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Low temperature constrains growth rates but not short-term ingestion rates of Antarctic ciliates

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Abstract Low environmental temperature is a major factor affecting the feeding activities, growth rates, and growth efficiencies of metazooplankton, but these features are poorly characterized for most protistan species. Laboratory experiments were conducted to examine the growth and ingestion rates of cultured herbivorous Antarctic ciliates. Three ciliates fed several algal species individually at 0 °C exhibited uniformly low growth rates (<0.26 day^{-1}), but the algae varied substantially in their ability to support ciliate growth. Specific ingestion rate (prey biomass consumed per unit ciliate biomass per unit time) was strongly affected by ciliate physiological state (starved vs. actively growing). Starved cells ingested many more prey than cells in balanced growth during short-term (minutes-to-hours) experiment but did not grow faster, indicating temperature compensation of ingestion rate but not growth rate. Field experiments were also conducted in the Ross Sea, Antarctica, to characterize the feeding rates of ciliates in natural plankton assemblages. Specific ingestion rates of two dominant ciliates were an order of magnitude lower than rates reported for temperate ciliates, but estimated rates were strongly affected by prey abundance. Our data indicate that short-term ingestion rates of Antarctic ciliates were not constrained by low environmental temperature although overall growth rates were, indicating the need for caution when designing experiments to measure the ingestion rates of these species at low environmental temperature. We present evidence that artifacts arising from estimating ingestion in short-term experiments may lead to errors in estimating feeding impact and growth efficiencies that are particularly large for polar protists.

Keywords Antarctic ciliates · Growth rates · Ingestion rate · Ross Sea · Heterotrophic protist

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Introduction

Polar microbial eukaryotes are highly diverse and abundant assemblages. The magnitude and breadth of these assemblages in the plankton and in sea ice have been well known for decades (Sullivan et al. 1982; Marchant 1985; Garrison et al. 1986; Stoecker et al. 1993; Archer et al. 1996; Smith and Gordon 1997; Umani et al. 1998; Gradinger et al. 1999), and recent gene-based surveys have continued to expand our knowledge of these assemblages (Gast et al. 2004; Lovejoy et al. 2006). These phototrophic, heterotrophic, and mixotrophic species play fundamental roles as primary producers, and as consumers of archaea, bacteria, cyanobacteria, and other protists.

Microzooplankton are key players in the top-down control of phytoplankton and the trophic transfer of energy in aquatic ecosystems (Gifford et al. 1995; Calbet and Landry 2004; Buitenhuis et al. 2010), and these species

have been implicated as the dominant herbivores in Antarctic coastal marine waters and other polar ecosystems (Laurion et al. 1995; Becquevort 1997; Caron et al. 2000; Lonsdale et al. 2000; Smetacek et al. 2004; Caron and Gast 2010). The permanently cold coastal waters surrounding the continent of Antarctica have been reported to have periodically high abundances of microzooplankton (Garrison 1991; Gowing and Garrison 1992; Garrison and Gowing 1993; Stoecker et al. 1995; Caron et al. 2000; Dennett et al. 2001; Smetacek et al. 2004). Seasonally high abundances of herbivorous protists have led to the deduction that herbivory by these taxa exerts a controlling influence on phytoplankton blooms in this region. The low palatability of many Antarctic phytoplankton as food for copepods and other metazoa has further implicated herbivorous protists as important trophic links at the base of the food web in these coastal ecosystems (Caron et al. 1997; Lonsdale et al. 2000).

The high diversity, large standing stocks, and key trophic activities of herbivorous protists in the plankton and sea ice ecosystems of polar regions have focused considerable attention in recent years on characterizing the diversity, basic physiology, and trophic activities of these assemblages at very low environmental temperature (Scott and Marchant 2005; Caron and Gast 2010). Low rates of microzooplankton community herbivory appear to be correlated with very low temperature (see Fig. 3b in Caron et al. 2000). One recent review of a large dataset of intrinsic growth rates of protists confirmed a differential effect of temperature on the maximal growth rates attainable by herbivorous and phototrophic protists at low environmental temperatures (Rose and Caron 2007). Protistan phytoplankton exhibited maximal growth rates in excess of their protistan consumers at polar temperatures (all other variables being non-limiting), an effect that may relate to key cellular or enzymatic functions unique to phototrophic or herbivorous protists (Caron and Rose 2008; López-Urrutia 2008). The differential effect of temperature on phototrophic and herbivorous protists may, in part, retard microzooplankton trophic activities in early austral summer and explain the high standing stocks of phytoplankton attained seasonally in this region (DiTullio and Smith 1996; Smith and Gordon 1997).

There is considerably less information regarding the effects of low temperature on other cellular processes of herbivorous protists such as gross growth efficiency. Protistan gross growth efficiency (GGE = % carbon from prey that is converted into consumer biomass) is an important aspect of protistan physiology that dictates the relative importance of phagotrophic protists as nutrient remineralizers and as trophic links in aquatic food webs (Calbet and Saiz 2005; Buitenhuis et al. 2010). GGEs that have been reported for protists are highly variable ($\approx$0 to >80 %; summaries in Caron et al. 1990; Straile 1997; Schmoker et al. 2011). Much of this variability has been attributed to the effects of prey abundance, suitability, and/or elemental stoichiometry (Elser et al. 2000; Jeong et al. 2004; Chen et al. 2010). There are few, and conflicting, data that specifically address the effect of low environmental temperature on protistan growth efficiency, but studies have reported decreases (Mayes et al. 1997), increases (Choi and Peters 1992), or constant GGE of protists (Rose et al. 2009) with decreasing temperature.

Estimates of GGE for protists have traditionally been determined by comparing estimates of the ingestion of prey biomass to the production of consumer biomass. Typically, the former feature is estimated from changes in prey abundance during a specified period of feeding by the predator, or via measurements of the short-term (minutes-to-hours) ingestion rates of the predator. An underlying assumption to this approach is that temperature should not uniquely affect the measurement of ingestion rates if the consumer population is acclimated, in balanced growth, and if the feeding activities of the protist are not temperature compensated (i.e., if ingestion rates are commensurate with rates of digestion and growth as constrained by temperature). However, short-term feeding rates of protists are strongly affected by prey abundance and could provide erroneously high estimates of prey ingestion if rates of digestion and ensuing anabolic processes are slower than rates of prey acquisition. In short, if low temperature creates a rate bottleneck for food processing but not for food capture, then overestimation of ingested prey could occur. Measurements of GGE in the latter situation would be erroneously low (i.e., less consumer biomass would appear to be produced per unit of prey consumed). Significantly, overestimations of short-term ingestion rates could also yield erroneously high estimates of the trophic impact of protists on natural phytoplankton assemblages.

The overall objective of this study was to characterize the effect of food quality and prey abundance on the ingestion rates and growth rates of Antarctic herbivorous ciliates at low environmental temperature ($\approx$0 °C). We hypothesized that low temperature was a major factor constraining the overall maximal growth rates of the ciliates, but that ingestion rates were more strongly affected by prey abundance than by temperature (i.e., growth rates were temperature constrained but ingestion rates were temperature compensated). Temperature compensation of ingestion could explain reports of very low gross growth efficiency that have been reported for some polar protists. Our hypothesis was examined by measuring the growth of three Antarctic ciliates in the laboratory when provided several algal prey species and the responsiveness of ingestion rate at low temperature when offered prey at different abundances. Field experiments were also carried

out in the Ross Sea to investigate ciliate ingestion rates in natural plankton assemblages and the responsiveness of ingestion rate to the abundance of surrogate prey employed to measure ingestion rate. Our results provide an explanation for some of the highly variable GGEs that have been reported for Antarctic protists, and they indicate the importance of performing feeding rate measurements at environmentally appropriate prey abundances.

Materials and methods

Cultures employed in laboratory studies

Cultures of Antarctic phototrophic and heterotrophic protists used in the experiments were isolated from samples of pack ice, seawater or pack ice meltwater at the ice/water interface at the surface of pack ice, collected over a series of research cruises in the Ross Sea, Antarctica. Three ciliate species were used in two experiments to investigate growth and feeding by herbivorous protists. Clonal cultures were established for each taxon, and identifications were accomplished based on morphological criteria (Petz et al. 1995; Lee et al. 2000; Scott and Marchant 2005) and 18S rDNA sequence similarity to the phylogenetically closest taxon in GenBank.

Euplotes sp. (clone I-99 Hypo) and *Strombidium* sp. (clone I-256 Ciliate) were isolated from ice cores collected in the pack ice region of the Ross Sea, Antarctica in January, 1999 at 68°07′S, 175°53′E and in January 2003 at 68°08′S, 175°33′E, respectively. Cores were obtained using a hand powered Sipre corer. Two sections (27–47 cm from the ice surface, and the bottom 15 cm) were slowly melted into 1-L sterile seawater in the dark at 0 °C over the course of several days. The melted core was enriched with F medium nutrient stock solutions (Guillard 1975) (*Euplotes* sp.) or K medium nutrient stock solutions (Keller et al. 1987) (*Strombidium* sp.) in order to stimulate growth of the natural phytoplankton assemblage. *Parauronema* sp. (clone SL-220 Scut) was enriched from meltwater at the ice/snow interface at the surface of pack ice from the same region in January, 1999. Clonal cultures of the ciliates were established once ciliate abundances increased in the phytoplankton enrichments. Individual cells were micropipetted, rinsed, and placed into sterile seawater, to which a few drops of cultured Antarctic algae were added. All manipulation and culturing was performed at 0 °C. Sequences subjected to BLAST searches against the GenBank database for *Euplotes* sp. were 96 % similar (with 2 gaps) to *Euplotes rariseta* (GenBank accession number KC287214), *Strombidium* sp. was 97 % similar to *Strombidium* species (AF399115) (Snoeyenbos-Wes et al. 2002), and *Parauronema* sp. was a scuticociliate 98 % similar to *Parauronema virginianum* (accession number KC287215).

Seven species of phototrophic protists were used as prey for the ciliates in the experiments. *Pyramimonas* sp. (clone RS-11) was isolated by dilution extinction from a water sample collected at 73°30′S, 176°50′W in November 1997 and enriched with nutrient stock solutions. The 18S sequence was 97 % similar to *Pyramimonas* sp. (accession numbers EF432515, EF432532, EF432516) and was identified as *Pyramimonas* cf. *tychotreta* by Daugbjerg (2000). *Fragilariopsis* sp. 1 (clone SL-64/78Cheetos) and *Fragilariopsis* sp. 2 (clone SL-64/78Frag) were isolated by dilution extinction from an enriched meltwater sample collected at 68°59′S, 164°59′W in January 1999. The 18S genes of both clones had 99 % similarity to *Fragilariopsis* (accession number EF432521 for clone SL-64/78Cheetos; accession number KC287216 for clone SL-64/78Frag). *Polarella glacialis* (clone RS-6) was isolated by dilution extinction from an enriched water sample collected at 76°37′S, 169°33′E in November 1997 and was identified as *Polarella* based on Montresor et al. (2003). The 18S sequence was 99 % similar to *P. glacialis* (accession number EF432528). *Chlamydomonas* sp. (clone I-155Chlamydomonas) was isolated by dilution extinction from an ice core collected at 71°59′S, 150°02′W in January 1999 (accession number EF432529). *Mantoniella antarctica* (clone SL-175) was isolated from an enriched meltwater sample collected at 68°59′S, 164°59′W in January 1999. The sequence of the isolate was 99 % similar to *M. antarctica* (accession numbers EF432530, EF432477, EF432411, EF432476). *Phaeocystis* sp. (clone W5-1Phaeo) was isolated from a water sample at 68°59′S, 164°59′W in January 1999 and had 99 % sequence similarity to *Phaeocystis antarctica* (accession number EF432545). *Mallomonas* sp. (clone I-76) was isolated by dilution extinction from an ice core collected at 68°05′S, 164°58′W in January 1999. The 18S gene of this clone was 94 % similar to *Mallomonas annulata* (accession number EF432525). All water and ice samples were collected and maintained at temperatures <2 °C, and all algae were enriched and isolated under the same conditions in order to prevent selection against heat-sensitive protists. All algal cultures were maintained in F/2 medium (Guillard 1975) at 0 °C on a 12:12 L:D cycle.

An unidentified Antarctic bacterial strain was isolated by streaking a sample from one of the protistan enrichments onto Marine Agar 2216 (BD Diagnostics), picking individual colonies, and culturing the isolates in Marine Broth 2216. Bacteria were stored on agar slants at 4 °C.

Laboratory experiments: growth rates of herbivorous ciliates

Experiments were carried out to examine the growth rates of the ciliates *Strombidium*, *Parauronema,* and *Euplotes*

when these ciliates were grown separately on several types of prey. Algae used as prey in these experiments were grown to early stationary growth phase in F/2 medium at 0 °C on a 12:12 h light/dark cycle. All prey were provided to the ciliates at high abundances to ensure that growth rates of the consumers were not constrained by prey abundance. The choice of prey was determined by cultures that were available at the time of the experiments. Experiments with *Euplotes* and *Parauronema* were conducted at the same time, and *Strombidium* was examined at a different time.

Growth of *Strombidium* sp. was examined when offered the following six algae individually:

1. *Fragilariopsis* sp. 1
2. *Fragilariopsis* sp. 2
3. *Pyramimonas* sp.
4. *P. glacialis*
5. *Chlamydomonas* sp.
6. *Mallomonas* sp.

Growth of the ciliates *Parauronema* and *Euplotes* was examined separately when offered the following prey individually:

1. *M. antarctica*
2. *Mallomonas* sp.
3. *Phaeocystis* sp.
4. *Pyramimonas cf. tychotreta*
5. A mixture of approximately equal quantities of the four algae above
6. Bacteria

Cultures of *Phaeocystis* sp. contained both free-living cells and small colonies. Bacteria were grown in Marine Broth with gentle shaking, centrifuged, rinsed, and resuspended in natural seawater. Ciliates were inoculated at low relative abundance (<50 mL^{-1}) into triplicate algal cultures of each prey species at the beginning of the experiments and grown for up to 26 days with sampling approximately every 2–4 days (an appropriate time interval for these species growing at 0 °C). *Strombidium* was acclimated to each prey type prior to examining growth on the different algae, while *Parauronema* and *Euplotes* were maintained on bacteria or *Geminigera cryophila*, respectively, and used as inocula when prey in the maintenance cultures were reduced to very low abundance to minimize transfer to experimental treatments. Experiments were performed in continuous darkness to prevent growth of algal prey.

Samples were removed and preserved with 2 % acid Lugols or 10 % formalin. All containers were sampled on ice in a 4 °C cold room during the experiment to avoid changes in temperature. Ciliate abundances were obtained from subsamples counted using a Sedgwick Rafter or Palmer-Maloney counting chamber (depending on the species and abundance) using light microscopy. Growth rates were calculated for all treatments based on the linear portion of a plot of natural log cell abundance versus time [i.e., logarithmic growth phase; linear portion in Fig. 1a)].

Laboratory experiments: short-term ingestion rates of *Strombidium* sp.

Laboratory studies were conducted to examine the relationship between the physiological state (starvation vs. balanced growth) of *Strombidium* sp. and its short-term rate of prey ingestion. Specifically, this experiment examined whether starved ciliates could exhibit high ingestion rates, or whether these rates were constrained by low environmental temperature. The diatom *Fragilariopsis* sp. (strain 1), grown to late exponential growth phase in F/2 medium, was used as prey because *Strombidium* sp. grew well on this alga, and the alga stained well using the vital stain CellTracker^{TM} Green (Molecular Probes, Eugene, OR) at 1 μM (Li et al. 1996). *Fragilariopsis* sp. also retained the stain inside and outside of microzooplankton food vacuoles after preservation with ice-cold 1 % glutaraldehyde. *Strombidium* sp. was grown to high abundance on the *Fragilariopsis* sp. culture until the alga was reduced to very low abundance and growth of the ciliate ceased. *Strombidium* sp. was then maintained under starvation conditions for 2 weeks prior to use as an inoculum for the experiment. Starvation of *Strombidium* sp. resulted in reduced cell volume but cells remained motile.

Fragilariopsis sp. cells stained with CellTracker Green were added to a dense, unstained culture of *Fragilariopsis* sp. at ~15 % of the abundance of the unstained cells. Starved *Strombidium* sp. were then added at ~300 cells mL^{-1} to triplicate cultures of the dense mixture of stained and unstained diatoms. Duplicate subsamples were removed from each of the triplicate cultures immediately and after 15, 30, 60, and 120 min and preserved with 1 % ice-cold glutaraldehyde (final concentration). Each subsample was examined using transmitted light and epifluorescence microscopy in a Sedgwick Rafter slide, and the number of ingested, stained algal cells was counted inside the first 30 ciliates encountered at each time point. The total number of ingested cells in each subsample was then calculated from the ratio of unstained:stained cells and the number of ingested stained cells. A regression of the total number of ingested prey in each of the triplicate cultures versus time was then used to determine ingestion rates in the cultures.

Starved *Strombidium* cells were also placed into dense cultures of the unstained diatom at the beginning of the experiment and maintained in a lighted incubator at 0 °C for 2 or 4 days to allow the ciliates to reestablish feeding

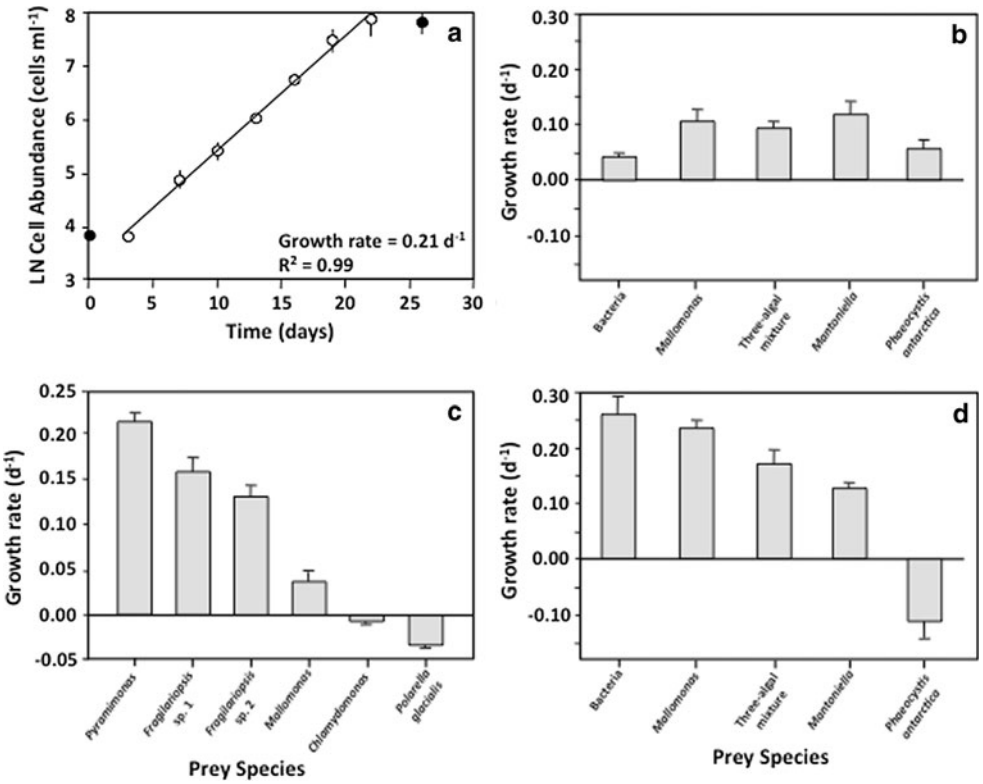

Fig. 1 Growth rates of herbivorous Antarctic ciliates in culture. **a** Example of the growth curve of *Strombidium* sp., fed the prasinophyte, *Pyramimonas* sp. *Open circles* indicate the data points used to generate the regression and calculate the growth rate of the ciliate. Growth rates of *Euplotes* sp. **b**, *Strombidium* sp. **c**, and *Parauronema* sp. **d** on unialgal cultures of several different species of Antarctic algae, a mixture of the three algal species or bacteria

and growth. Aliquots were removed after 2 or 4 days, placed into the mixture of stained and unstained diatoms, and rates of ingestion of the diatoms were determined as described above. Initial abundances of *Fragilariopsis* in these cultures at day 2 and 4 were not substantially different from abundances at day 0.

Field experiments: short-term ingestion rates of microplankton in natural assemblages

Experiments were conducted in the Ross Sea polynya, Antarctica aboard the RVIB *Nathaniel B. Palmer* to determine the short-term ingestion rates of microplankton (predominantly aloricate and loricate ciliates and the heterotrophic dinoflagellate *Protoperidinium* sp.) from natural plankton assemblages using cultures of Antarctic algae (*Fragilariopsis* sp. 1) stained with CellTracker Green as described above. Four experiments were carried out during austral summer (late December 2004 and January 2005) using water collected from a depth of 5 m using Niskin bottles. The stations were located at 77°00′S, 180°00′E (station 1), 74°30′S, 173°30′E (station 2, occupied twice, separated in time by approximately 2 weeks), and 75°00′S, 167°00′E (station 3).

All experiments were carried out in the dark at 0 °C and consisted of a series of subsamples of a natural water sample contained in 150-cm^2 polystyrene tissue culture flasks to which stained diatoms were added at three abundances ranging from 10^6 to 10^8 cells L^{-1}. The maximal abundances of *Fragilariopsis* sp. that could be added to each mixed plankton assemblage were limited by the abundance of *Fragilariopsis* sp. that could be attained in the algal culture, and the amount of CellTracker Green that could be added to whole seawater without the risk of fluorescently staining algae and microzooplankton present in the natural plankton assemblages. An aliquot of the fluorescently stained algal culture was filtered (0.2 μm) and the filtrate was added to an aliquot of natural seawater to ensure that the plankton assemblage did not take up the fluorescent stain directly from solution. All experimental and control (filtrate) treatments were performed in triplicate.

Feeding by microzooplankton was assessed in 125 mL samples removed immediately and after 30 min (treatments with $\geq 10^7$ algal cells L^{-1} added) or 120 min (treatments with $\leq 5 \times 10^6$ algal cells L^{-1} added). Ingestion of diatoms by the natural microzooplankton assemblage was assumed to be linear during this time period based on the results of experiments conducted with *Strombidium* sp. in the laboratory (see "Results"). Samples were preserved with 1 % ice-cold glutaraldehyde (final concentration), and microzooplankton were concentrated by settling each sample for at least 24 h in the dark, siphoning off the top approximately 200 mL, and then centrifuging the remaining 50 mL in a clinical centrifuge at top speed for 10 min. The top 45 mL was then siphoned

off, and the remaining 5 mL was examined in 1 mL aliquots by light microscopy using a Sedgwick Rafter counting chamber. The supernatant was examined periodically to ensure that microzooplankton were not lost in this process.

The first 30 microzooplankton encountered were examined by epifluorescence microscopy for ingested stained algal cells. The rate of uptake of stained diatoms and the ratio of stained to total algal abundance were used to calculate community ingestion rates. The rates for two common tintinnid ciliate genera were also determined by examining either the first 30 cells of each taxon encountered or the number of cells encountered in an entire 1-mL aliquot. Rates reported here for these two genera are those for which at least 15 cells were observed per experiment. Ingestion rates could not be determined for *Protoperidinium* sp. because the natural apple-green fluorescence of the dinoflagellate interfered with visualization of ingested fluorescently labeled algae. However, since *Protoperidinium* sp. did not numerically dominate the microzooplankton assemblage during any of the experiments, we believe this did not significantly affect our estimations of ingestion by the total microzooplankton assemblage.

Microplankton standing stock and trophic impact

Starting abundances of the natural microzooplankton assemblages (ciliates and identifiable heterotrophic dinoflagellates) were determined from samples collected at the time of each experiment and preserved with 10 % acid Lugols solution. Only cells in the microplankton size range (20–200 μm) were counted because the size of the labeled prey used to investigate ingestion precluded ingestion by most nanoplanktonic protists (i.e., <20 μm). Samples (100 mL) were settled in settling chambers for at least 18 h and counted at 200× magnification using inverted microscopy (Utermöhl 1958). Ciliates and the heterotrophic dinoflagellates *Protoperidinium* and *Gyrodinium* were enumerated but other dinoflagellate genera were not recorded because their morphologies were not distinctive and the Lugols preservative made it impossible to distinguish phototrophic from heterotrophic taxa.

The trophic impact of the microzooplankton at the time of the field experiments was estimated from total standing stock of the microplankton assemblage and their measured ingestion rates, as described above. Standing stock of phytoplankton was estimated from chlorophyll determined fluorometrically from 100 to 500 ml samples filtered onto Whatman® GF/F glass fiber filters (Parsons et al. 1984), assuming an average carbon:chlorophyll ratio of 111 for Ross Sea phytoplankton (Smith et al. 1996). The total phytoplankton carbon consumed per day was estimated by multiplying the average ingestion rates measured for the microzooplankton assemblage (g C d^{-1}) by the total abundance of microzooplankton. The percentage of phytoplankton standing stock grazed per day was then estimated by dividing the total phytoplankton carbon grazed per day by the phytoplankton standing stock.

Specific ingestion rates of Antarctic ciliates

Short-term ingestion rates (algal cells ingested ciliate^{-1} h^{-1}) of *Strombidium* sp. (clone I-256 Ciliate) and two dominant tintinnid ciliates from the field experiments were converted to specific ingestion rate (h^{-1}). Specific ingestion rate normalizes the ingestion rates of the consumer to the size of the consumer (Hansen et al. 1997). The dimensions of ciliates and algae in culture and in natural plankton assemblages, preserved in 10 % Lugols solution, were determined using digital photographs of the protists taken using phase contrast microscopy and an inverted microscope (Leica DM IRB) at 200× (ciliates) and 400× (algae) magnification. Measurements of the length and width of preserved cells were performed on the digital photographs using the software program Openlab 3.5.1. Linear dimensions were converted to volume based on standard equations. Biovolumes of the ciliates were converted into g C cell^{-1} assuming a conversion factor of (0.2 × wet weight in grams) = dry weight and (0.4 × dry weight) = g C (Beers and Stewart 1971; Beers et al. 1975; Gifford and Caron 1999). Algal cell volumes were converted into g C based on the equation, log $C = -0.314 + 0.712x$ (log volume) from Strathmann (1967). Specific ingestion rate was calculated as (pg C ingested ciliate^{-1} h^{-1})/(pg C ciliate^{-1}).

Results

Laboratory experiments

Growth rates of the three Antarctic ciliates were low on all algae tested, but varied significantly among the algal genera used as prey (Fig. 1). Growth rates of the ciliate *Strombidium* sp. varied from −0.03 day^{-1} (i.e., cell death in the treatment with *P. glacialis*) to 0.21 day^{-1} (when *Pyramimonas* sp. was used as food; Fig. 1c). Positive growth of the ciliate was supported by *Pyramimonas* sp., *Fragilariopsis* sp. 1 and sp 2., and *Mallomonas* sp.. Neither *Chlamydomonas* sp. nor *P. glacialis* supported positive growth of *Strombidium* sp.. Growth rates were similar when the *Strombidium* sp. was fed two strains of *Fragilariopsis* sp. (0.16 and 0.13 day^{-1}). Growth rates of *Euplotes* sp. ranged from 0.045 day^{-1} when bacteria were offered as prey to 0.12 day^{-1} when grown with *M. antarctica* as prey (Fig. 1b) but all prey types yielded positive

growth of this flagellate. Intermediate values were obtained when this ciliate was fed *P. antarctica* (0.057 day^{-1}), an algal mix (0.094 day^{-1}) or *Mallomonas* sp. (0.11 day^{-1}). *Parauronema* sp. exhibited the highest growth rate of the three ciliates and the most variable for the prey examined, with values ranging from −0.11 day^{-1} using *P. antarctica* as prey to 0.26 day^{-1} when bacteria alone were offered as prey (Fig. 1d). *M. antarctica*, *Mallomonas* sp., and the mixture of four algal types all yielded intermediate growth rates of *Parauronema* sp. (0.13, 0.23, and 0.18 day^{-1}, respectively).

The ingestion rates of *Strombidium* sp. varied greatly depending on whether cultures were starved prior to being offered prey or were in balanced growth (Fig. 2). Ciliates starved for approximately 2 weeks prior to additions of high abundances of *Fragilariopsis* sp. averaged 12 ingested algal cells $ciliate^{-1}$ only 15 min after prey addition and attained a maximum of approximately 18 ingested algae $ciliate^{-1}$ at 60 min (Fig. 2, closed circles). Digestion of stained cells had begun in many of the ciliates by 120 min, and individual prey could not always be reliably enumerated in food vacuoles within each ciliate at that time. Therefore, the results from this time point were not included.

Considerably lower rates of ingestion of the ciliate were obtained when starved ciliates were incubated with high densities of *Fragilariopsis* sp. for 2 or 4 days prior to the performance of the ingestion rate measurements (Fig. 2, open circles, inverted triangles). Fifteen minutes after the addition of labeled prey in ciliates exposed to high abundances of prey for 2 days, the average total number of ingested algal cells was 2.3 $ciliate^{-1}$ (Fig. 2, open circles), a nearly eightfold lower rate than observed for starved ciliates. The number of ingested cells appeared to be still increasing at 120 min, at which time the number of ingested algal cells was 10.8 $ciliate^{-1}$. Ingestion rates of *Strombidium* sp. fed *Fragilariopsis* sp. at high density for 4 days prior to the performance of the ingestion rate measurements were similar overall for ciliates fed for 2 days (Fig. 2, inverted triangles). Fifteen minutes after the addition of labeled cells, the average number of ingested algae was 1.8 $ciliate^{-1}$. The number of ingested prey $ciliate^{-1}$ approached a maximum after 60 min, and the number of ingested algal cells was the same at 120 and 180 min (7.7). Ingestion rates from these three experiments were 32.9, 8.94, and 6.23 algal prey ingested $ciliate^{-1}$ h^{-1} for the starved, fed 2 days, and fed 4 days treatments, respectively.

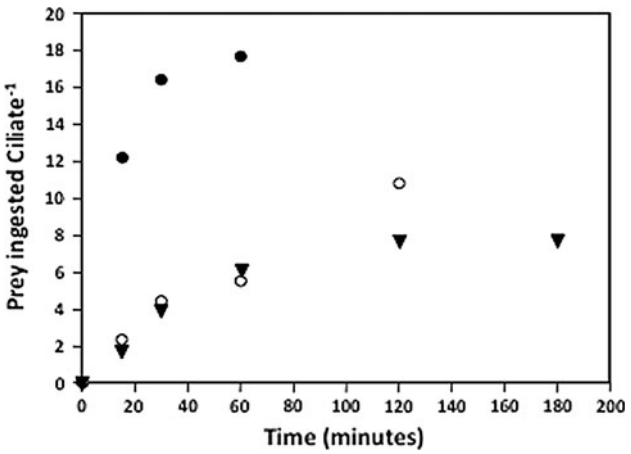

Fig. 2 Ingestion rates of the cultured Antarctic ciliate *Strombidium* sp. fed the Antarctic diatom *Fragilariopsis* sp. after starvation (*closed circle*), after 2 days of feeding on a dense culture of *Fragilariopsis* sp. (*open circle*) and after 4 days of feeding on the diatom (*closed inverted triangle*)

Specific ingestion rate (rate of ingestion of prey biomass per unit of consumer biomass per unit time) of the starved *Strombidium* sp. was 0.187 h^{-1}. The specific ingestion rate decreased markedly for the cultures in balanced growth to 0.0510 h^{-1} after 2 days of feeding on a high abundance of prey, and 0.0354 h^{-1} after 4 days of feeding. These lower specific ingestion rates represent approximately four- to fivefold decreases from the short-term rate measured for starved ciliates.

Field experiments

Phytoplankton and microzooplankton abundances were relatively high at the time of all four experiments in the Ross Sea, and evidence of herbivory by microplanktonic ciliates was not uncommon (Fig. 3). Total phytoplankton abundances at the time of the experiments ranged from 3.0×10^7 to 6.2×10^7 cells L^{-1} (Table 1), and the assemblage was numerically dominated at all stations by a genus of small pennate diatoms, *Nitzschia*. *P. antarctica* was the second most common taxon at abundances ranging from 5×10^5 to 5×10^6 cells L^{-1}.

Total microzooplankton abundances increased throughout the study period (Table 1). These assemblages were dominated at two of the stations by a mixed assemblage of aloricate ciliates, while loricate ciliates dominated at two stations. The dominant loricate ciliates at these stations belonged to the genera *Salpingella* and *Codonellopsis*. The abundances of both of these ciliate genera were substantially different between the three stations, $0.25–1.2 \times 10^3$ cells L^{-1} for *Codonellopsis* and $0.18–12 \times 10^3$ cells L^{-1} for *Salpingella*. Dramatic changes occurred at station 2 during the 2 weeks between the two experiments performed there. Morphologically identifiable heterotrophic dinoflagellates constituted a minor but significant component of the microplankton assemblages and were dominated by *Protoperidinium* sp. (*Gyrodinium* sp. were observed only extremely rarely).

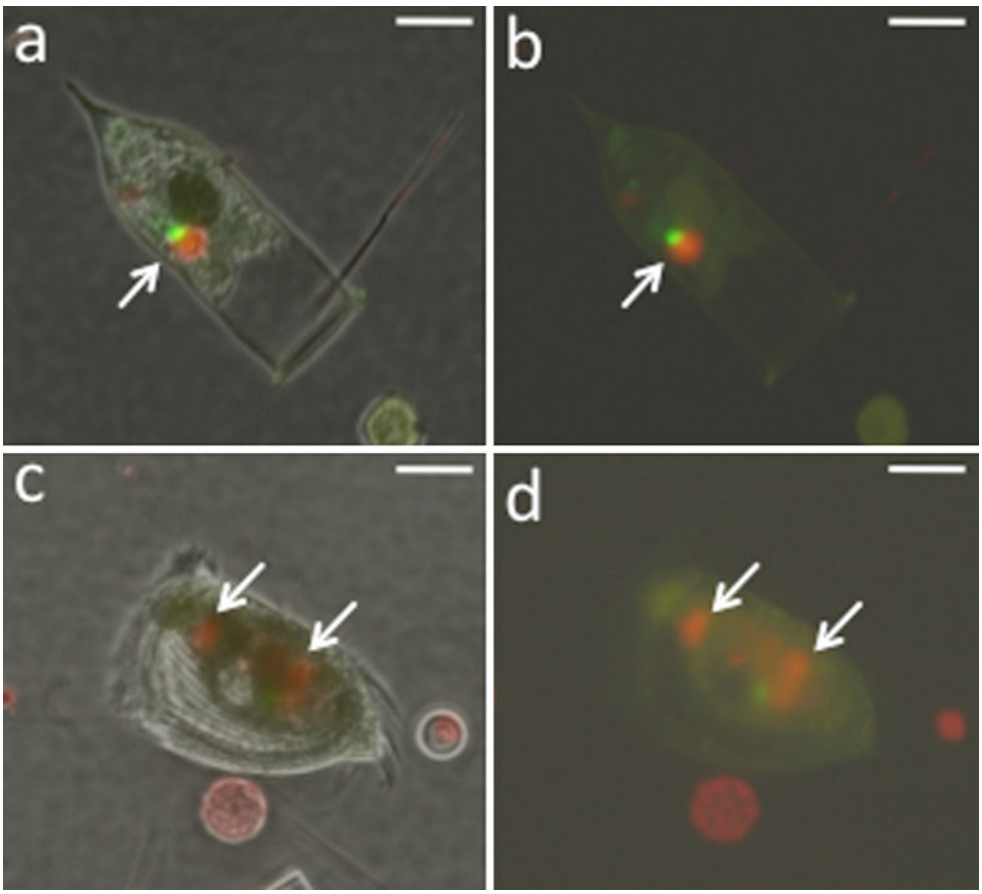

Fig. 3 Combined transmitted light and epifluorescence micrographs showing naturally occurring autofluorescing ingested photosynthetic prey in a tintinnid ciliate **a**, **b** and a hypotrich ciliate **c**, **d** from the Ross Sea, Antarctica. The amount of transmitted light has been reduced in **b** and **c** to allow better visualization of prey. *Arrows* indicate ingested prey. *Marker bars* are 20 μm **a**, **b** and 40 μm **c**, **d**

Table 1 Chlorophyll concentration, total phytoplankton and microzooplankton abundances, and abundances of subgroups of microzooplankton at the time of experiments conducted at four stations in the Ross Sea, Antarctica during late austral summer

Station number and location	Chlorophyll concentration (μg L^{-1})	Phytoplankton abundance (cells L^{-1})	Total microzooplankton abundance (cells L^{-1})	*Salpingella* sp. (cells L^{-1})	*Codonellopsis* sp. (cells L^{-1})	Aloricate ciliates (cells) L^{-1}	*Protoperidinium* sp. (cells L^{-1})
(1) 77°00′S 180°00′E	4.79	4.6×10^7	6.6×10^3	180	250	5,500	600
(2) 74°30′S 173°30′E	4.28	5.5×10^7	5.1×10^3	2,000	190	2,600	290
(3) 75°00′S 167°00′E	3.27	6.2×10^7	1.3×10^4	8,000	530	3,200	810
(4) 74°30′S 173°30′E	3.00	3.0×10^7	1.9×10^4	12,000	1,200	5,100	560

Values represent averages for triplicate measurements

Measurements carried out with natural assemblages of microzooplankton revealed that community-level ingestion rates increased with increasing abundances of stained *Fragilariopsis* sp. added to samples at all stations (Fig. 4). The number of ingested prey microzooplankton^{-1} after 30 min appeared to approach a maximum at the highest

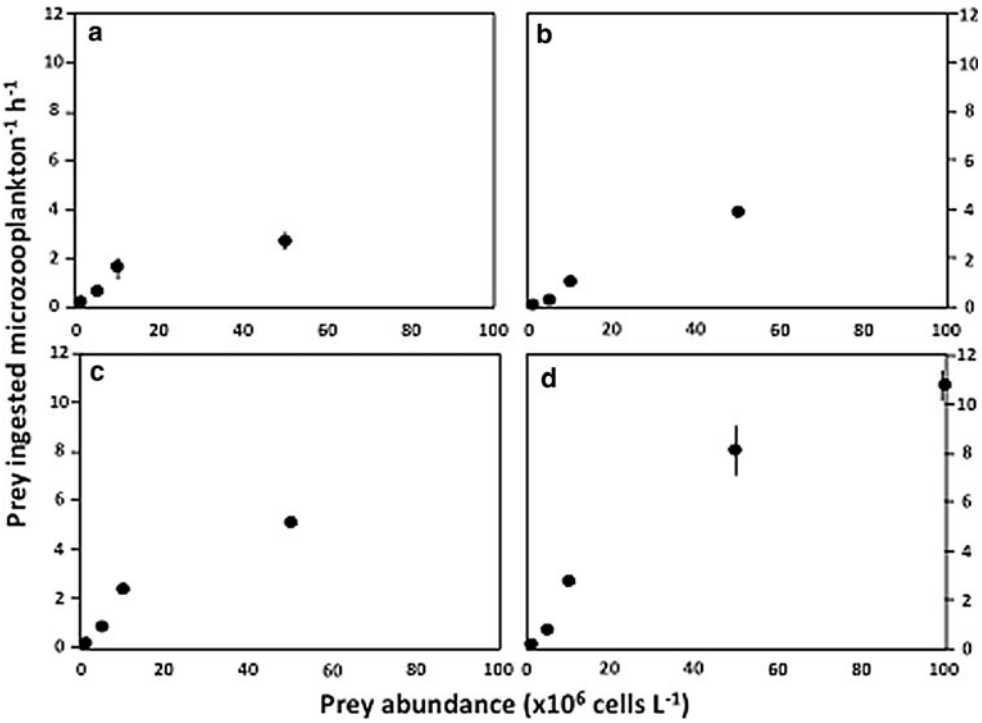

Fig. 4 Average number of ingested fluorescently stained *Fragilariopsis* cells microzooplankton^{-1} obtained using different abundances of stained algae with natural assemblages of herbivorous microzooplankton at four stations in late austral summer in the Ross Sea, Antarctica. Incubations were conducted for 30 min, and values represent the number of prey ingested during that time. Station locations are provided in the "Materials and methods" section

abundance of added *Fragilariopsis* sp., but definitive maximal ingestion rates were not observed at any station. Higher abundances of *Fragilariopsis* sp. could not be added due to limitations imposed by the staining method. The highest number of average ingested algal cells was 10.8 cells h^{-1} (station 3), when 10^8 stained *Fragilariopsis* sp. L^{-1} were added to the natural plankton assemblage. Values ranged from 2.7 to 8.1 cells h^{-1} across all stations when diatoms were added at 5×10^7 cells L^{-1}. The number of ingested prey microzooplankton^{-1} was more similar (and lower) among stations when prey were added at tracer levels and ranged from 0.07 to 0.1 cells h^{-1} at all four stations when *Fragilariopsis* sp. was added at 10^6 cells L^{-1}.

Ingestion rates of two dominant ciliate genera were calculated for the experiments carried out at station 2 (Table 2). Rates measured for *Salpingella* sp. varied from 0.06 to 7.2 cells h^{-1} when *Fragilariopsis* sp. was added at abundances ranging from 10^6 to 10^8 cells L^{-1}. Ingestion rates for *Codonellopsis* sp. were generally much higher than those observed for *Salpingella* sp. (Table 2). *Codonellopsis* sp. ingested 0.13–40.3 cells h^{-1}, when diatoms were added at abundances of 10^6–10^8 cells L^{-1}.

There was a general trend of increasing ingestion rate at high abundances of added prey for all experiments. This trend was consistent for both the total microzooplankton (Fig. 4) as well as the two most common ciliate genera (Table 2). At 5×10^7 *Fragilariopsis* sp. L^{-1}, values for the total microzooplankton assemblages were 2.7, 3.8, 5.1, and 8.0 ingested prey microzooplankton^{-1} h^{-1} for the four experiments conducted.

Specific ingestion rates of the dominant tintinnids in the natural assemblages of microzooplankton increased at higher abundances of *Fragilariopsis* sp. added as prey (Table 2). Overall, these rates were low relative to rates published for temperate ciliates at even the highest abundances of *Fragilariopsis* sp. in our experiments, with one exception (Fig. 5). The short-term-specific ingestion rates measured for the starved ciliate at 0 °C were comparable to rates reported for temperate ciliates at appropriate temperatures for those environments (0.187 for *Strombidium* was within the range 0.1–0.481 of temperate ciliates, Fig. 5). In contrast, *Strombidium* sp. fed for 2 or 4 days prior to measuring its rate yielded values that were much lower, and more similar to the maximal specific ingestion rates of *Salpingella* sp. and *Codonellopsis* sp. observed in the natural plankton assemblages in this study (0.0510 and 0.0354 for *Strombidium* vs. 0.0135 and 0.0252 for *Salpingella* and *Codonellopsis*, respectively; Table 2; Fig. 5).

The data obtained in the laboratory and field experiments of the present study were used to estimate growth rates of *Strombidium* sp., *Salpingella* sp., and *Codonellopsis* sp. by assuming a gross growth efficiency of 30 % (Gismervik 2005; Rose et al. 2009). These estimated growth rates were plotted against the maximal growth rate regression of Rose and Caron (2007) which reported the maximal growth rates of herbivorous protists in relation to environmental or culture temperature. The resulting growth rates for all three ciliates in all treatments of this study

Table 2 Abundances of labeled prey (fluorescently labeled diatoms, *Fragilariopsis* sp.), ingestion rates, and specific ingestion rates of two Antarctic ciliates, *Salpingella* sp. and *Codonellopsis* sp., in natural plankton assemblages at station 2 in the Ross Sea, Antarctica (see "Materials and methods" section)

Tintinnid ciliate genus	Experiment	Prey abundance added (cells l^{-1})	Ingestion rate (cells ingested ciliate^{-1} h^{-1})	Specific ingestion rate (h^{-1})
Salpingella sp.	1	5×10^7	2.30	0.0043
		10^7	0.68	0.0013
		5×10^6	0.25	0.0005
		10^6	0.07	0.0001
	2	10^8	7.23	0.0135
		5×10^7	7.05	0.0131
		10^7	2.43	0.0045
		5×10^6	0.69	0.0013
		10^6	0.06	0.0001
Codonellopsis sp.	1	5×10^7	10.7	0.0067
		10^7	2.12	0.0013
		5×10^6	0.51	0.0003
		10^6	0.12	0.0001
	2	10^8	40.3	0.0252
		5×10^7	21.1	0.0132
		10^7	5.41	0.0034
		5×10^6	1.03	0.0006
		10^6	0.13	0.0001

The experiments were conducted 2 weeks apart

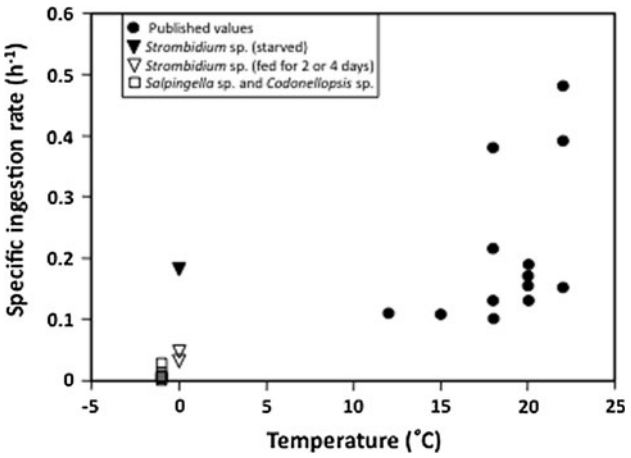

Fig. 5 Comparison of specific ingestion rates measured in this study to values for temperate ciliates reported in Hansen et al. (1997). Cultures of the Antarctic ciliate, *Strombidium* sp. (*open inverted triangle*), populations of *Salpingella* sp and *Codonellopsis* sp. from natural samples (*open square*), and literature values for cultures of temperate ciliates (*open circle*)

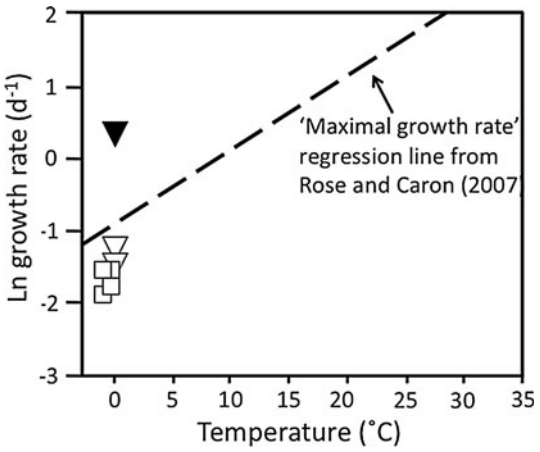

Fig. 6 Growth rates of the Antarctic ciliates *Strombidium* sp. (*open inverted triangle*) from the laboratory experiments, and *Salpingella* sp. and *Codonellopsis* sp. from the field experiments (*open square*), estimated from the rate of ingestion of fluorescently labeled algae measured in this study, assuming a gross growth efficiency of 30 %. The *filled triangle* was obtained for the starved *Strombidium* sp. while the *open triangles* were values obtained for *Strombidium* sp. in balanced growth. The *dotted line* is the regression line from Rose and Caron (2007) indicating the maximal growth rates of herbivorous protists obtained in a large meta-analysis. Note that growth rate estimated for the starved *Strombidium* sp. exceeds any known growth rates for herbivorous protists at low environmental temperatures

were below the maximal rates predicted by Rose and Caron (2007) with one exception (Fig. 6). The maximal growth rate estimated from the short-term ingestion rate of the starved *Strombidium* sp. was far in excess of any growth rate previously reported for any herbivorous protists growing at low environmental temperature.

Discussion

Growth and ingestion rates of herbivorous Antarctic protists in culture

The maximal growth rates of the three ciliate species examined in the laboratory (Fig. 1) were consistently low relative to the maximal growth rates that have been reported for similar taxa from warmer ecosystems. These findings are consistent with the seminal work of Fenchel and Lee (1972) that reported no evidence for temperature compensation of the growth rates of Antarctic protists, and with a recent summary and analysis of growth rates for a large number of herbivorous protists across a wide spectrum of environmental and culture temperatures (Rose and Caron 2007). The latter study indicated that the maximal growth rate predicted for herbivorous protists at 0 °C is approximately 0.37 day^{-1} (Fig. 6). The values observed in this study are to our knowledge the first data reporting the

growth rates of Antarctic herbivorous protists growing at this low environmental temperature, they are consistent with the maximum predicted by Rose and Caron (2007), and they confirm that low temperature constitutes a strong constraint on the growth rates of heterotrophic protists across a range of potential prey species.

Ciliate growth rates were constrained overall by the low temperature employed in this study, but the prey types still had a very significant and species-specific effect on these herbivores, even though prey were offered in excess in all treatments (Fig. 1b–d). These prey types represent the dominant algal taxa in Antarctic coastal waters (Scott and Marchant 2005). *Strombidium* sp. and *Parauronema* sp. attained similar maximal growth rates (0.22 and 0.26 day^{-1}, respectively) but these rates were attained when fed different prey. *Pyramimonas* sp. supported maximal growth of *Strombidium* sp. for the six algae tested (Fig. 1c), while bacteria and *Mallomonas* sp. supported maximal growth of *Parauronema* sp. (Fig. 1d). Negative growth (i.e., net mortality) was observed for *Strombidium* fed *Chlamydomonas* sp. or *P. glacialis* and for *Parauronema* fed *P. antarctica*. The maximal growth rate attained by *Euplotes* sp. was approximately half the values observed for the other two ciliates, a result that was not surprising given the larger size of this hypotrichous ciliate. Maximal or near-maximal rates were obtained when *Euplotes* sp. was fed *Mallomonas* sp., *Mantoniella* sp., or a mixture of three algal species (Fig. 1b). All prey types supported positive growth of this ciliate.

Differences in the ability of the algal species to support growth of the three ciliates in this study were not unexpected. Selectivity based on prey size (Fenchel 1980; Jonsson 1986), food quality (Montagnes 1996; Chen et al. 2010; Apple et al. 2011), or the production of chemicals that might render prey unpalatable or toxic (Strom et al. 2003) has been identified as important factors affecting the feeding behaviors and growth of these species. The poor suitability of *Phaeocystis* species as food for herbivores has been intensively studied (Netjstgaard et al. 2007). These factors and the widely differing cell sizes and feeding behaviors of the ciliates examined in this study could also explain the range of responses to the prey offered as food.

Feeding by *Strombidium* sp. in the laboratory was strongly affected by its physiological state. The short-term ingestion rates of this ciliate were much higher when it was starved for 2 weeks prior to the addition of prey, relative to rates measured after the ciliate fed and grew for 2–4 days (i.e., when the ciliate was in balanced growth; Fig. 2). This result was surprising in that the short-term specific ingestion rate of the starved ciliate was within the range of maximal specific ingestion rates reported for temperate ciliates at much higher temperatures (Fig. 5). In contrast, the specific ingestion rates of the ciliate in balanced growth were 19–27 % of the rate of the starved ciliates fed the same prey, and nearly an order of magnitude lower than rates reported for similar species of ciliates at higher temperatures. Moreover, the specific ingestion rates of *Strombidium* sp. in balanced growth in the laboratory were comparable to rates measured for *Salpingella* sp. and *Codonellopsis* sp. from natural plankton assemblages (Table 2).

The high ingestion rates observed for the cultured, starved *Strombidium* sp. did not translate into a higher growth rate of the ciliate. Starved ciliates quickly became engorged when prey were added to the cultures, but the number of ingested prey rapidly reached a maximum, and food processing (presumably digestion and subsequent anabolic processes) became the factor limiting the feeding activity of the ciliate. Moreover, when the ingestion rates measured for the starved ciliate were used to estimate growth rate (assuming a gross growth efficiency of 30 %), the resulting value (filled inverted triangle in Fig. 6) exceeded all maximal growth rates observed for herbivorous protists at low temperature (Rose and Caron 2007). However, growth rates of the ciliate calculated from its ingestion rates measured after 2–4 days of feeding at high prey abundances were consistent with maximal rates compiled for herbivorous protists growing at low environmental temperature (open inverted triangles in Fig. 6). The very high ingestion rate of the starved ciliate appears to indicate temperature compensation of food capture (but not food processing or growth) because that ingestion rate is roughly equivalent to rates reported for temperate ciliates at much higher temperatures (Jonsson 1986; Montagnes 1996; Hansen et al. 1997).

Our results indicate that, although growth rate was constrained by low temperature, the short-term ingestion rate of *Strombidium* sp. was not, resulting in overestimation of its feeding activity observed in short-term uptake experiments and indicating that caution is particularly warranted in the performance of these experiments with natural assemblages of polar protists. Estimation of the trophic impact of protistan assemblages using the uptake of fluorescently labeled prey has become a commonly employed method in microbial ecology (Sherr et al. 1987; Sherr et al. 1991). This method relies on the addition of labeled cells (bacteria or algae) to natural samples or cultures followed by monitoring the rate of uptake of labeled cells that serve as tracers for particle uptake by phagotrophic protists. In practice, the addition of the labeled prey is generally a compromise between the desire to add true tracer abundances (to avoid a functional response of the predator to increased prey abundance) and the need to observe sufficient ingested prey to allow a robust calculation of ingestion rate. Employing low abundances of labeled prey can be problematic in studies of natural

assemblages of microzooplankton because the feeding rates of these species can be relatively low. This practical problem is often counterbalanced by the addition of labeled cells at relatively high abundances in order to enable the observation of sufficient numbers of ingested, labeled prey. Increased ingestion in response to increased prey availability is not unique for protists feeding at low temperature, but our results indicate that temperature compensation of the ingestion rates of polar protists may enable these consumers to be highly responsive to changes in prey abundance, leading to overestimation of their trophic impact.

Ingestion rates and trophic impact of natural protistan assemblages

Ingestion rates of the total microzooplankton assemblage were similar for all four experiments performed using fluorescently labeled *Fragilariopsis* sp. as prey at tracer-level abundances ($\leq 5 \times 10^6$ cells L^{-1}; ≤ 10 % of the natural phytoplankton abundance; Fig. 4). Rates ranged from 0.07 to 0.21 *Fragilariopsis* sp. ingested microzooplankton^{-1} h^{-1} when *Fragilariopsis* sp. were added at 10^6 cells L^{-1} and from 0.3 to 0.8 *Fragilariopsis* sp. ingested microzooplankton^{-1} h^{-1} when prey were added at 5×10^6 cells L^{-1}. These data were used to calculate ingestion rates on the total phytoplankton assemblage using the ratio of labeled prey to total phytoplankton at each station. This calculation yielded values ranging from 2 to 10 ingested algal cells microzooplankton^{-1} h^{-1}.

The percent standing stock of phytoplankton grazed per day by the microzooplankton assemblage was then calculated from the information obtained for microzooplankton abundances and ingestion rates (Table 1; Fig. 4) and the total phytoplankton standing stock. These calculations yielded estimates of 3, 1, 7, and 3 % of the phytoplankton standing stock consumed by the microzooplankton assemblage per day in the four experiments, respectively. These rates are modest, suggesting that microzooplankton generally did not exert strong grazing pressure on the total phytoplankton assemblage during this study even though they were abundant at all stations.

The findings of our experiments with natural microzooplankton assemblages are consistent with low rates of herbivory that have been reported using the dilution method in the Ross Sea (Caron et al. 2000) and in Arctic ecosystems (Sherr et al. 2009). The former authors reported rates that were significantly different than zero in only 13 of 51 dilution experiments carried out during austral spring, summer, and fall in the Ross Sea polynya. The highest rate reported for experiments in that study was 0.26 day^{-1}, and the authors speculated that low temperature and poor phytoplankton food quality were the likely causes. Our ingestion rates were measured using an algal species that does not have the same morphological features such as large size and colony formation that have been hypothesized to make common Antarctic diatom species and *P. antarctica* unpalatable to some microzooplankton grazers. The low ingestion rates observed in the present study were thus more likely due to the constraints of extreme low temperature in the Ross Sea, Antarctica, on microzooplankton feeding or growth rate than by poor food quality of the fluorescently labeled algae.

The short-term ingestion rates of *Codonellopsis* sp. and *Salpingella* sp. were variable but consistently low among our experiments when *Fragilariopsis* sp. was added at tracer abundances ($\leq 5 \times 10^6$ cells L^{-1}; Table 2). Specific ingestion rates for these ciliates in these treatments were also consistently low between experiments and between genera, ranging from 0.0001 to 0.0013 h^{-1}. Differences between the two ciliates were likely due to differences in size of the two ciliates, as lorica size for *Salpingella* sp. averaged 26×127 μm, while *Codonellopsis* sp. averaged 55×238 μm.

The feeding response of the tintinnid ciliates investigated from natural microzooplankton assemblages was highly similar to the response of starved *Strombidium* sp. in culture. It is tempting to conclude that these results indicate that feeding rates of these natural assemblages of microzooplankton in the Ross Sea were not prey saturated and that increased algal abundances would result in substantial increases in microzooplankton growth rates. However, accurate interpretation of the field results is complicated by the potential that short-term feeding rates may be decoupled from the constraints of low temperature, and the latter factor could still restrict prey digestion and therefore overall growth rates of these species. Given our results of the experiment with *Strombidium* sp., it cannot be assumed that these responses in the short-term ingestion rates of the Ross Sea microzooplankton necessarily imply that growth rates of these species would increase dramatically in response to increased food availability.

Implications for estimates of protistan gross growth efficiency at low temperature

The gross growth efficiencies of heterotrophic protists have proven to be difficult but important physiological parameters to measure. GGEs are generally obtained by monitoring the growth of a protist in culture (in order to determine the production of consumer biomass) and the consumption of prey (biomass consumed) either by monitoring changes in prey abundance during a specified time period or by measuring ingestion rates of the consumer. Experiments investigating the GGEs of protists at polar temperatures have been particularly problematic. The typically slow growth rates of these species present difficulties

for measuring the biomass of prey consumed via changes in their abundances during incubations because prey can often grow faster than their consumers at low temperature. Short-term ingestion rates provide a means of measuring prey consumption in these situations, and various adaptations have been employed for estimating GGE and the trophic activities of polar protists (Sherr et al. 1997; Roberts and Laybourn-Parry 1999; Scott et al. 2001; Bell and Laybourn-Parry 2003; Moorthi et al. 2009).

Errors in the determination of ingestion rates of protists, however, would introduce significant error into the estimation of prey biomass consumed. Such errors provide one possible explanation for the highly variable growth efficiencies that have been reported for protists growing at low temperature. For example, Mayes et al. (1997) reported that the GGEs of two Antarctic amoebae grown at 0–2 °C were nearly zero (0.8–1.2 %). In contrast, Choi and Peters (1992) reported very high GGEs for the heterotrophic bacterivorous flagellate, *Paraphysomonas imperforata,* cultured at −1.5 °C (60 and 70 %). A recent study of an Antarctic strain of *P. imperforata* acclimated and grown at a variety of temperatures indicated a strong influence of temperature on growth rate but no apparent effect on gross growth efficiency (Rose et al. 2009). A summary of available data for *P. imperforata* indicated very high variability in individual GGEs reported for this protist, but an overall average GGE for this species across a wide range of environmental temperatures of approximately 40 %. Other factors such as prey abundance, prey nutritional status, and presumably the prevalence of methodological artifact in estimating this value appeared to have a greater impact on measurements of growth efficiency (see Fig. 5b in Rose et al. 2009).

We speculate that methodological artifacts in the estimation of the short-term ingestion rates of protists have confounded our understanding of the potential effect of temperature on gross growth efficiency. In particular, the probability of exceptionally low GGEs for polar protists as a consequence of low environmental temperature seems unlikely. Protists constitute a diverse and abundant assemblage that supports a complex community of higher organisms in polar ecosystems. It is difficult to reconcile the existence of these vibrant food webs with extremely low efficiencies of trophic transfer among species that constitute the base of these food webs. Moreover, protistan species are poikilothermic organisms that typically exhibit exceptionally low basal metabolism (Fenchel 1986; Caron et al. 1990), so there is no a priori reason for high energetic cost and low growth efficiency of these species at low temperature.

The gross growth efficiencies of protists are undoubtedly responsive to changing prey concentration and food quality, but there is little substantive evidence that low environmental temperature is a major determinant of that variability. Rivkin and Legendre (2001) presented evidence for an increase in bacterial gross growth efficiency with decreasing environmental temperature, although covariation of temperature with the availability and quality of bacterial substrates may confound the relationship. There is also limited information indicating a similar relationship for protists (Li et al. 2011). Nonetheless, a number of studies have invoked low GGE of protists as a means of reconciling unexpected results in field studies. For example, First and Hollibaugh (2008, 2010) hypothesized low GGE of bacterivorous protists in a benthic ecosystem as a means of explaining the unexpectedly low standing stocks of the protists. Conversely, some modeling approaches have assumed that the growth efficiencies of heterotrophic microbes are independent of temperature or nearly so (Sherr and Sherr 2002; Allen et al. 2005). This latter approach seems more appropriate for considering the effect of temperature on gross growth efficiency until more accurate methods for estimating this parameter are developed and a better understanding of this feature of protistan physiology is attained.

Conclusions

The maximal growth rates of three herbivorous Antarctic ciliates were strongly affected by prey type when offered different species of Antarctic algae. Low temperature had an overriding effect on growth rate, however, and maximal rates for the three consumers examined in this study did not exceed 0.26 day^{-1}. Surprisingly, short-term ingestion rates of the ciliate *Strombidium* sp. were not constrained by low temperature and exhibited maximal rates comparable to those of congeners isolated and cultured at substantially higher temperatures, although other aspects of the metabolism the Antarctic ciliate constrained overall maximal growth rate. Measurements of the ingestion rates of natural assemblages of microzooplankton in the Ross Sea, estimated from the rate of uptake of fluorescently labeled algae, indicated modest trophic impact of herbivorous protists on the algal assemblage. Moreover, abundances of surrogate prey in the experiments strongly influenced the rates observed, presumably because ingestion rates were temperature compensated as observed in the laboratory experiments. Our results indicate that caution is needed in performing experimental determinations of the feeding rates of polar protists in short-term experiments because temperature compensated feeding rates by these species can result in overestimation of the importance of these species to community-level herbivory and result in erroneous estimates of the gross growth efficiencies of polar protists.

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