mesocosm seawater

The FlowCAM was run in autoimage-mode, using 4x magnification to analyze particles ranging between 15 and 1000 µm. The water samples were kept in dim light at 4°C until analyzed, within 4 h after sampling. Each sample was run for ca. 30 min, corresponding to 5.7 ml of analyzed volume. The relevant context capture property chosen to do the analysis was distance to nearest neighbor = 50 µm. All the image collages were manually post-analyzed in order to identify the particles in question. Particle sizes were estimated from area-based diameter determined by the FlowCAM VISP 2.2 software following Jakobsen & Carstensen (2011). Colonies of *P. pouchetii* are composite clusters of cells spaced in colonies of various sizes. When the colonies are forming dense blooms, the FlowCAM cannot distinguish whether a cluster is a part of colony or if a cluster is a part of a neighbouring colony. The FlowCAM therefore captures clusters of colonies. *S. marinoi* and *P. pouchetii* are colonial structures of multiple cells, and we developed scaling relationships between colony area and cell number by manually counting the number of cells in either a diatom chain or a *P. pouchetii* colony ($n > 100$) and related the cell number to particle area. We further developed scaling relationships between cluster areas by manually counting the number of cells in *P. pouchetii* clusters ($n > 100$). The same procedure was used for estimating *S. marinoi* cells, where we manually counted the number of cells per chain. The cell numbers were related to either cluster area (*P. pouchetii* clusters) or chain length (*S. marinoi)*, and the relationships were then used to estimate the total number of *P. pouchetii* or *S. marinoi* cells in colonies or chains, respectively, by sample. Cell concentrations were estimated by correcting for the analyzed volume. The resulting regression equation used for estimation the number of *P. pouchetii* cells in colonies and colony fragments was $\text{Cells} = \text{ABD}^{1.834} * 0.028$ ($R^2 = 0.83$), where ABD (area base diameter) is an output parameter from FlowCam analysis.

Cloning and sequencing

Phytoplankton cultures were obtained from the University of Bergen culture collection maintained by the Department of Biology. Cells in one milliliter of an exponential-phase culture of *Phaeocystis pouchetii* (Hariot) Lagerheim strain AJ01 were collected by centrifugation and resuspended in sterile water. Five adult female *Calanus* copepods were collected from Raunefjorden and used to obtain a local full-length *Calanus* SSU rRNA gene sequence. Amplification of the full-length SSU ribosomal RNA gene sequence from *P. pouchetii and Calanus* was performed with primers UnivF-15 and UnivR-1765S (Table 1 in main article) modified from Frischer et al. (2000). Fifty-microliter reactions consisted of 1x HotStar Taq master mix (QIAGEN, Düsseldorf, Germany), 500 nM each primer, 1x Coralload buffer (QIAGEN) and 5 µl of cultured cell suspension. Amplification was performed with a C-1000 thermocycler (Bio-Rad) at 95°C for 15 min, 30 cycles of 94°C for 30 s, 50°C for 45 s and 72°C for 2 min, followed by a final elongation at 72°C for 10 min. Agarose gel electrophoresis demonstrated single products of approx 1750 bp in length. DNA was extracted from excised gel slices using an Illustra GFX PCR gel band purification kit (GE Healthcare, Piscataway, New Jersey). The eluted PCR products were cloned using the StrataClone pSC-A-Amp/Kan kit (Agilent Technologies, Santa Clara, California) according to the manufacturer's protocol for blue/white selection of transformants. Transformants were further checked for correct insert size, and those clones with insert sizes matching that of the target sequence (approx. 1750 bp + approx. 250 bp vector sequence) were sequenced in both directions using the BigDye v3.1 Cycle Sequencing kit (Life Technologies Ltd, Paisley, UK). Ten microliter sequencing reactions contained 1µl BigDye sequencing mix, 1 µl buffer, 320 nM primer M13F or M13R and 20–50 ng template according to kit instructions. Primer walking using primers designed from the initial clone sequences was necessary to close the gap between forward and reverse sequencing directions. Sanger sequencing was performed in-house at the University of Bergen core sequencing facility. Sequence chromatograms were manually edited to remove vector sequence using FinchTV v1.X (Geospiza, Inc., Seattle, Washington) and aligned, and the partial SSU rRNA gene sequences for *P. pouchetii* and *S. marinoi* were submitted to GenBank (accession numbers KR091066 and KR091067, respectively).

Optimization of 5'-nuclease (TaqMan) assay

A cloned *P. pouchetii* SSU rRNA gene sequence (accession number) obtained from *P. pouchetii* strain AJ01 culture from the culture collection at University of Bergen, Department of Biology, was aligned with full-length *P. pouchetii* (X77475.1, AF182114 and AJ278036) sequences present in GenBank using ClustalW2 (Larkin et al. 2007). A cloned *Skeletonema marinoi* SSU rRNA gene sequence isolated from the same culture collection was aligned with 145 *Skeletonema* spp. sequences found in the Silva SSU database (Pruesse et al. 2007) using Clustal W2. Primer sequences were checked against this alignment to ensure high homology. Stretches of high homology in the *P. pouchetii* and *Skeletonema* alignments were further examined for promising primer (*P. pouchetii*) and probe (*P. pouchetii and Skeletonema*) candidates.

Specificity testing

Purified plasmid containing cloned *P. pouchetii* or *S. marinoi* SSU rRNA genes were used as positive controls for assay optimization. Temperature gradient qPCR was performed to determine optimal annealing temperature for elongation, which was found to be 56°C for *P. pouchetii* and 55.8°C for *S. marinoi*. Optimal primer and probe concentrations were determined to be 900 nM (probe) and 250 nM (primer) for *P. pouchetii*, while 250 nM was found to be optimal for both primers and probe for the *S. marinoi* assay. Specificity of the *P. pouchetii* TaqMan assay was tested using plasmids containing cloned *P. pouchetii* SSU rRNA gene sequence (see above) and with genomic DNA (gDNA) from *P. pouchetii*, an adult female *Calanus* copepod, the diatom *Chaetoceros* sp., the appendicularian *Oikopleura dioica*, and the cryptophytes *Rhodomonas* sp. and *Rhinomonas* sp.. Specificity of the *S. marinoi* TaqMan assay was tested using a plasmid with cloned

S. marinoi SSU rRNA gene fragment and with genomic DNA from *O. dioica*, the tunicate *Ciona intestinalis*, the blue mussel *Mytilus edulis*, an adult female *Calanus* sp. copepod, the haptophytes *P. pouchetii*, *Isochrysis* sp. and *Emiliania huxleyi*, the cryptophytes *Rhodomonas* sp. and *Rhinomonas* sp., the diatoms *Chaetoceros* sp., *Ditylum brightwellii* and *Thalassionema nitzchioidies*, the chlorophytes *Pyramimonas* sp. and *Tetraselmis* sp. Results of specificity tests for *P. pouchetii* and *S. marinoi* TaqMan assays demonstrated that the assays were specific for the target phytoplankton only (data not shown).

Sensitivity testing

The sensitivity of TaqMan assays was determined using 10-fold serial dilutions of plasmids containing cloned target genes from *P. pouchetii* or *S. marinoi*. For the *P. pouchetii* TaqMan assay, sensivity was determined to be linear from 10^2–10^8 plasmid copies (regression: $y(x) = -3.41x + 41.39$, $R^2 = 0.999$, PCR efficiency = 96.45%), while the linear range for the *S. marinoi* TaqMan assay was determined to be 10^2–10^7 plasmid copies (regression: $y(x) = -3.409x + 39.19$, $R^2 = 0.996$, PCR efficiency = 95.50%).

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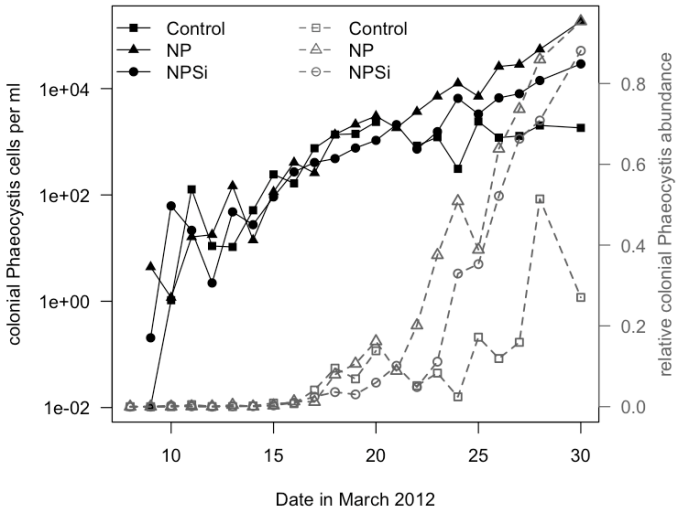
A



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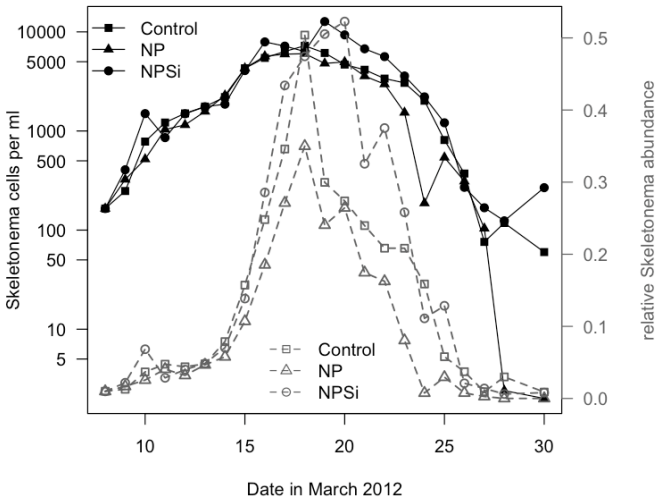


Fig. S1. Absolute and relative abundances of (A) *Phaeocystis pouchetii* and (B) *Skeletonema marinoi* in Control (M1, squares), NP (M2, triangles) and NPSi (circles) mesocosms as determined by microscopy. Black symbols and solid black lines show absolute abundances on left-hand y-axis while open symbols and dashed grey lines show abundances relative to total microeukaryote abundance on the right-hand y-axis. Note that no samples from Raunefjorden were counted by microscopy.

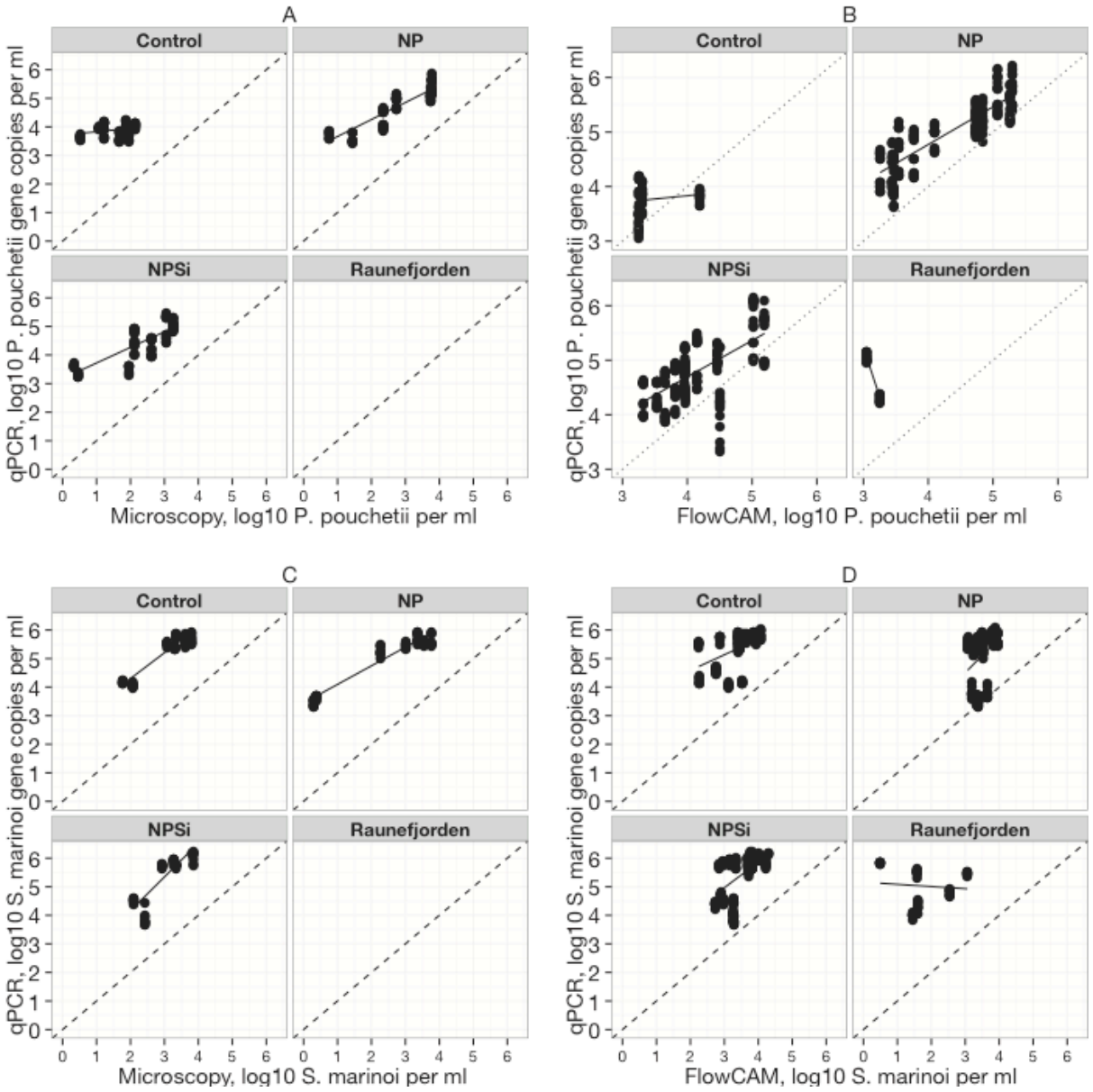
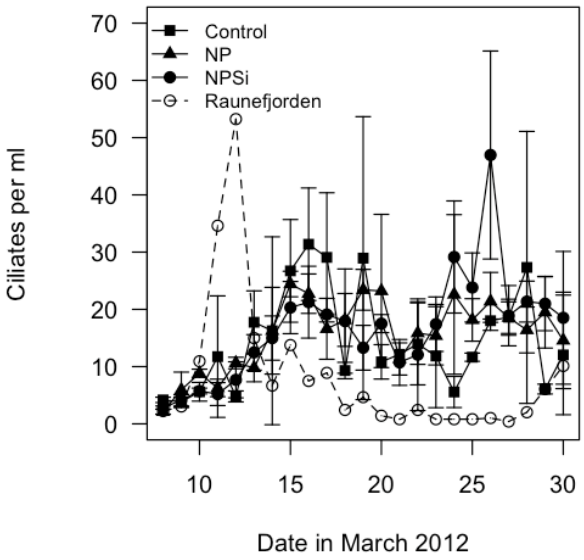


Fig. S2. Log-log plots showing correspondence of quantification methods for (A,B) *Phaeocystis pouchetii* and (C,D) *Skeletonema marinoi*. Panels A and C show qPCR results (target gene copies per ml) on the *y*-axis and microscopy results (cells per ml) on the *x*-axis. Panels B and D show qPCR results on the *y*-axis and FlowCAM results (cells per ml) on the *x*-axis. Dashed lines indicate slope = 1, intercept =0. Solid lines indicate linear regressions.

A



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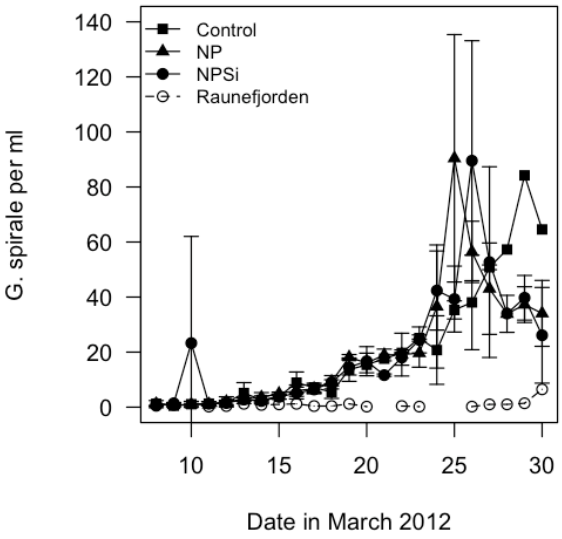

Fig. S3. Abundance of (A) ciliates and (B) the dinoflagellate *Gyrodinium spirale* in mesocosm bags and in Raunefjorden as assessed by FlowCAM analysis. Plotted points represent mean abundances per treatment. Error bars indicate one standard deviation from the mean.