



Structure-dependent, protistan grazing and its implication for the formation, maintenance and decline of plankton patches

Susanne Menden-Deuer^{1,*}, Kerri Fredrickson²

¹Graduate School of Oceanography, University of Rhode Island, Rhode Island 02882, USA

²Shannon Point Marine Center, Western Washington University, Washington 98221, USA

ABSTRACT: Plankton distributions are frequently patchy and phytoplankton patches have long been suggested as important resources in otherwise nutritionally dilute environments. The present study confirms this hypothesis empirically in a coastal fjord with naturally forming phytoplankton patches and examines the implications for plankton distributions, abundance and rates of patch formation, maintenance and decline. Phytoplankton patches were identified using a CTD mounted fluorometer and sampled with a horizontally mounted 2 l Niskin bottle in 3 separate field seasons in summer 2007 and 2008 and spring 2009. We quantified chl *a* and macronutrient concentrations as well as phytoplankton growth and zooplankton (<200 μm) grazing rates. Average phytoplankton growth was equal inside and outside of patches ($0.34 \pm 0.07 \text{ d}^{-1}$) and there was no indication of nutrient limitation to phytoplankton growth. Average grazing rate inside patches ($0.25 \pm 0.03 \text{ d}^{-1}$) was significantly higher than outside of patches ($0.09 \pm 0.03 \text{ d}^{-1}$). Grazing pressure was not simply a function of prey availability; there was no significant relationship between grazing rate and initial chl *a* concentration. Protistan grazing consumed on average 65% of primary production within and 26% outside of patches. Model predictions of population dynamics suggest that protistan grazing focused within patches and more rapid phytoplankton accumulation outside of patches eroded layer structures within hours to days. Formation of plankton patches due to phytoplankton growth was not supported by the data. Averaging rates, irrespective of phytoplankton distribution, greatly overestimated layer persistence and minimally underestimated primary production and its availability to higher trophic levels. These results emphasize empirically the importance of predator–prey interactions to the ubiquitous phenomenon of plankton patchiness and ultimately microbial food web dynamics.

KEY WORDS: Plankton patches · Spatial ecology · Heterotrophic protists · Grazing · Food web dynamics

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Localized concentrations of planktonic organisms (e.g. patches and layers) have long been described from a wide range of coastal and oceanic environments (e.g. Eppley et al. 1968, Donaghay et al. 1992, Cowles 2004, McManus et al. 2003, 2005). Layers are frequently associated with stratification and there appear to be distinct associations between the physical structure of the water column and the intensity of plankton layers (Dekshe-

nieks et al. 2001). These layers alter the optical and acoustical properties of the water column (Holliday et al. 2003) and have been shown to have far reaching ramifications in marine ecosystems, including for the feeding of whales (Baumgartner et al. 2003). Plankton layers can be horizontally extensive, spreading over km, and can last for several days (Rines et al. 2002, Menden-Deuer 2008). The frequency, intensity and distribution of these layers determine their effects on water column properties and ecological processes.

*Email: smenden@gso.uri.edu

The processes that drive plankton patch formation and magnitude are subject to ongoing debate (see recent discussion, Birch et al. 2009, Stacey et al. 2009). Layer formation requires that dispersive forces (e.g. turbulent diffusion) are less than coherent forces (Stacey et al. 2007). Both physical and biological processes have been identified as variables in layer formation and persistence. Franks (1992, 1995) has identified particle buoyancy and shearing as important mechanisms. Formation of a diatom layer was recently observed in regions of maximum shear (Velo-Suárez et al. 2010) as predicted for phytoplankton based on modeling studies (Birch et al. 2008). High degrees of correlation between chlorophyll (chl) *a*-derived fluorescence signals and physical water column properties suggest that phytoplankton patches are frequently passive tracers of fluid flow (Abraham 1998). In the ocean, swimming and aggregative behaviors have been shown to concentrate phytoplankton (Kiefer & Lasker 1975, Bjørnsen & Nielsen 1991) and zooplankton (Gallager et al. 2004, Genin et al. 2005). The relative importance of physical vs. biological processes to patch formation cannot be determined *a priori* and may shift in magnitude among events or over time (Stacey et al. 2007).

Surprisingly, plankton population dynamics, namely the rates of change of phytoplankton cell number due to growth and mortality, have found little attention in the discussion on patch formation mechanisms. Typical growth rates of microplankton that constitute the majority of layer forming organisms (Alldredge et al. 2002, Menden-Deuer 2008, Rines et al. 2010) are on the order of 1 doubling d^{-1} , suggesting that population size inside layers can change within hours. Phytoplankton growth rates that exceed grazing and other losses (e.g. dispersive/mixing) will result in an accumulation of phytoplankton biomass, and thus would promote layer formation and persistence. Conversely, grazing rates that exceed or equal phytoplankton growth rates would result in a reduction in phytoplankton biomass or prevent its buildup, and thus would prevent layer formation or induce decline.

Heterotrophic protists are major consumers of phytoplankton biomass, and have growth rates that match or exceed those of their phytoplankton prey (Strom & Morello 1998, Jeong et al. 1999, Sherr & Sherr 2007). Previous field studies have shown that protist predator and phytoplankton prey abundances are sometimes, but not always, positively correlated (Stoecker et al. 1984, Menden-Deuer 2008). Benoit-Bird et al. (2010) acoustically determined zooplankton and phytoplankton signals and concluded that their association was greatest when at least 18% of the phytoplankton biomass was concentrated inside layers. The spatial and temporal extent of these layers often exceeds km-

scales and several days, which provides sufficient persistence for predator biomass to accumulate either through growth or aggregative behaviors and exploit the biomass inside layers (Grünbaum 2002, Menden-Deuer & Grünbaum 2006). Association of herbivores with phytoplankton layers implies that trophic interactions occur and that they may play an important role in dictating patch dynamics. Mullin & Brooks (1976) hypothesized that concentrated phytoplankton patches are needed to support observed zooplankton production rates in the otherwise nutritionally dilute pelagic. Similar suggestions have been made for virtually all planktonic consumers (e.g. Menden-Deuer & Grünbaum 2006, Paffenhöfer et al. 2007, Stocker et al. 2008). To our knowledge, this hypothesis has never been tested in the ocean.

Heterogeneous distributions have long been recognized as potentially important factors in altering phytoplankton abundance and productivity estimates. In the present study, we confirm this expectation empirically. The goal of this work was (1) to quantify the magnitude of phytoplankton growth and grazer induced mortality rates as a function of phytoplankton distribution, and (2) to estimate whether trophic and demographic processes can lead to layer formation, persistence, or decline.

MATERIALS AND METHODS

Study site and sampling design. East Sound is a temperate fjord within the San Juan Archipelago in the northeastern Pacific ($48^{\circ} 38.61' N$, $122^{\circ} 52.75' W$). The fjord has a north–south extent of approximately 10 km and east–west width varies between 1 and 2 km. Depth ranges from <20 m at the northern end to >40 m at the southern end; mean depth is 30 m. Circulation and exchange with the tidally well-mixed Harney Channel to the south is restricted by a partial sill at the southwestern terminus of the fjord. A longitudinal transect of 5 stations, including Stn 1 just outside East Sound, was regularly sampled (Fig. 1). To increase the spatial resolution of layer distribution, up to 15 additional stations were profiled. Stations were between 0.3 and 2 km apart. Up to 3 sampling trips were carried out each week, with visits separated by 1 to 2 d. A total of 29 1-d cruises were conducted: 15 in 2007, 8 in 2008 and 6 in 2009. At each station the water column was profiled with a SeaBird 19+ CTD mounted with a WetLabs WetStar fluorometer. In 2007 a hand-held light meter (Li-Cor, LI-1400) with an underwater spherical quantum sensor (SPQA 3585) was used to acquire water column light profiles. After 2007, a SeaBird C-Star measuring beam transmission/attenuation over 25 cm at 660 nm and a Biospherical QSP-2300L 4π

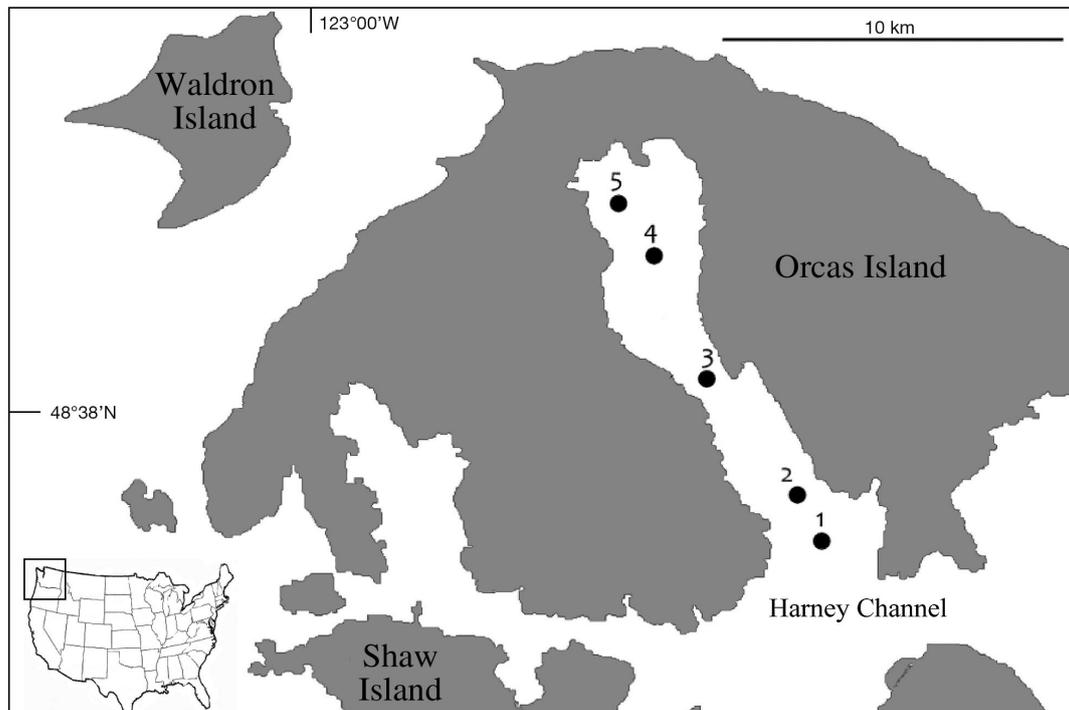


Fig. 1. East Sound within the San Juan Archipelago, northeastern Pacific, and the 5 major sampling stations. An additional 15 stations are not shown. The small inset shows the approximate location of the study area in the northwestern United States (not to scale)

PAR sensor were added. The mean descent rate of the CTD was 0.2 m s^{-1} and the vertical resolution was 0.1 m. Real-time measurements of water column profiles, including chl *a*-induced fluorescence, were displayed shipboard and used to identify the presence and depth of distinct layers. For the purpose of shipboard identification and choice of target depths, a sharp gradient in fluorescence that exceeded the overall fluorescence of the remainder of the water column was termed a layer. The observed structures were evident in repeat casts at the same location, and there was excellent agreement between down- and up-casts as well as repeated casts. All data presented are based on down-cast profiles. We followed thin layer identification criteria in Ryan et al. (2008), namely a layer intensity that exceeds background by 3-fold and agreement with layer presence in subsequent profiles. However, some of our layers exceeded the maximum 5 m thickness criterion. Since we were interested in these layers as a potential prey source in an otherwise dilute medium, we included thicker layers, as long as a distinct patchiness was observable by the presence of steep vertical gradients. In Menden-Deuer (2008) the term 'Plankton Rich Layers' (PRLs) was used to describe layers of high plankton biomass with variable vertical extent that were bordered by steep gradients in plankton concentrations. The same approach to identifying plankton layers was used here. In our verti-

cal profile, heterogeneous phytoplankton distributions appear as vertically restricted layers. High-resolution horizontal sampling showed that these layers were also laterally bound and can be considered patches. The terms layers and patches are used interchangeably here.

Based on the real-time water column profiles, whole water samples were collected from at least 2 depths, one within the fluorescence maximum and one 2 m shallower or deeper using a horizontally mounted, 2 l Niskin bottle. Larger volumes, such as for grazing experiments, were sampled with a 10 l horizontally mounted Niskin bottle. In 2009, water samples for grazing experiments were collected with 5 l rosette mounted Niskin bottles. The samples were stored in the dark and cooled by surface water during the 45 min transport to the laboratory.

Chl *a* and nutrient measurements. In the laboratory the concentration of extracted chl *a* was determined from triplicate, size-fractionated samples ranging in volume between 50 and 500 ml depending on phytoplankton abundance (Lorenzen 1966, Strickland & Parsons 1972). Centrifuge tubes and chl *a* bottles were triple rinsed with sample volume before filling. In 2007, triplicate GF/F filtered chl *a* extractions were made at 3 size fractions (>0.7 , >5 and $>20 \mu\text{m}$). In 2008 and 2009, chl *a* extractions were made at 2 size fractions (>0.7 and $>20 \mu\text{m}$). Average coefficient of varia-

tion (CV) for triplicate chl *a* measurements was 11.2%. Nutrient analysis followed Parsons et al. (1984). Dissolved inorganic macronutrient concentrations (phosphate, nitrate + nitrite [nitrate hereafter] and silicic acid) were drawn from each sampling bottle and immediately frozen at -20°C in VWR 50 ml acid-washed, sample rinsed centrifuge tubes. Silicic acid and phosphate concentrations were measured spectrophotometrically, whereas nitrate concentrations were measured with an Alpkem AutoAnalyzer. In 2007, triplicate nutrient samples were taken. When CV was found to be 3, 1 and 3% for nitrate, silicic acid and phosphate, respectively, only single nutrient measurements were made because sampling error was much smaller than the variation amongst most samples. In 2007, dissolved inorganic macronutrient concentrations were measured on both 0.45 μm Metricel membrane filtered and unfiltered samples. There was no significant effect of filtration on nitrate and silicic acid concentrations ($p = 0.98$ and $p = 0.67$, respectively, ANOVA). Phosphate concentrations were 3% or 0.06 μM higher in unfiltered samples ($p = 0.002$, ANOVA). Macronutrient concentrations were measured in unfiltered samples after 2007. Samples were frozen for up to 3 wk and thawed in the dark at 4°C for at least 2 d before analysis. There was no significant effect of freezing for up to 4 wk on silicic acid concentration between 10 and 50 μM ($p = 0.88$, ANOVA).

Plankton community composition. On each sampling day, whole, live seawater samples from inside layers were examined on dissecting and compound microscopes and the qualitative species composition recorded. Whole seawater (100 ml) was preserved with acid Lugol's iodine (Thronsdon 1978) at a final concentration of 2%. Counts of dominant phytoplankton and heterotrophic protist species $>5 \mu\text{m}$ in diameter were made with a Sedgwick-Rafter slide (volume 1 ml) at 100 and 200 \times on an inverted Zeiss microscope. Species identification was based on Horner (2002) and Butcher (1959). Additional species identifications were kindly provided by Dr. Rita Horner, University of Washington, USA.

Growth and grazing rate measurements. Phytoplankton growth rates and protistan grazer induced mortality rates were measured using the dilution method (Landry & Hassett 1982) in a 2-point modification (Landry et al. 2008, Strom & Fredrickson 2008). A total of 11 dilution experiments were done, 8 in 2007 and 3 in 2009, with 3 to 6 replicates at each dilution level. Of these 11 dilution experiments, 7 were conducted with samples taken outside of plankton layers and 4 were captures within plankton layers. Whole seawater (20 l) was gently screened through a 200 μm Nitex mesh to remove macro-zooplankton. The volume was gently inverted and well mixed in a 25 l polycar-

bonate carboy and 10 l were 0.2 μm gravity filtered using a filtered seawater rinsed filter cartridge to generate filtered seawater (FSW) necessary for diluting whole seawater (WSW). In 2007 each dilution experiment had 2 dilution levels of 5 and 100% WSW in triplicate each. In 2009, the lower dilution contained 20% WSW. The appropriate ratio of FSW and WSW were gently siphoned into triplicate 1.2 l incubation bottles. Triplicate samples between 100 and 500 ml were withdrawn from each treatment to measure initial, size-fractionated chl *a* concentrations of >20 and $<20 \mu\text{m}$. Each experiment, except for on July 13, 2007, was run both with and without added nitrate (5 μM) and phosphate (0.3 μM) to account for potential nutrient limitation on rate estimates. Silicone tubing and polycarbonate bottles were used to avoid toxicity effects on heterotrophic protists. The clear bottles were placed in neutral density mesh screen bags to reproduce the light level reduction at sampling depth. Incubations (24 h) took place in an outdoor incubator that was cooled by surface seawater (12°C) and illuminated at ambient light levels. Triplicate, size-fractionated chl *a* samples were withdrawn from each bottle after 24 h. In 2009, only duplicate chl *a* samples were taken. Additional samples were taken for fixation and analysis of community composition. Net phytoplankton growth rate (k , d^{-1}) was calculated as:

$$k = 1/t \times \ln(C_t/C_0) \quad (1)$$

where C_t and C_0 are the final and initial chl *a* concentration, respectively, and t is the time elapsed in days. k was calculated separately for total chl *a* as well as the <20 and $>20 \mu\text{m}$ size fractions. Previous analyses showed that estimates for the intrinsic phytoplankton growth rate (μ , d^{-1}) were statistically indistinguishable from k (d^{-1}) of the lowest dilution level regardless of whether estimates were based on the 2-point method or on a fully resolved dilution series of ≥ 4 dilution levels (Strom et al. 2006, Strom & Fredrickson 2008). Based on these findings, k (d^{-1}) presented here is assumed to be equivalent to μ (d^{-1}). It is noteworthy that the observed lack of significant difference between the 2-point method and the fully resolved dilution series is due to the immense variability in the measurements, and not due to a true lack of difference. Therefore, all measurements made here were obtained from at least triplicate, independent incubations. Furthermore, the 2-point method was developed using open-ocean and early spring phytoplankton communities, which were at much lower concentrations than the phytoplankton concentrations during the spring of 2009. The interpretation of growth rates needs to be done cautiously for the 2009 experiments, as the 20% dilution level may have underestimated μ . Grazing rate (g d^{-1}) was calculated as the difference between

the growth rates measured in both dilution levels. This rate represents the grazing rate by heterotrophic protists because samples were 200 μm pre-screened to exclude metazoan predators. Growth and grazing rates for each replicate were calculated based on total chl *a* and for each size fraction separately. Negative grazing rates were observed, which is known from dilution experiments (Agis et al. 2007), although they are theoretically impossible. For this reason, it is sometimes recommended to set negative grazing rates to 0 (e.g. Calbet & Landry 2004). In the present study, all statistical tests were run both on the untreated data set and with negative grazing rates set to 0. The presented results and conclusions were identical, irrespective of approach used. There was no significant difference in growth rate estimates in either size-fractionated or total chl *a* rates for nutrient-amended or non-amended experiments (paired *t*-test) for all but one experiment. Growth rate estimates for one dilution experiment on July 18, 2007 (Stn 4, 12 m depth), were negative (-0.58 d^{-1}) in the non-amended treatments and these rates were excluded from the analysis.

Data analysis. Effects of freezing and filtration on nutrient concentrations and effect of nutrient addition on phytoplankton growth rate were estimated using a 2-way ANOVA that took spatial variation (e.g. station and depth) as a factor into account. Comparisons of inside and outside of layer growth and grazing rates were done with a 2-tailed *t*-test. The goal was to conduct a comparison of plankton population dynamics across a broad spectrum of conditions and include diverse types of plankton distributions and communities, rather than contrast rates inside and outside of specific layers as would have been addressed by a paired design. Replication across depth or stations was insufficient for the 11 experiments to be examined statistically. A multidimensional scaling analysis was performed followed by an Analysis of Similarity (ANOSIM) to test whether date, depth, station, season or initial chl *a* co-varied significantly with grazing rate. None of these factors could explain the observed patterns. This analysis was done in Primer v5 using

Euclidean distance to calculate the similarity coefficients. Regression analyses were based on a linear, type-II model (i.e. both variables measured with error). Population dynamics were modeled assuming exponential growth:

$$dP/dt = P \times (\mu - r) \quad (2)$$

where *P* is chl *a* concentration ($\mu\text{g l}^{-1}$), μ is phytoplankton growth rate (d^{-1}) and *r* is phytoplankton mortality rate (d^{-1}). The equation was solved using the Matlab ODE solver function *dsolve*. All analyses were carried out in Matlab 7.9 and assigned statistical significance at $p < 0.05$.

RESULTS

Water column structure and fluorescence distribution

Seven distinct layer events were observed in the 3 different field seasons (Table 1). Although layer events were observed at each station within the sound, fluorescence intensity generally increased northward (data not shown). Initiation of layer formation was associated with a shoaling of the isopycnals (Fig. 2). Layers were associated with stratification and occurred around the pycnocline but were not associated with a characteristic temperature or salinity. During the summer months of 2007 and 2008, distinct layers lasted several days. During the spring of 2009, the same phytoplankton community persisted for the entire 3 wk sampling season, in a largely mixed water column. Consistent increases in phytoplankton biomass resulted in a diatom bloom that briefly occupied the entire water column on May 5, 2009 (Day 125). For the purpose of our analysis, data from May 5 were not considered 'layered' because the fluorescence profile was nearly uniform throughout the water column and no patchiness was observable. For the remainder of the 2009 spring bloom, fluorescence remained variable with depth.

Table 1. Date of peak occurrence, dominant phytoplankton species, sampling depth, maximum chl *a* concentration, % chl *a* in the $>20 \mu\text{m}$ size fraction, temperature and salinity of plankton layers

Date	Dominant species	Depth (m)	Chl <i>a</i> ($\mu\text{g l}^{-1}$)	Chl <i>a</i> $>20 \mu\text{m}$ (%)	Temp. ($^{\circ}\text{C}$)	Salinity
July 2, 2007	<i>Pseudo-nitzschia</i> spp.	5	5.1	84	11	29.7
July 16, 2007	<i>Heterosigma akashiwo</i>	0.5	16.7	6	15.5	27.8
July 18, 2007	$<5 \mu\text{m}$ flagellate, unidentified	1	3.5	5	16.1	27.8
July 14, 2008	<i>Pyramimonas</i> cf. <i>grossi</i>	9	14.9	38	14.8	26.3
July 18, 2008	<i>Scrippsiella trochoidea</i>	4	7.0	70	12.4	28.8
July 30, 2008	<i>Coscinodiscus</i> spp. & <i>Pseudo-nitzschia</i> sp.	4	20.7	85	12.4	29.6
April 30, 2009	<i>Nitzschia acicularis</i>	7	26.6	90	10	30.5

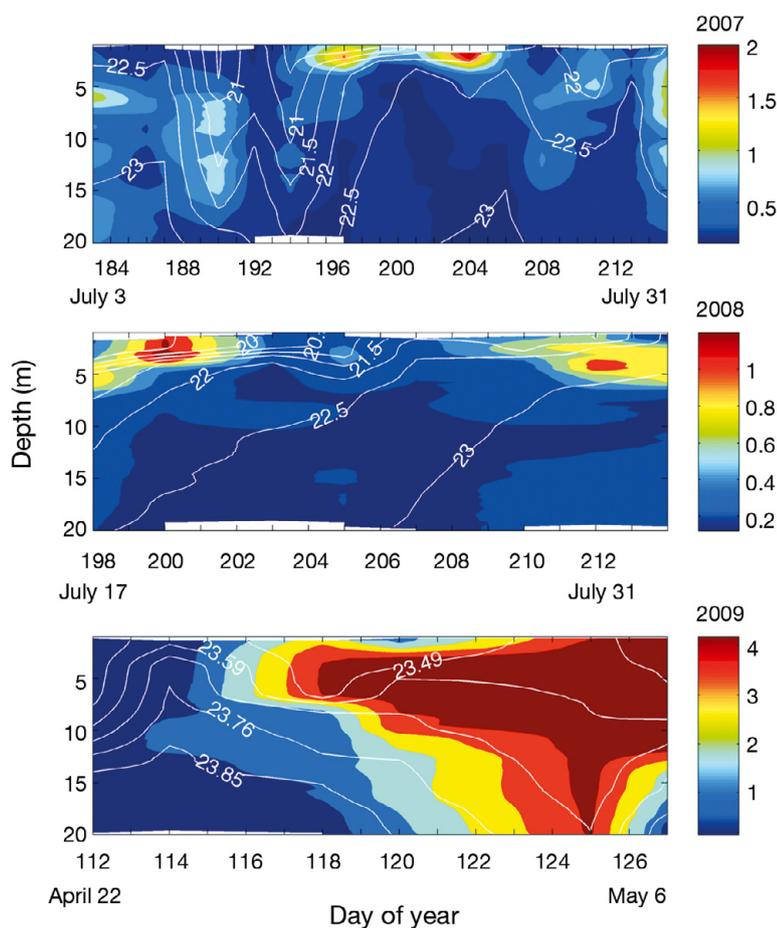


Fig. 2. Chl *a* induced fluorescence (volts on scale bar) at Stn 3 during each of the 3 sampling seasons. The x-axis indicates day of year in 2007 through 2009. Note difference in volt ranges. Isopycnals (σ_θ) are overlain to indicate water column structure

Fluorescence signal and chl *a* concentrations

Over the 3 sampling seasons, extracted total chl *a* concentration ranged from 0.55 to 26.5 $\mu\text{g l}^{-1}$. Chl *a* in the $>20 \mu\text{m}$ size fraction contributed as little as 5% during a bloom of the raphidophyte alga *Heterosigma akashiwo* (Hada) on July 16, 2007, and as much as 90% during a bloom of the pennate diatom *Nitzschia acicularis* (Küzing) in late April and early May 2009. A significant, positive regression was observed between the fluorescence signal measured with the CTD mounted fluorometer and the extracted chl *a* concentration (Fig. 3). Over two-thirds of the variability between the 2 proxies for phytoplankton abundance were explained by this relationship. A significant regression for these 2 proxies of phytoplankton abundance was also observed for all 3 years separately

(data not shown), despite differences in the dominant phytoplankton type, community composition, or season.

Nutrient concentrations

Dissolved inorganic macronutrients were measured to assess the effect of nutrient limitation on observed patterns of phytoplankton growth and trophic interactions. Macronutrient concentrations were generally plentiful with few exceptions, ranging from non-detectable up to 32, 65 and 2.5 μM for nitrate, silicic acid and phosphate, respectively. Silicic acid was significantly lower in spring 2009, with a mean concentration of 15 μM compared to 39 and 37 μM in summer 2007 and 2008, respectively (Table 2). In 2007 and 2008, silicic acid concentrations remained above 20 μM , and did not show pronounced fluctuations. Similarly, phosphate was significantly higher ($p = 0.0018$) in 2007 and 2008 than in 2009. Nitrate concentrations peaked in 2008 and differed significantly between all years. Nitrate concentrations were the most variable, with fluctuations as high as 10 μM between stations or depths. There was a persistent gradient with increasing nutrient concentrations from south to north, suggesting an influx to East Sound from Harey Channel, which borders the mouth of East Sound in the south. In 2007 and 2008, there were no discernable trends in nutrient concentrations with time. In 2009, there was a 3- to 5-fold decrease of nitrate and silicic acid concentrations between April 24 and May 5, 2009. The average Si:DIN ratio was 7 and DIN:P ratio was 9.8 over the 3 years. This deviation from Redfield stoichiometry may reflect differences in the nutrient sources. For spring 2009 it almost certainly reflects a difference in consumption rates as the phytoplankton community was then dominated by silica-dependent diatoms.

Table 2. Average macronutrient concentration during the 3 sampling seasons. Nitrate concentration includes nitrite. Standard deviations of the mean are given in parentheses; n: sample size

Year	Nitrate (μM)	Silicic acid (μM)	Phosphate (μM)	n
2007	12.4 (5.8)	39.5 (8.4)	1.30 (0.48)	112
2008	9.7 (6.8)	37.6 (8.0)	1.13 (0.53)	74
2009	14.6 (7.7)	15.2 (7.7)	0.96 (0.28)	31

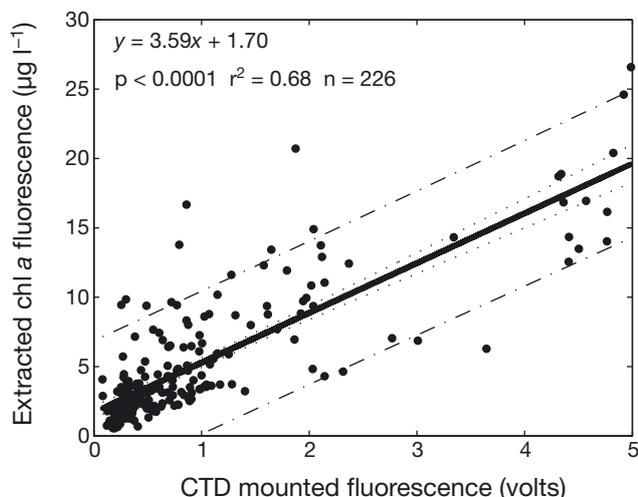


Fig. 3. Linear regression (type II) of fluorescence measured with the CTD mounted fluorometer against the concentration of extracted chl *a*. Solid line shows line of best fit, dotted lines show the confidence interval about the slope and the dashed-dotted lines show the prediction interval. The regression equation, probability (*p*), r^2 and sample size (*n*) are stated. CTD measured fluorescence was a significant predictor of extracted chl *a* concentration

Nutrient concentrations were significantly lower inside phytoplankton layers than outside ($p < 0.001$, Fig. 4). There were no significant differences in nutrient concentrations among samples taken outside of plankton patches, either below, above or at stations without plankton layers. Nitrate concentrations inside layers were, on average, 43% lower than outside: $7.0 \mu\text{M} (\pm 0.77)$ inside compared to $>12.2 \mu\text{M} (\pm 0.8)$ outside. Silicic acid concentrations were on average 22% lower: $<30 \mu\text{M} (\pm 1.4)$ inside layers and $>37 \mu\text{M} (\pm 0.99)$ outside of layers. Phosphate concentrations inside layers were on average 33% lower: $0.88 \mu\text{M} (\pm 0.06)$ inside and $1.31 \mu\text{M} (\pm 0.08)$ outside of layers.

Plankton community composition

Although no quantitative analysis of the species composition and abundance was conducted, the most abundant layer forming phytoplankton species were taxonomically identified for each event (Table 1). Phytoplankton inside layers were taxonomically diverse, representing broad size ranges as well as motile and non-motile species. However, each layer was dom-

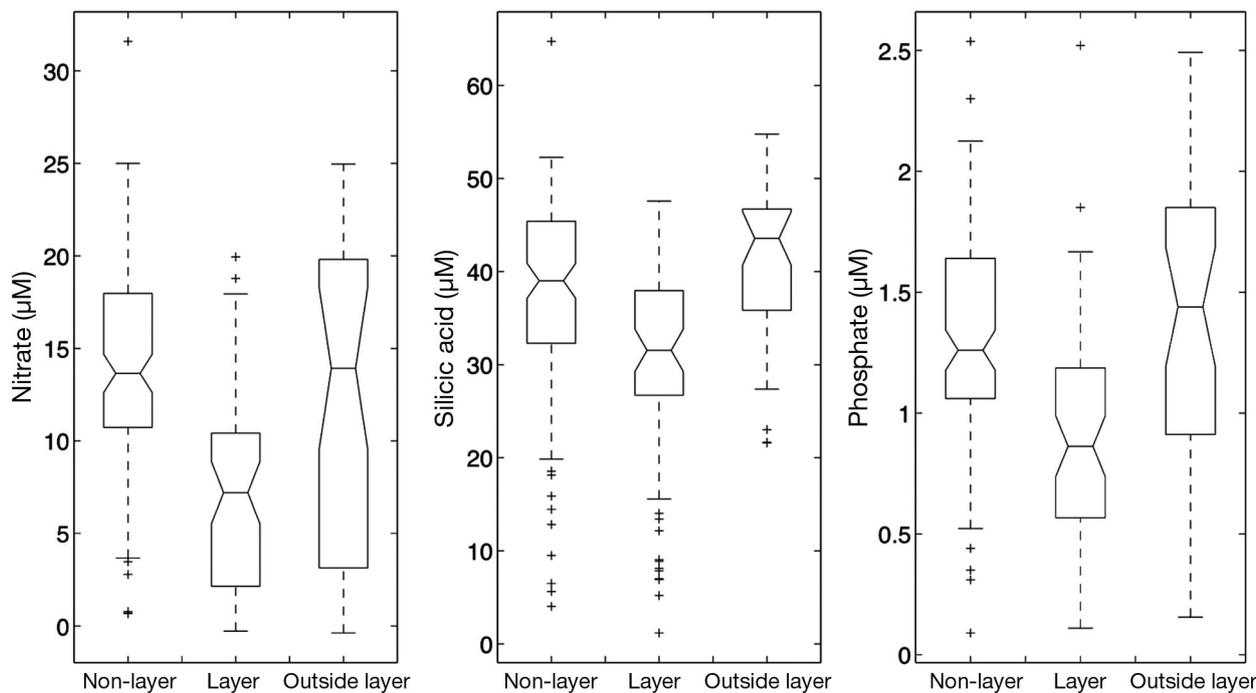


Fig. 4. Concentrations of dissolved nitrate, silicic acid and phosphate taken from stations without distinct layers (Non-layer), from inside layers (Layer) and below or above layers (Outside layer). Boxplots represent all data collected over the 3 sampling seasons. On each box, the central mark is the median, the edges of the box are the 25th and 75th percentiles, the whiskers contain 95% data range. Crosses indicate data beyond the 95% data range. Concentrations of all macronutrients were significantly lower inside plankton rich layers, indicating active uptake and temporal coherence of patch water masses

inated numerically by 1 and, at most, 2 phytoplankton species. For example, at Stn 4, *Heterosigma akashiwo* concentrations peaked at 3700 cells ml⁻¹ on July 16, 2007, and *Nitzschia acicularis* reached 3055 cells ml⁻¹ on April 28, 2009. All samples contained a diverse set of heterotrophic protists, including thecate dinoflagellates, and loricate and aloricate ciliates.

Demographic and trophic rates

Mean phytoplankton growth rates in nutrient-amended and non-amended experiments were 0.36 d⁻¹ (n = 24). There was no significant difference in growth rate estimates for nutrient-amended and non-amended dilution experiments based on either size fraction or on total chl a (p > 0.5, paired *t*-test). Thus, rate measurements from experiments with and without nutrient addition were combined for the analysis. Mean growth rate calculated based on changes in total chl a inside layers was 0.37 (±0.04 SE) and for outside layers was 0.34 ± 0.07 d⁻¹ (Table 3). There was no significant difference between net phytoplankton growth rates measured in or outside of layers for either the total or >20 µm size fractions (p > 0.25). However, growth for the smaller size fraction was significantly greater outside layers (0.32 ± 0.08 d⁻¹) than within (0.16 ± 0.06 d⁻¹; p < 0.001).

Phytoplankton growth rates were variable, ranging from 0 to 0.99 d⁻¹ across different phytoplankton communities, depths and stations (Table 3). The negative growth rate observed on July 13, 2007, at the shallow depth is unlikely due to nutrient limitation, as all macronutrient concentrations at 1 m depth were

higher than at 9 m depth, where maximal growth rates were observed. Higher phytoplankton growth rates were associated with flagellate dominated layers in July and August 2007, whereas the diatom bloom in spring 2009 grew less rapidly (~0.24 d⁻¹). Nitrate and silicate concentrations decreased by 80 and 70%, respectively, between April 24 and May 5, 2009, and low growth rates on May 5, 2009, may have been due to nutrient limitation. Nutrient-amended growth for that sample was comparable to prior rates (0.23 d⁻¹), suggesting that the low growth rate was due to nutrient limitation. This was observed, although the difference between nutrient-amended and non-amended dilution experiments was not statistically significant.

Grazing rates on total chl a were significantly higher inside layers (0.25 ± 0.03 d⁻¹) compared to outside of layers (0.09 ± 0.03 d⁻¹; p < 0.01; Fig. 5). There were substantive differences in grazing pressure among size fractions outside of layers, whereas all size fractions were consumed equally inside layers. Grazing pressure on all size fractions was lower outside of layers than inside. Grazer induced mortality on the >20 µm size fraction was almost identical inside and outside of layers (0.18 ± 0.05 and 0.23 ± 0.04 d⁻¹, respectively). Only the >20 µm size fraction appears to have been subject to grazing pressure outside of layers. However, average grazing rate on the <20 µm size fraction inside layers (0.23 ± 0.04 d⁻¹) was significantly higher (p < 0.0001) than non-layer samples (0.06 ± 0.14 d⁻¹), suggesting that grazing pressure was concentrated on the smaller size fraction inside layers.

Concentrated grazing pressure inside layers was not a function of total prey availability as measured by chl a concentration (Fig. 6). Average chl a concentra-

Table 3. Date, station, depth (m), sample size (n), initial chl a concentration (µg l⁻¹), growth and grazing rates (d⁻¹) measured for the total and >20 µm and <20 µm size fractions, outside and inside phytoplankton layers. Data in parentheses give one standard error of the mean. Data were averaged across nutrient-amended and non-amended rates, as they were not significantly different

Date	Stn	Depth (m)	Sample size	Initial chl a (µg l ⁻¹)	Growth rate (d ⁻¹)			Grazing rate (d ⁻¹)		
					Total	>20 µm	<20 µm	Total	>20 µm	<20 µm
Outside layers										
Jul 13, 2007	1	3	3	3.93	0.51 (0.06)	0.50 (0.14)	0.51 (0.09)	0.16 (0.06)	0.24 (0.14)	0.13 (0.04)
Jul 13, 2007	3	3	3	4.67	-0.11 (0.11)	0.14 (0.13)	-0.14 (0.10)	0.01 (0.01)	0.26 (0.00)	0.00 (0.02)
Jul 13, 2007	3	9	3	3.61	0.99 (0.07)	0.90 (0.05)	1.00 (0.08)	0.00 (0.02)	0.65 (0.04)	0.00 (0.02)
Jul 18, 2007	4	12	3	1.97	0.18 (0.07)	-0.05 (0.10)	0.25 (0.07)	0.12 (0.01)	0.00 (0.04)	0.21 (0.02)
Jul 18, 2007	4	1	6	3.78	0.18 (0.02)	0.12 (0.06)	0.19 (0.03)	0.07 (0.02)	0.27 (0.06)	0.05 (0.02)
Aug 1, 2007	4	12	6	2.06	0.66 (0.14)	0.63 (0.14)	0.71 (0.13)	0.08 (0.03)	0.11 (0.03)	0.01 (0.03)
May 5, 2009	5	5	6	19.00	0.08 (0.08)	0.09 (0.09)	-0.11 (0.06)	0.20 (0.09)	0.10 (0.15)	0.19 (0.06)
Average				6.39 (1.23)	0.34 (0.07)	0.32 (0.07)	0.32 (0.08)	0.09 (0.03)	0.18 (0.05)	0.06 (0.03)
Inside layers										
Jul 18, 2007	3	1	6	3.53	0.45 (0.11)	0.41 (0.05)	0.45 (0.14)	0.37 (0.06)	0.17 (0.06)	0.38 (0.07)
Aug 1, 2007	4	5	6	2.30	0.54 (0.03)	0.94 (0.03)	0.10 (0.03)	0.21 (0.01)	0.35 (0.04)	0.16 (0.04)
Apr 24, 2009	5	9	6	5.89	0.23 (0.08)	0.29 (0.09)	-0.11 (0.09)	0.26 (0.08)	0.33 (0.09)	0.28 (0.09)
Apr 28, 2009	6	5	6	16.84	0.24 (0.03)	0.24 (0.03)	0.22 (0.05)	0.15 (0.06)	0.06 (0.07)	0.11 (0.09)
Average				7.14 (1.14)	0.37 (0.04)	0.47 (0.06)	0.16 (0.06)	0.25 (0.03)	0.23 (0.04)	0.23 (0.04)

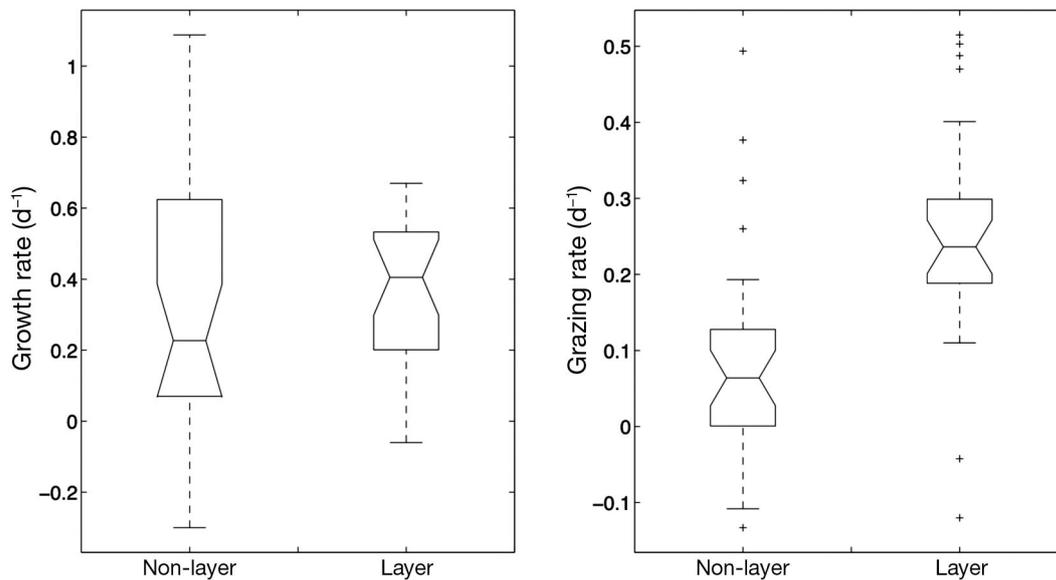


Fig. 5. Phytoplankton growth and heterotrophic protist grazing rates for all replicates in all dilution series of plankton communities captured outside of plankton layers (Non-layer) and inside (Layer) based on total chl *a*. Box plot set up as in Fig. 4. There was no significant difference between phytoplankton growth rates inside or outside of layers. Grazing rates within layers were significantly higher than outside of layers ($p < 0.01$)

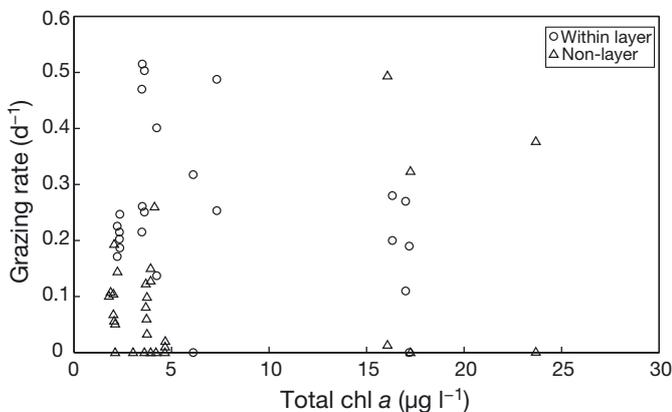


Fig. 6. Measured protistan grazing rates (d^{-1}) versus total initial chl *a* concentrations ($\mu g l^{-1}$; an indicator of prey availability) from dilution experiments using water from inside and outside phytoplankton layers. No significant relationship was found ($p = 0.49$)

tion outside of layers was comparable (median 6.39 , range 2.02 to $19.00 \mu g l^{-1}$) to concentrations inside layers (median 7.14 , range 2.22 to $23.68 \mu g l^{-1}$). There was no significant relationship between initial chl *a* concentration and protistan grazing rate.

DISCUSSION

The relative roles of biological, chemical and physical processes in the formation, maintenance and decline of plankton rich layers remain elusive. The

data presented here provide empirical measurements confirming that phytoplankton patches were sites of intense biological activity, including evidence of increased nutrient uptake by phytoplankton and grazing by heterotrophic protists. Empirically measured protistan grazing rates inside phytoplankton patches were on average 2-fold higher than grazing rates outside patch structures. This concentrated grazing pressure has implications for phytoplankton patchiness and the trophic dynamics of marine microbial food webs. These results were obtained from a wide range of community types and environmental conditions, in summer and spring, suggesting that patches serve as important trophic hotspots for protistan predators in a broad range of conditions. Together with recent findings on the ubiquity and frequency with which phytoplankton patches and layers are observed (e.g. McManus et al. 2005) these findings highlight the importance of considering phytoplankton distributions in assessing food web dynamics.

Phytoplankton growth

Growth rates for taxonomically diverse phytoplankton species were variable and on average lower than expected for temperate regions. Calbet & Landry's (2004) meta-analysis found average phytoplankton growth rates of $\sim 0.7 d^{-1}$ for temperate and $\sim 0.4 d^{-1}$ for polar regions. The rates measured here are thus more comparable with rates measured for polar than tem-

perate regions. Water temperature in the dilution incubations was on average 12°C, reflecting the range of temperatures measured in the field (9 to 16.1°C). Temperature values were not provided in the Calbet & Landry (2004) meta-analysis, but we expect that 12°C would be considered temperate. The *in situ* light intensities used for the incubations may have been a limiting factor, particularly in the spring season.

Phytoplankton growth was, with one exception, not limited by macronutrient availability. Sufficient nutrient availability for the system studied here was indicated by both direct measurements of ample nutrient concentrations, even within patches, and the fact that nutrient addition to all but one dilution experiment did not yield enhanced growth rates. Although concentrations were not limiting to growth, macronutrient concentrations were significantly lower inside phytoplankton patches. In previous seasons, there were no significant differences in phytoplankton community composition between layer samples and adjacent waters (Menden-Deuer 2008). Similarity in community composition throughout the water column could have been the result of frequent exchanges of either organisms or water between layer and non-layer depths. The fact that layer samples contained significantly lower macronutrient concentrations suggests that water masses were separated for substantial amounts of time, on the order of a few days at minimum, so that nutrient uptake from the more concentrated phytoplankton community inside layers was reflected in lower nutrient concentrations.

Protist grazing pressure

Average grazing rates measured here were substantially lower (on average less than half) than grazing rates characteristic of coastal or temperate systems identified in a meta-analysis of all available dilution experiment data (Calbet & Landry 2004). The low average grazing rates observed here may have been due to low overall grazer biomass, activity or unsuitable prey species. Lower than average predation rates are not unprecedented (Strom et al. 2007, Fredrickson & Strom 2009) and have at times been attributed to predation by heterotrophic protists upon each other (Calbet & Landry 1999, Hansen & Jensen 2000).

Grazing rates measured inside phytoplankton patches were on average significantly higher than outside of such structures. This does not mean that no grazing was observed outside of patches and on a few occasions, substantive grazing was measured. Interestingly, increased grazing pressure inside layers was not a function of absolute prey concentration, as measured chl *a* concentrations ranged from low to high values for both layer and

non-layer samples. The highest grazing rates were observed at chl *a* concentrations of $<4 \mu\text{g l}^{-1}$. This lack of functional relationship between potential prey abundance and grazing rate has been observed previously in northern Puget Sound and the Gulf of Alaska (Strom et al. 2001). A possible explanation is that chl *a* is a poor indicator of phytoplankton species composition, prey palatability and suitability to protistan predators that are known to be highly selective feeders. Interpretation of predator–prey dynamics through the dilution method makes the generalization that chl *a* abundance is indicative of prey availability and needs to be done cautiously. Although we have shown that chl *a* concentrations within East Sound were positively correlated with phytoplankton carbon biomass (Menden-Deuer 2008), taxonomic differences are not revealed in this abundance approach. There is no gross measure of phytoplankton abundance currently available that properly reveals taxonomic diversity. Microscopic analyses, on the other hand, provide insight into the actual species present but statistically adequate samples to track predator–prey specificity are difficult to obtain, even from controlled mesocosm incubations (e.g. Rose et al. 2009). Application of molecular methods that track predation through prey-specific probes may be a fruitful addition to the dilution method (e.g. Durbin et al. 2008). The observed independence of grazing pressure with respect to prey density challenges the widely practiced approach in coupled models that make grazing rate a function of the instantaneous concentration of the phytoplankton abundance.

The dilution method has rightfully been criticized for numerous methodological shortcomings, recently reviewed by Agis et al. (2007). Many of these shortcomings apply to the experiments presented here. For example, whole plankton samples were screened through a 200 μm mesh to remove mesozooplankton (which are typically lower in abundance), because they would not adequately be represented numerically in the liter-scale bottles reducing replicability amongst bottles. However, Nejtgaard et al. (2001) have shown that mesozooplankton exert a significant top-down control by selectively feeding on heterotrophic protists, and can significantly alter the rates obtained. If mesozooplankton were a significant component of the plankton and selectively feeding on heterotrophic protists, then grazing rates would be overestimated. On the other hand, protists may significantly alter their feeding behavior as a function of prey concentration, which varied by over an order of magnitude among experiments. Thus, especially at high prey concentrations, grazing rates may have been saturated and thus underestimated. Variations in the experimental design, including the deliberate addition of mesozooplankton could shed light on whether these artifacts apply to layer dynamics and at what magnitude. Most

importantly, experimental artifacts may have spatial and temporal biases. The incubation of the plankton community for 24 h within a bottle, without the possibility of emigration or immigration may have significantly altered the exposure of predator and prey species. Some mesozooplankton have been observed to forage in the vicinity of plankton layers (Leising et al. 2005) and some heterotrophic protists are capable of exploiting prey layers through behavioral modifications (Menden-Deuer & Grünbaum 2006). Thus, immigration and emigration may alter the frequency of exposure of predator and prey and ultimately their trophic interactions.

Implications for trophic dynamics and productivity estimates

Measured grazing rates accounted for a significant fraction of phytoplankton productivity. Phytoplankton growth exceeded grazing rates both inside and outside layers but by a much smaller margin inside layers. The ratio of protistan grazing rate to phytoplankton growth rate, as an indicator of primary productivity consumed, was 0.65 inside and 0.26 outside of patches (Table 4). Thus, grazing accounted for a loss of two-thirds of phytoplankton productivity inside plankton layers but only one-quarter outside. Given these balances of growth and grazing rates, phytoplankton production could accumulate more rapidly outside of layers, due to lower predation pressure. When all grazing rates were averaged, irrespective of whether measurements were made inside or outside of layers, the grand average grazing rate was 0.16 d^{-1} . This average grazing rate would account for 45% of primary productivity, overestimating predation pressure outside of layers and underestimating predation pressure inside layers.

These differences in structured vs. averaged rates have been hypothesized to have implications for phytoplankton biomass and productivity estimates. The rates measured here provide the opportunity to

Table 4. Average protist grazing (g) and phytoplankton growth (μ) rates inside and outside of plankton patches as well as the hypothetical average of all rates, irrespective of plankton distribution. The mortality ratio ($g:\mu$) of grazing: growth rates indicates fraction of primary production consumed by predators

Sample source	g (d^{-1})	μ (d^{-1})	$g:\mu$
Outside patch	0.09	0.34	0.26
Inside patch	0.24	0.37	0.65
Hypothetical average	0.16	0.35	0.46

explore the implications of patch-focused grazing rates on biomass and water column productivity estimates. Using a simple, exponential growth model, we contrasted predictions of biomass and productivity after 24 h, following 2 procedures, based on assuming either spatially uniform or spatially structured rates: (1) calculating biomass based on the grand averages of all measured growth and grazing rates, and applying those rates to the entirety of the water column and (2) calculating biomass with the same model, but using spatially structured rates measured inside and outside of layers. Phytoplankton biomass and distribution within the water column were estimated using the conversion regression between fluorescence and chl *a* established here. Estimates were calculated for 15 vertical profiles that had taxonomically diverse phytoplankton layers with a mean vertical extent of 3.8 m at the base. This assessment did not make further assumptions about advection, nutrient or light limitation. These factors would presumably affect productivity predictions equally, irrespective of whether structured or spatially uniform rates were assumed.

The results of this comparison showed that use of spatially averaged growth and grazing rates lead to a systematic but small bias in biomass estimates and underestimated total water column productivity, compared to results obtained using structured rates. Net phytoplankton growth, using average rates, was predicted to be 0.18 d^{-1} throughout the water column. In contrast, inside and outside layer growth was predicted to be 0.10 and 0.28 d^{-1} , respectively. Assuming average rates throughout the water column resulted in an overestimate of biomass of about $4 \mu\text{g chl } a \text{ l}^{-1}$ inside layers (range 0.8 to 8.25) and an underestimate of about $1.7 \mu\text{g chl } a \text{ l}^{-1}$ outside layers (range 0.15 to 6.28). Extrapolating these differences over a 20 m water column and an average layer thickness of 3.8 m would result in an underestimate of about $12 \mu\text{g chl } a \text{ m}^{-2}$ or about 10% of total biomass. Although the ranges in estimates show that deviations can be substantial in some cases, the average difference is minimal compared to the often order of magnitude variation in measured productivity rates. Therefore, as long as growth inside and outside of layers is similar, patchiness has little effect on overall biomass and productivity estimates.

Formation, persistence and decline of plankton patches

It remains unclear which factors control the formation, persistence and decline of plankton patchiness. The data gathered here can provide insight into the relative importance of plankton population dynamics. Nutrient concentrations were lower inside than outside

of layers, indicating active uptake and phytoplankton growth inside those layers. Since phytoplankton biomass was at least 3-fold higher inside patches, higher levels of nutrient uptake could be expected, and possibly also competition for nutrients. All but one layer studied here dissipated after a few days, such that competition for nutrients was unlikely a limiting factor to phytoplankton growth. However, prior work (Rines et al. 2002, Menden-Deuer 2008) and the diatom bloom observed in spring 2009 showed that some layers can remain intact for several weeks, sometimes forming a phytoplankton bloom. Indeed, significant nutrient draw down was observed at the end of the spring 2009 sampling season. This suggests that, infrequently, nutrient limitation may terminate patches. Some predators have been shown to benefit from declining physiological status of nutrient limited phytoplankton prey and attained maximal grazing rates on decaying phytoplankton (Menden-Deuer et al. 2005). Such increased grazing pressure would further increase the rates of decline of layer intensity.

Since phytoplankton growth exceeded grazing inside layers, it may appear intuitive that layer formation and persistence would be supported by phytoplankton growth and that decline of layers due to grazing would be impossible. However, simulation of layer dynamics over 72 h suggests otherwise. Using the same exponential growth model as above, we examined the development of layer intensity over time and predicted phytoplankton distribution in the water column (Fig. 7). This comparison is meant as an illustration of how predator–prey interactions could affect layer dynamics. This comparison is not meant to suggest past or future dynamics of the specific layers observed, as relative growth and mortality rates, along with phytoplankton and protist population sizes, were likely not static over time. Continuous records of patch dynamics over time require a Lagrangian approach, such as was used by Landry et al. (2008).

Our model predictions show that the key to understanding and predicting layer dynamics lies in the relative rates of net phytoplankton growth inside and out-

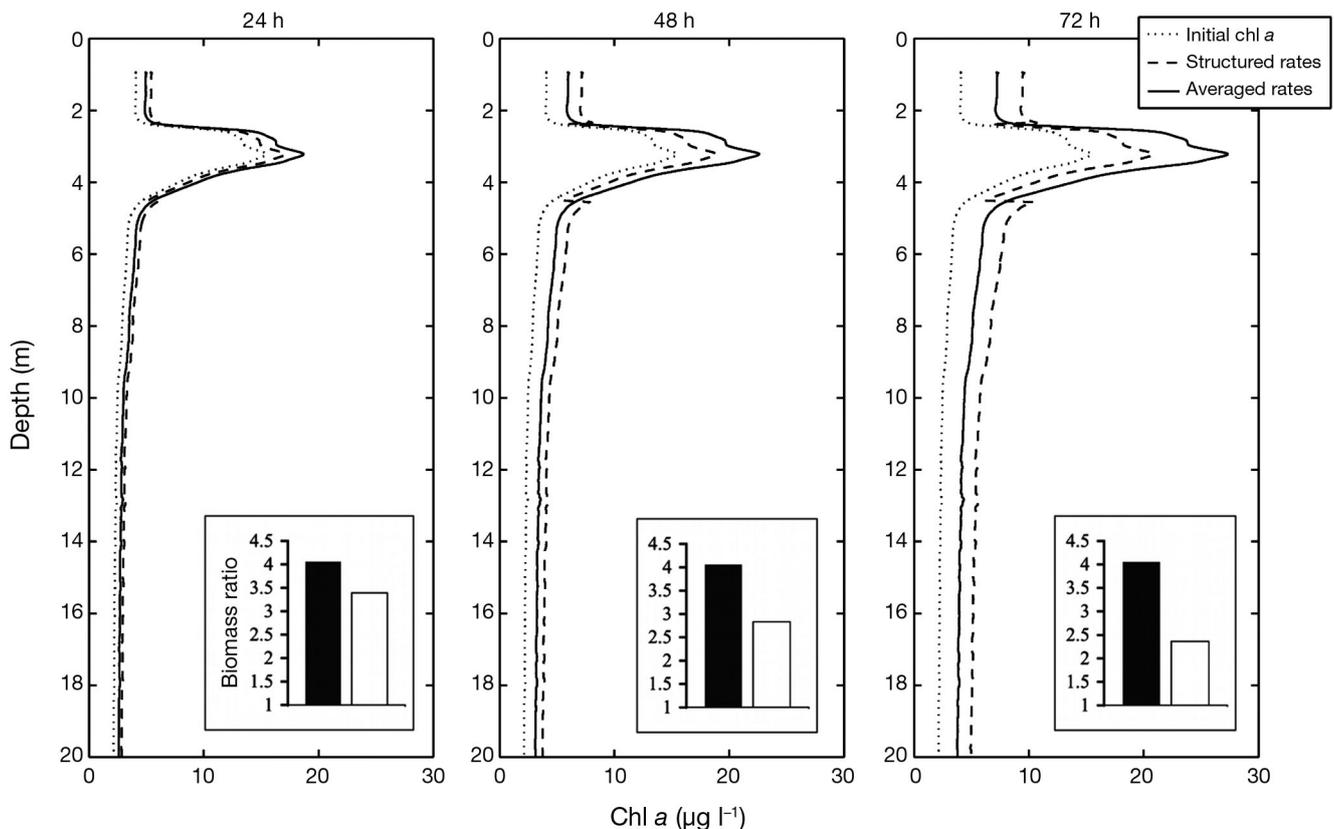


Fig. 7. Predicted development of a phytoplankton layer over 3 consecutive days assuming averaged protistan grazing rates throughout the water column and structure-dependent grazing rates. Growth rates were equal in the 2 scenarios. Inset shows the ratio of biomass inside the layer, relative to the same volume outside the layer for the average (solid bars) and structured rate models (open bars). Assumption of averaged rates throughout the water column predicts equal accumulation of biomass inside and outside of layers and layer persistence. Assumption of the observed, structure-dependent grazing rates predicts more rapid biomass accumulation outside layers and a decrease in layer intensity over time. These differential biomass accumulation rates lead to patch erosion after 48 h compared to persisting layers assuming uniform and averaged grazing rates. Note reversal of peak biomass accumulation inside and outside of layers depending on whether averaged or structured rates were assumed

side of layers. Grazing consumed on average 65% of phytoplankton productivity inside layers but only 26% of phytoplankton productivity outside of layers. Based on this imbalance, phytoplankton biomass outside of layers is predicted to accumulate more rapidly than inside layers. Simultaneously, more intense protist grazing pressure inside layers reduces the rate of biomass accumulation and thus layer intensity. It is the balance between the higher rate of biomass accumulation outside of layers and more rapid removal of biomass inside layers that leads to an erosion of sharp, vertical gradients in chl *a*. This result does not imply an overall decrease in biomass because layers are vertically restricted and occupy a narrow depth range compared to non-layer depths. In most layers examined, predicted phytoplankton biomass after 24 h was only twice as large as background abundance in the water column. Based on the criterion of 3-fold above background signals used here (Dekshenieks et al. 2001, Ryan et al. 2008) the layer would no longer be considered a patchy structure after 24 h. Thus, structure-dependent protist grazing has the potential to limit the persistence of layers and initiate their decline within hours to days.

Phytoplankton growth appeared to be an unlikely driver of layer formation or maintenance, due to the significant grazing pressure inside layers. Based on the rates measured here, phytoplankton biomass would have accumulated due to growth and reduced grazing outside of layers more rapidly rather than inside layers. However, if the measured growth were not balanced by grazing, in cases where predator biomass or feeding effort was low at the onset of layer formation, for example, then growth could very well result in the formation of plankton rich layers. Most of the layers observed were short lived (<1 wk), whilst the diatom layer in spring 2009 preceded a diatom bloom that encompassed the entire water column. Our results suggest that this latter bloom likely formed due to growth outside the initial patch. These results do not rule out layer formation due to other biological (e.g. behavior or aggregation) or biophysical interactions (e.g. aggregations at shear-boundaries; Durham et al. 2009).

Clearly, the trophic dynamics of plankton patches extend beyond phytoplankton–herbivore interactions. Benoit-Bird et al. (2009) used acoustics to show associations between phytoplankton, zooplankton and fish with plankton layers, and concluded that variations in shape were caused by trophic interactions. Plankton patches are likely sites of increased biological activity, providing enhanced resources for prokaryotes as well as higher trophic levels. Aggregation of any trophic level may act as a resource concentration for a higher trophic level, which implies increased risk of predation. The risk of predation due to aggregation is higher

for metazoan than protozoan predators due to their longer generation times, as Tiselius (1992) suggested based on a modeling study. Based on Tiselius' model, heterotrophic protists, and presumably also other species with hour-scale generation times, are expected to derive the greatest benefit from patch exploitation. The rate measurements presented here show that resource exploitation by heterotrophic protists was indeed enhanced in phytoplankton patches. Extension of this type of study over multiple trophic levels would provide insight into the importance of phytoplankton distributions to the magnitude of energy and matter transfer in pelagic food webs.

CONCLUSIONS

Phytoplankton patches were sites of intense biological activity that were isolated and coherent for at least a few days. The structure-dependent grazing rates strongly suggest that phytoplankton patches serve as important prey resources for heterotrophic protists. Moreover, the balance of phytoplankton growth and protist grazing implies that these biological drivers can promote layer decline, but formation of layers due to phytoplankton growth was not suggested by the data. Many previous studies have shown that heterotrophic protists are important consumers of phytoplankton production. Here we showed that accounting for the structure dependence in grazing pressure altered predictions of phytoplankton abundance and distributions. Averaging rates, irrespective of resource distributions, overestimated patch intensity and longevity and systematically underestimated primary production and its availability to higher trophic levels or the benthos, albeit by a small amount. The observed prey density-independent grazing pressure is a noteworthy challenge to the practice of scaling grazing pressure to instantaneous prey concentration. Grazing pressure may need to incorporate additional modulators, including predator selectivity. These results underline the importance of predator–prey interactions to the ubiquitous phenomenon of patchiness. These biological factors need to be quantified as a function of physical factors, including mixing and advection rates to estimate their relative importance in the formation and persistence of phytoplankton patches.

Acknowledgements. We thank the faculty and staff at the Shannon Point Marine Center, Western Washington University, especially S. Strom. RV 'Zoea' Skippers G. McKeen and N. Schwarck are gratefully acknowledged as well as the many graduate and undergraduate students who have helped with the research. Comments from 3 anonymous reviewers improved the manuscript considerably. This research was supported through funding from the Office of Naval Research

(N000140710136 and N000141010124) and a research fellowship from the German National Science Foundation (Deutsche Forschungsgemeinschaft).

LITERATURE CITED

- Abraham ER (1998) The generation of plankton patchiness by turbulent stirring. *Nature* 391:577–580
- Agis M, Granda A, Dolan JR (2007) A cautionary note: examples of possible microbial community dynamics in dilution grazing experiments. *J Exp Mar Biol Ecol* 341:176–183
- Allredge AL, Cowles TJ, MacIntyre S, Rines JEB and others (2002) Occurrence and mechanisms of formation of a dramatic thin layer of marine snow in a shallow Pacific fjord. *Mar Ecol Prog Ser* 233:1–12
- Baumgartner MF, Cole TVN, Campbell RG, Teegarden GJ, Durbin EG (2003) Associations between North Atlantic right whales and their prey, *Calanus finmarchicus*, over diel and tidal time scales. *Mar Ecol Prog Ser* 264:155–166
- Benoit-Bird KJ, Au WWL, Wisdom DW (2009) Nocturnal light and lunar cycle effects on diel migration of micronekton. *Limnol Oceanogr* 54:1789–1800
- Benoit-Bird KJ, Moline MA, Waluk CM, Robbins IC (2010) Integrated measurements of acoustical and optical thin layers I: Vertical scales of association. *Cont Shelf Res* 30:17–28
- Birch DA, Young WR, Franks PJS (2008) Thin layers of plankton: formation by shear and death by diffusion. *Deep-Sea Res I* 55:277–295
- Birch DA, Young WR, Franks PJS (2009) Plankton layer profiles as determined by shearing, sinking and swimming. *Limnol Oceanogr* 54:397–399
- Bjørnsen PK, Nielsen TG (1991) Decimeter scale heterogeneity in the plankton during a pycnocline bloom of *Gyrodinium aureolum*. *Mar Ecol Prog Ser* 73:263–267
- Butcher RW (1959) An introductory account of the smaller algae of British coastal waters. Part I: Introduction and Chlorophyceae. *Fishery Investigation Series IV*. Ministry of Agriculture, Fisheries and Food, London
- Calbet A, Landry MR (1999) Mesozooplankton influences on the microbial food web: direct and indirect trophic interactions in the oligotrophic open-ocean. *Limnol Oceanogr* 44:1370–1380
- Calbet A, Landry MR (2004) Phytoplankton growth, microzooplankton grazing and carbon cycling in marine systems. *Limnol Oceanogr* 49:51–57
- Cowles TJ (2004) Planktonic layers: physical and biological interactions on the small scale. In: Seuront L, Strutton P (eds) *Handbook of scaling methods in aquatic ecology: measurement, analysis, simulation*. CRC Press, Boca Raton, FL, p 31–49
- Deksheniaks MM, Donaghay PL, Sullivan JM, Rines JEB, Osborn TR, Twardowski MS (2001) Temporal and spatial occurrence of thin phytoplankton layers in relation to physical processes. *Mar Ecol Prog Ser* 223:61–71
- Donaghay PL, Rines HM, Sieburth JM (1992) Simultaneous sampling of fine scale biological, chemical and physical structure in stratified waters. *Ergeb Limnol* 36:97–108
- Durbin EG, Casas MC, Rynearson TA, Smith DC (2008) Measurement of copepod predation on nauplii using qPCR of the cytochrome oxidase gene. *Mar Biol* 153:699–707
- Durham WM, Kessler JO, Stocker R (2009) Disruption of motility by shear triggers formation of thin phytoplankton layers. *Science* 323:1067–1070
- Eppley RW, Holm-Hansen O, Strickland JDH (1968) Some observations on the vertical migration of dinoflagellates. *J Phycol* 4:333–340
- Franks PJS (1992) Sink or swim: accumulation of biomass at fronts. *Mar Ecol Prog Ser* 82:1–12
- Franks PJS (1995) Thin layers of phytoplankton: a model of formation by near-inertial wave shear. *Deep-Sea Res I* 42: 75–79
- Fredrickson KA, Strom SL (2009) The algal osmolyte DMSP as a microzooplankton grazing deterrent in laboratory and field studies. *J Plankton Res* 31:135–152
- Gallager SM, Yamazaki H, Davis CS (2004) Contribution of fine-scale vertical structure and swimming behavior to formation of plankton layers on Georges Bank. *Mar Ecol Prog Ser* 267:27–43
- Genin A, Jaffe JS, Reef R, Richter C, Franks PJS (2005) Swimming against the flow: a mechanism for zooplankton aggregation. *Science* 308:860–862
- Grünbaum D (2002) Predicting availability to consumers of spatially and temporally variable resources. *Hydrobiologia* 480:175–191
- Hansen BW, Jensen F (2000) Specific growth rates of protozooplankton in the marginal ice zone of the central Barents Sea during spring. *J Mar Biol Assoc UK* 80:37–44
- Holliday DV, Donaghay PL, Greenlaw CF, McGehee DE, McManus MA, Sullivan JM, Miksis JL (2003) Advances in defining fine- and micro-scale pattern in plankton. *Aquat Living Resour* 16:131–136
- Horner RA (2002) A taxonomic guide to some common marine phytoplankton. *Biopress*, Bristol
- Jeong HJ, Shim JH, Lee CW, Kim JS, Koh SM (1999) Growth and grazing rates of the marine planktonic ciliate *Strombidinopsis* sp. on red-tide and toxic dinoflagellates. *J Eukaryot Microbiol* 46:69–76
- Kiefer DA, Lasker R (1975) Two blooms of *Gymnodinium splendens*, an unarmored dinoflagellate. *Fish Bull* 73: 675–678
- Landry MR, Hassett RP (1982) Estimating the grazing impact of marine microzooplankton. *Mar Biol* 67:283–288
- Landry MR, Brown SL, Rii YM, Selph KE, Bidigare RR, Yang EJ, Simmons MP (2008) Depth-stratified phytoplankton dynamics in Cyclone Opal, a subtropical mesoscale eddy. *Deep-Sea Res II* 55:1348–1359
- Leising AW, Pierson JJ, Cary S, Frost BW (2005) Copepod foraging and predation risk within the surface layer during night-time feeding forays. *J Plankton Res* 27:987–1001
- Lorenzen CJ (1966) A method for the continuous measurement of the in vivo chlorophyll concentration. *Deep-Sea Res* 13:223–227
- McManus MA, Allredge AL, Barnard AH, Boss E and others (2003) Characteristics, distribution and persistence of thin layers over a 48 hour period. *Mar Ecol Prog Ser* 261:1–19
- McManus MA, Cheriton OM, Drake PJ, Holliday DV, Storzlazzi CD, Donaghay PL, Greenlaw CF (2005) Effects of physical processes on structure and transport of thin zooplankton layers in the coastal ocean. *Mar Ecol Prog Ser* 301:199–215
- Menden-Deuer S (2008) Spatial and temporal characteristics of plankton-rich layers in a shallow, temperate fjord. *Mar Ecol Prog Ser* 355:21–30
- Menden-Deuer S, Grünbaum D (2006) Individual foraging behaviors and population distributions of planktonic predators aggregating to phytoplankton layers. *Limnol Oceanogr* 51:109–116
- Menden-Deuer S, Lessard EJ, Satterberg J, Grünbaum D (2005) Growth and starvation survival capacity of three species of the pallium feeding thecate dinoflagellate genus *Protoperidinium* (Peridiniaceae, Dinophyceae) distributions. *Aquat Microb Ecol* 41:145–152

- Mullin M, Brooks E (1976) Some consequences of distributional heterogeneity of phytoplankton and zooplankton. *Limnol Oceanogr* 21:784–796
- Nejstgaard JC, Naustvoll J, Sazhin A (2001) Correcting for underestimation of microzooplankton grazing in bottle incubation experiments with mesozooplankton. *Mar Ecol Prog Ser* 221:59–75
- Paffenhöfer G, Sherr BF, Sherr EB (2007) From small scales to the big picture: persistence mechanisms of planktonic grazers in the oligotrophic ocean. *PSZNI: Mar Ecol* 28:243–253
- Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis, Elsevier, New York, NY
- Rines JEB, Donaghay PL, Deksheniaks MM, Sullivan JM, Twardowski MS (2002) Thin layers and camouflage: hidden *Pseudo-nitzschia* spp. (Bacillariophyceae) populations in a fjord in the San Juan Islands, Washington, USA. *Mar Ecol Prog Ser* 225:123–137
- Rines JEB, McFarland MN, Donaghay PL, Sullivan JM (2010) Thin layers and species-specific characterization of the phytoplankton community in Monterey Bay, California, USA. *Cont Shelf Res* 30(1):66–80
- Rose JM, Feng Y, Gobler CJ, Gutierrez R, Hare CE, Leblanc K, Hutchins DA (2009) Effects of increased pCO₂ and temperature on the North Atlantic spring bloom. II. Microzooplankton abundance and grazing. *Mar Ecol Prog Ser* 388: 27–40
- Ryan JP, McManus MA, Paduan JD, Chavez FP (2008) Phytoplankton thin layers caused by shear in frontal zones of a coastal upwelling system. *Mar Ecol Prog Ser* 354:21–34
- Sherr EB, Sherr BF (2007) Heterotrophic dinoflagellates: a significant component of microzooplankton biomass and major grazers of diatoms in the sea. *Mar Ecol Prog Ser* 352:187–197
- Stacey MT, McManus MA, Steinbeck JV (2007) Convergences and divergences and thin layer formation and maintenance. *Limnol Oceanogr* 52:1523–1532
- Stacey MT, McManus MA, Steinbeck JV (2009) Response to 'Plankton layer profiles as determined by shearing, sinking, and swimming' by D. A. Birch, W. R. Young, and P. J. S. Franks. *Limnol Oceanogr* 54:400
- Stocker R, Seymour JR, Samadani A, Hunt DE, Polz M (2008) Rapid chemotactic response enables marine bacteria to exploit ephemeral microscale nutrient patches. *Proc Natl Acad Sci USA* 105:4209–4214
- Stoecker DK, Davis LH, Anderson DM (1984) Fine scale spatial correlations between planktonic ciliates and dinoflagellates. *J Plankton Res* 6:829–842
- Strickland JDH, Parsons TR (1972) A practical handbook of seawater analysis, Bull 167. Fisheries Research Board of Canada, Ottawa
- Strom SL, Fredrickson KA (2008) Intense stratification leads to phytoplankton nutrient limitation and reduced microzooplankton grazing in the southeastern Bering Sea. *Deep-Sea Res* 55:1761–1774
- Strom SL, Morello TA (1998) Comparative growth rates and yields of ciliates and heterotrophic dinoflagellates. *J Plankton Res* 20:571–584
- Strom SL, Brainard MA, Holmes JL, Olson MB (2001) Phytoplankton blooms are strongly impacted by microzooplankton grazing in coastal North Pacific waters. *Mar Biol* 138: 355–368
- Strom SL, Olson MB, Macri EL, Mordy CW (2006) Cross-shelf gradients in phytoplankton community structure, nutrient utilization, and growth rate in the northern coastal Gulf of Alaska. *Mar Ecol Prog Ser* 328:75–92
- Strom SL, Macri EL, Olson MB (2007) Microzooplankton grazing in the coastal Gulf of Alaska: variations in top-down control of phytoplankton. *Limnol Oceanogr* 52:1480–1494
- Thronsen J (1978) Preservation and storage. In: Sournia A (ed) *Phytoplankton manual*. UNESCO, Paris, p 69–74
- Tiselius P (1992) Behaviour of *Arcatia tonsa* in patchy food environments. *Limnol Oceanogr* 37:1640–1651
- Velo-Suyrez L, Fernand L, Gentien P, Reguera B (2010) Hydrodynamic conditions associated with the formation maintenance and dissipation of a phytoplankton thin layer in a coastal upwelling system. *Cont Shelf Res* 30(2): 193–202

Editorial responsibility: Hans Heinrich Janssen, Oldendorf/Luhe, Germany

Submitted: March 20, 2010; Accepted: September 29, 2010
Proofs received from author(s): December 6, 2010