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ORIGINAL ARTICLE

Effect of Temperature and Prey Type on Nutrient Regeneration by an Antarctic Bacterivorous Protist

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Abstract Experimental studies were carried out on an Antarctic isolate of the heterotrophic nanoflagellate *Paraphysomonas imperforata* to examine the efficiency of incorporation and remineralization of nitrogen and phosphorus from bacterial prey. Experiments were carried out over a temperature range from ambient Antarctic temperature (0 °C) to 10 °C. Temperature had a marked effect on the maximal growth rate of the phagotrophic nanoflagellate. Growth rate in the presence of high prey abundance ranged from 0.6 day^{-1} at 0 °C to 2.6 day^{-1} at 10 °C. In contrast, temperature had no discernable effect on the efficiencies of incorporation and remineralization of major nutrients by *P. imperforata*. The efficiencies of phosphorus and nitrogen incorporation from prey biomass averaged over the temperature range examined were 58 and 39%, respectively, for the two elements. Ammonium and phosphate were the dominant forms of dissolved nitrogen and phosphorus appearing in the culture medium during the grazing phase of the experiments. Overall, dissolved organic nitrogen and phosphorus constituted minor components of these elements released by the grazing activities of the protist. The results of this study indicated that incorporation/remineralization of nitrogen and phosphorus contained in prey was relatively unaffected by culture temperature in this heterotrophic nanoflagellate, although low temperature significantly depressed its growth rate. This finding has important implications for energy utilization and elemental cycling in perennially cold ecosystems and is at odds with conclusions that have been reached in some previous studies regarding the growth efficiency of phagotrophic Antarctic protists.

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Introduction

Remineralization of major nutrients (nitrogen/phosphorus) via the grazing activities of phagotrophic protists is an important source of nutrients fueling phytoplankton growth in the ocean [31, 35]. Nutrients not incorporated into protistan biomass are excreted after feeding on bacteria, phytoplankton, or other heterotrophic protists, and thereby recycled to primary producers. These trophic activities can be important contributors to total nutrient remineralization. This conclusion is based on the high weight-specific metabolic activities of protists, their cellular composition (relative to the composition of their prey), and their predominance as consumers in many aquatic ecosystems [3, 11, 32]. Moreover, heterotrophic bacteria may at times compete with phytoplankton for dissolved inorganic nutrients and thus may be a sink rather than a source for these substances [7, 9, 36]. Characterizing protistan nutrient remineralization and the factors controlling this process are fundamental aspects for understanding nutrient dynamics in marine ecosystems.

Heterotrophic protists excrete a variety of dissolved nitrogen and phosphorus compounds, but ammonium and

phosphate are the dominant forms released by most species [1, 3, 11, 14]. Concentrations of dissolved organic phosphorus (DOP) and a variety of dissolved organic nitrogen (DON) compounds (e.g., amino acids and polypeptides, urea, uric acid, hypoxanthine, dihydrouracil, adenine, and guanine) are significant but typically secondary in absolute amount to the inorganic forms [19, 23, 24, 32, 33]. Similarly, the release of undigested particulate material by protists constitutes a significant albeit minor portion of the total amount of ingested prey biomass [6, 34].

Laboratory experiments with heterotrophic protists have demonstrated their ability to remineralize a substantial percentage of the phosphorus and nitrogen that they ingest as prey (i.e., nutrient remineralization efficiency), especially when feeding on bacteria [1, 4, 5, 11, 14, 30]. The C/N and C/P ratios of bacterial biomass are generally much lower than those of heterotrophic protistan biomass. Thus, bacterivorous protists maintain appropriate intracellular C/N/P ratios by excreting the excess nitrogen and phosphorus [2]. Intracellular stoichiometric balance in heterotrophic protists via the excretion of excess N or P in P- or N-limited bacteria and diatoms has been experimentally demonstrated [1, 14].

Excretion rates are also substantial for small, heterotrophic protists. Weight-specific excretion rates of zooplankton have been shown to be inversely related to organismal size [15, 16]. The relatively small size of many heterotrophic protists implies a potential for extremely high weight-specific excretion rates in comparison to metazoa. Metazoan-based equations for nutrient excretion rates have been extrapolated to the size range of heterotrophic protists and compared to information from laboratory studies of cultured heterotrophic protists [3, 11]. These analyses have indicated higher maximal phosphorus excretion rates and comparable maximal nitrogen excretion rates compared to rates predicted by the metazoan-based equations. These results indicate that protists are capable of the highest weight-specific excretion rates of aquatic consumers and that they may play a particularly important role in phosphorus remineralization in some aquatic ecosystems.

Most experimental studies of nutrient remineralization by heterotrophic protists have been performed with cultures established and examined at relatively warm environmental temperatures (15–25 °C). One exception [30] examined ammonium excretion by a temperate heterotrophic flagellate (identified as *Monas* sp.) at four temperatures (range= 3–30 °C). The authors observed lower gross growth efficiencies (based on carbon) and higher ammonium excretion rates at the two temperature extremes compared to efficiencies and rates observed at intermediate temperatures of 18 and 23.5 °C. However, they noted that the lowest and highest temperatures were clearly outside the optimal physiological temperature range for the isolate.

To our knowledge, no other study has examined nutrient remineralization (rates or efficiencies) of protistan strains isolated and studied at ambient polar temperatures. Therefore, it is not clear whether the extrapolation of these parameters obtained for temperate heterotrophic protists is appropriate to describe the excretion rates of heterotrophic protists from permanently cold environments growing at environmentally pertinent temperatures. Moreover, available information on the effect of temperature on carbon based growth efficiency (which is physiologically linked to nutrient remineralization) does not help resolve the issue whether protists growing at extremely low environmental temperature would exhibit nutrient remineralization efficiencies that are lower, higher, or unchanged from these species growing at higher temperatures. Studies have reported both positive and negative relationships between temperature and protistan remineralization efficiencies, as well as no relationship [4, 8, 21]. It has been proposed that there may be a high energetic cost for growth at low temperature resulting in low growth efficiency (and thus high remineralization efficiency), but the validity of this hypothesis has not been tested directly.

This study was the first to examine the effect of temperature (including environmentally appropriate temperatures; 0–10 °C) and prey type on nutrient excretion rates and remineralization efficiencies of a polar heterotrophic nanoflagellate. *Paraphysomonas imperforata* was selected as the study organism because the genus has a global distribution [12], is an important bacterial consumer in aquatic ecosystems, and temperate conspecific strains of this species have been extensively studied. Our results indicated no effect of prey type on either nutrient remineralization rates or overall nutrient remineralization efficiency at a given temperature. Higher rates of nutrient remineralization by the nanoflagellate were observed at higher temperatures, but total nutrient remineralization efficiencies were unaffected over the range of temperatures examined.

Methods

Cultures

P. imperforata (clone RS-4–2) was isolated from an enriched 30 ml water sample obtained from the Ross Sea, Antarctica (76°01.383′ S, 165°24.459′ W). Water was collected and the natural bacterial flora was enriched with 0.01% sterilized yeast extract and a single sterile rice grain. Cultures were maintained at temperatures below 2 °C at all times to avoid selection against heat-sensitive protists. *P. imperforata* was isolated by dilution extinction after growing in the enriched water sample for one year, and clonal (uniprotistan) cultures were identified based on the

structure of scales on the surface of the flagellate according to Preisig and Hibberd [26] at 10,000× magnification using the transmission electron microscope JEOL 100CX. Two unidentified bacterial strains were isolated from an enriched water sample also obtained from the Ross Sea, Antarctica (76°30S, 169°33E). Enriched water was plated onto marine agar (Marine Agar 2216, Difco Laboratories, Sparks, MD), and single colonies were picked and streaked repeatedly on marine agar plates to ensure purity. A temperate bacterial strain, *Halomonas halodurans*, was also used in the nutrient regeneration experiments.

Nutrient Regeneration Experiments

Subcultures of the Antarctic *P. imperforata* were acclimated to three temperatures (0, 5, and 10 °C) for several months before the beginning of experiments. Subcultures of the Antarctic *P. imperforata* at high protistan and low bacterial density were inoculated into pure cultures of the three bacterial strains for several transfers before the beginning of experiments to minimize the contribution of other strains of bacteria as prey for the protist. Experiments were run at 0 °C with the protist growing on all three bacterial strains and at 5 and 10 °C with the protist growing only on Antarctic bacterial strain A.

All bacterial strains were grown to late stationary growth phase on marine broth (Difco Laboratories), harvested by centrifugation and rinsed and resuspended three times in sterile seawater to remove residual organics from their culture medium. The two strains of Antarctic bacteria were grown at 5 °C, and *H. halodurans* was grown at 20 °C. Bacteria were then added to 600 ml sterile seawater at a starting concentration of approximately 10^7 cells ml^{-1}, and flasks containing bacterized seawater were brought to the appropriate experimental temperatures in separate temperature-controlled incubators.

Protists were grown to high abundance (~5×10^5 cells ml^{-1}, stationary growth phase) and inoculated into each of the experimental treatments at low starting abundance (~10^3 cells ml^{-1}) to minimize the transfer of organic material and bacteria in the inoculum. Protist-free controls of bacterized seawater were incubated in duplicate for each temperature and each prey type to monitor changes in prey abundances and nutrient concentration not associated with protistan grazing activity. All experimental treatments were performed in triplicate and were kept in continuous darkness. The 5 and 10 °C treatments were sampled in a 5 °C cold room, and the 0 °C treatment was sampled on ice in a cold room to avoid heat-shock of the organisms.

Sample Analysis

Samples were removed periodically and fixed with 2% lugols solution for enumeration of protists by light microscopy using a Palmer Maloney counting chamber. Samples were also fixed with 1% formalin for enumeration of bacteria by flow cytometry according to the protocol of del Giorgio et al. [10]. The biomass of protistan and bacterial cells at the beginning and end of the experiment was determined based on volume measurements. Samples for volume estimates of protists and bacteria were preserved with 1% glutaraldehyde (final concentration) and stored at 5 °C until analysis. Digital photographs of protists and bacteria were taken using phase contrast microscopy and an inverted microscope (Leica DM IRB) at 1000× magnification. Protistan diameter and bacterial length and width were measured on the digital photographs using the software program Openlab 3.5.1. Protistan and bacterial volumes were calculated using standard equations for spheres and cylinders, respectively. The volumes of at least 25 protists and 50 bacteria from each sample were averaged for each treatment. Volumes were multiplied by abundances to obtain biomass estimates. The percentage biomass of protists or bacteria at a time point was calculated by dividing protistan or bacterial biomass at that time point by the total biomass of protists and bacteria combined.

Samples of 20 ml volume were collected onto pre-combusted GF/F glass fiber filters (Gelman) for determinations of particulate carbon and nitrogen. Samples were kept frozen at −20 °C until analysis by the MSI Analytical Lab, Santa Barbara, CA. Filtrates from the particulate carbon and nitrogen samples were collected and kept frozen at −20 °C for analysis of NH_4 concentration according to Parsons et al. [25]. Samples of 10 ml volume were collected onto pre-combusted GF/F glass fiber filters for analysis of particulate phosphorus according to a modification of the method by Menzel and Corwin [22]. We experienced problems with partial disintegration of the GF/F filters during the autoclaving step of the protocol, which increased the turbidity of the sample solution. This turbidity was not effectively removed by centrifugation and resulted in extremely high spectrophotometric absorbance readings for all samples, including blank filters used as controls. Therefore, all samples including filter blanks and phosphorus standards were filtered through a 0.22-μm syringe filter after autoclaving to remove particulate material before reading the sample on the spectrophotometer. New filters and syringes were used for each sample to prevent cross-contamination of samples with phosphorus. Controls were performed to ensure that phosphorus was not introduced by this additional manipulation. Filtrates from the particulate phosphorus samples also were collected for analysis of soluble reactive phosphorus (SRP) according to Parsons et al. [25] and total dissolved phosphorus (TDP) according to Menzel and Corwin [22]. Both filters and filtrates were kept frozen at −20 °C until analysis. DOP concentrations were calculated for each sample by subtracting the SRP values from the TDP concentrations.

Rates of loss of particulate nutrients and increases (remineralization) of dissolved nutrients were calculated for each of the treatments. Rates of loss of particulate carbon and nitrogen and accumulation of ammonium were calculated during the exponential growth phase of the protist when protistan abundance was sufficient to impact nutrient concentration. Rates of loss of particulate phosphorus and accumulation of SRP were calculated during the early stationary phase growth of the flagellate. The phosphorus rates were calculated during this phase growth because phosphorus constituents did not change until the very end of exponential growth phase. These results with phosphorus were consistent with observations for the same species of flagellate as reported in Andersen et al. [1].

Statistics

Rates of loss and remineralization were compared among the five treatments using a percentile bootstrap method for comparing slopes as described in Wilcox [38]. This method was chosen over the standard analysis of covariance (ANCOVA) test because the percentile bootstrap test does not have the assumptions of normality or heteroscedasticity and does not assume that the regression lines are parallel. Initial C/N and C/P ratios were compared among the three bacterial prey treatments using a percentile bootstrap test for multiple comparisons as described in Wilcox (2003). This method was chosen over the standard analysis of variance (ANOVA) test because the percentile bootstrap test has higher power and no assumptions of normality or heteroscedasticity. This same test was used to compare nitrogen and phosphorus regeneration efficiencies among temperature and bacterial prey treatments. The range of nitrogen regeneration efficiencies for the temperate and Antarctic strains of *P. imperforata* were compared using a percentile bootstrap test for trimmed means as described in Wilcox [38]. This method was chosen over the standard Student's *t* test for comparing two independent groups because it has higher power and does not have the assumptions of normality or heteroscedasticity.

Results

Total biomass in the cultures was dominated by bacterial biomass at the beginning of the experiment but by protistan biomass by the end of the exponential growth phase. Bacteria comprised an average of 99.9% of total biomass (range= 99.8–99.9%) at the initial time point in all five treatments. At the time of maximal protistan abundance, an average of 95% of the total biomass was present in the protists (range=88–98%), whereas an average of 5% of the total biomass was present in the bacteria (ranging from 2–12%).

Particulate carbon (PC) concentrations in all five treatments examined in this study decreased during the period when *P. imperforata* was actively growing and bacteria were being consumed (Fig. 1). PC concentrations decreased more rapidly at higher temperature (Fig. 1C), although the difference between the rates of decrease at 5 and 10 degrees was not significant ($p>0.05$; 0 vs 5 and 0 vs 10 both $p<0.05$). Loss rates calculated for the three temperature treatments were 0.34, 0.25, and 0.10 day^{-1} for the 10, 5, and 0 °C treatments, respectively. The concentrations of PC at the time of maximal abundance of *P. imperforata* showed no discernable trend with either incubation temperature or prey type (Table 1).

Particulate nitrogen (PN) concentrations also decreased during the period of active grazing by *P. imperforata* in all five treatments (Fig. 2, solid symbols). Increases in ammonium concentrations in each treatment temporally lagged behind increases in protistan abundance, but ammonium concentrations increased rapidly as the cultures reached stationary growth phase (Fig. 2, open symbols). The rates of loss of PN and ammonium accumulation increased with increasing temperature. PN loss rates for the three temperature treatments were 0.34, 0.24, and 0.10 day^{-1} for the 10, 5, and 0 °C treatments, respectively. Rates of ammonium accumulation were 0.94, 0.72, and 0.27 day^{-1} for the 10, 5, and 0 °C treatments. The rates observed in the 0 °C treatment were significantly different than either the 5 or 10 °C treatment (all $p<0.05$), but rates were not significantly different between the 5 and 10 °C treatment ($p>0.05$). Rates of loss of PN and ammonium accumulation were unaffected by prey type at 0 °C (Fig. 2f, all $p>0.05$). The total amount of ammonium regenerated was similar at all temperatures and prey types (Fig. 2f). Final ammonium concentrations in all treatments ranged from 118 to 148 μM. Only minor changes in ammonium concentration were observed between the initial and final time points in the controls. On average, there was a 4.0 μM increase in the five control treatments as compared to an average 84 μM increase in ammonium in the five experimental treatments.

Changes in particulate and dissolved phosphorus showed similar but less pronounced patterns to those for nitrogen constituents (Fig. 3). Particulate phosphorus (PP) concentrations decreased (Fig. 3, solid symbols) concomitant with increases in dissolved phosphorus concentrations (Fig. 3, open symbols). The dissolved phosphorus constituents primarily consisted of SRP (open circles), with DOP (open triangles) comprising 10–20% of the TDP (open squares) in each treatment by the end of exponential growth phase of the protist. Similar to nitrogen dynamics, the rates of PP loss and SRP release increased with increasing temperature, and there were significant differences between the rate of PP loss in the 0 vs 10 °C treatments ($p<0.05$). The loss rates were 1.3 and 0.14 day^{-1} for the 10 and 0 °C

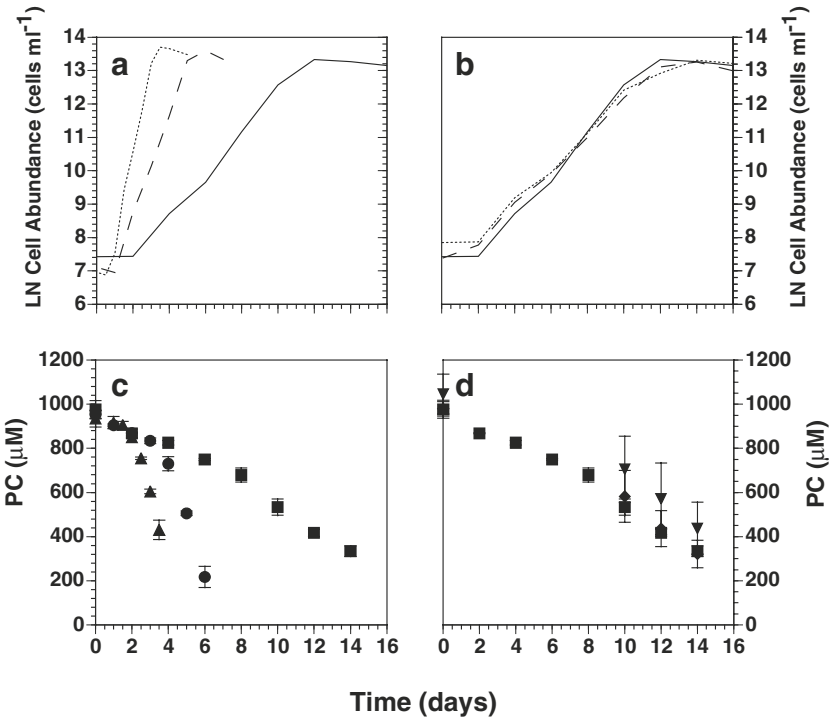

Figure 1 Changes in protistan abundance (*lines* in **a**, **b**) and particulate carbon concentrations (*symbols* in c, d) in cultures of *Paraphysomonas imperforata* grown at three temperatures (**a**, **c**) and on three different strains of bacteria at a single temperature (**b**, **d**).). Treatments included: growth at 0 °C on Antarctic bacterial strain a (*solid lines* and *squares*, shown in all four panels for comparison); growth at 5 °C on Antarctic bacterial strain a (dashed line and *circle*, in **a** and **c**); growth at 10°C on Antarctic bacterial strain a (*dotted line* and *triangles* in **a** and **c**); growth at 0°C on Antarctic bacterial strain b (*dashed line* and *diamonds* in **b** and **d**); growth at 0 °C on *Halomonas halodurans* (*dotted line* and *inverted triangles* in **b** and **d**). *Error bars* represent one standard deviation

treatments. The rate of loss of PP in the 5 °C treatment could not be calculated due to measurement error. The difference in rates of SRP accumulation in the 0 vs 5 and 0 vs 10 °C treatments were significant ($p<0.05$), although the rates of SRP accumulation in the 5 vs 10 °C treatments were not significantly different ($p>0.05$). The rates of SRP release were 1.4, 1.2, and 0.07 day^{-1} in the 10, 5, and 0 °C treatments, respectively. Also similar to nitrogen dynamics, rates of PP loss and SRP accumulation were unaffected by prey type (Fig. 3f, all $p>0.05$). The total amount of SRP released also mimicked nitrogen dynamics in that it was not affected by either temperature or prey type. Final SRP concentrations in four of the five treatments were 6–8 μM. One treatment, *P. imperforata* grazing on *H. halodurans* at 0 °C, not only had higher final SRP concentrations (12 μM), but also had higher initial TDP concentrations (3.3 μM vs 0.4–1.0 μM in the other treatments). Only minor changes were observed in PP and SRP in the control (ungrazed) bacterial cultures over the course of the experiment. An average decrease of 0.35 μM PP and

Table 1 Changes in concentration of particulate carbon and particulate and dissolved nitrogen and phosphorus before and after bacterial grazing by *Paraphysomonas imperforata*

	0 °C Antarctic bacterial strain A	5 °C Antarctic bacterial strain A	10 °C Antarctic bacterial strain A	0 °C Antarctic bacterial strain B	0 °C *Halomonas halodurans*	Average
Initial PC (μM)	977	952	935	979	1046	978
Final PC (μM)	416	217	430	436	571	414
Initial PN (μM)	213	208	205	216	247	218
Initial NH_4 (μM)	4.7	2.2	4.3	5.2	6.0	4.5
Final PN (μM)	89.6	43.0	92.1	93.0	123	88.1
Final NH_4 (μM)	86.1	101	84.7	82.5	88.4	88.5
Initial PP (μM)	8.6	9.6	9.3	10.4	15.8	10.7
Initial SRP (μM)	0.1	0.2	0.2	0.4	1.9	0.6
Final PP (μM)	6.8	3.5	7.2	7.1	8.9	6.7
Final SRP (μM)	2.1	4.6	2.4	2.7	7.8	3.9

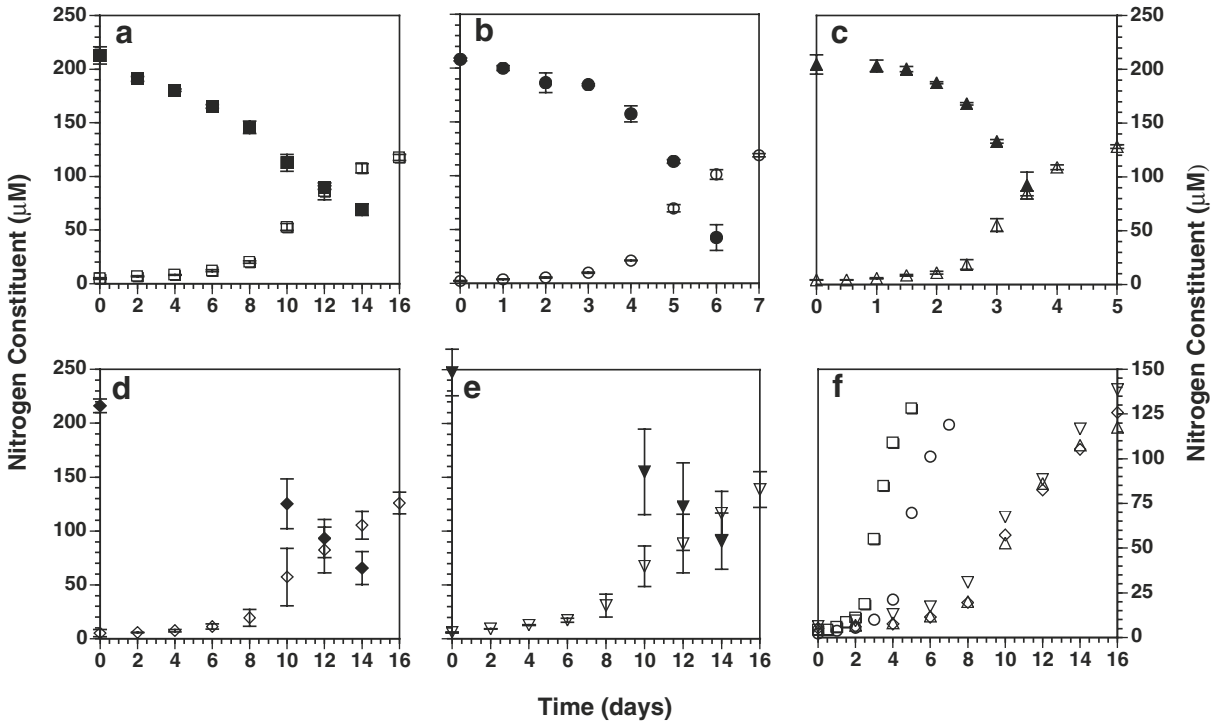

Figure 2 Changes in particulate nitrogen (*solid symbols*) and ammonium (*open symbols*) during bacterial grazing by *Parapyhysomonas imperforata* on Antarctic bacterial strain a at 0 °C (**a**), 5 °C (**b**), and 10 °C (**c**), and at 0 °C on Antarctic bacterial strain b (**d**) and *Halomonas halodurans* (**e**). Changes in ammonium concentration in all treatments (**f**). Note different ranges for *x*- and *y*-axes in (**a**–**f**). *Error bars* represent one standard deviation

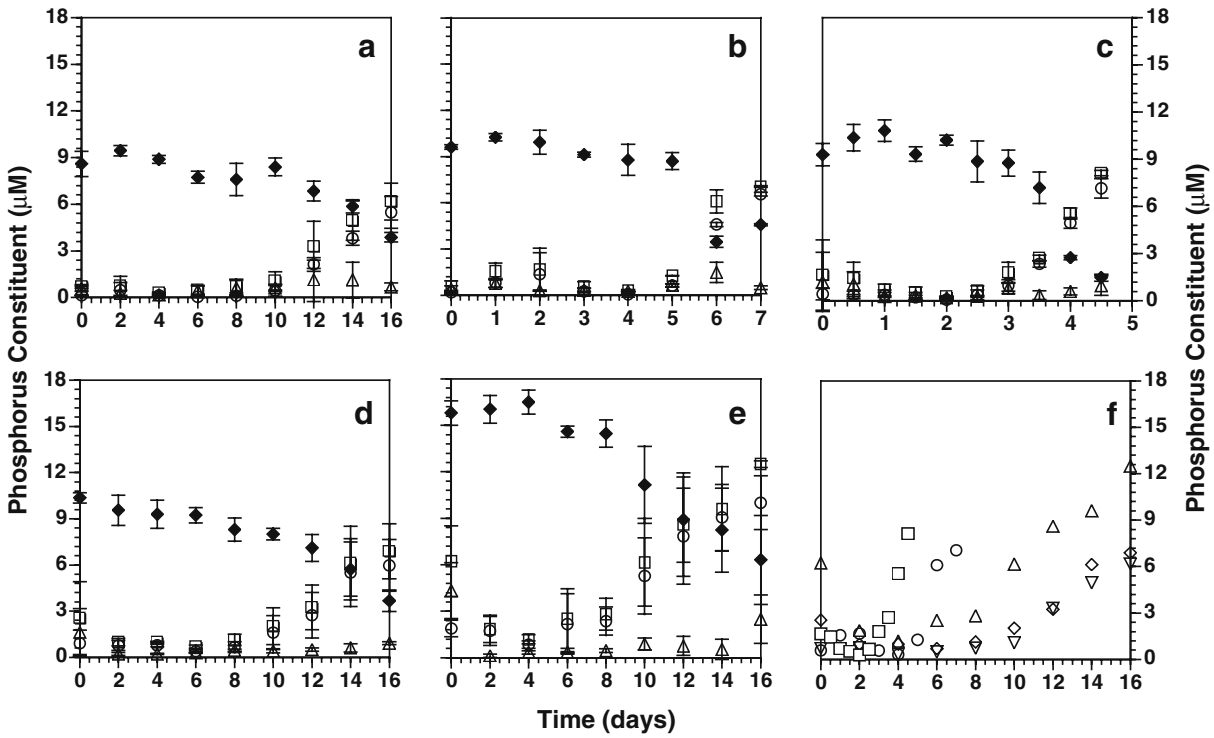

Figure 3 Changes in particulate phosphorus (*solid symbols*) and dissolved phosphorus (*open symbols*), including dissolved organic phosphorus (*open triangle*), soluble reactive phosphorus (*open circle*), and total dissolved phosphorus (*open square*; **a**–**e**). Grazing by *Paraphysomonas imperforata* on Antarctic bacterial strain a at 0 °C (**a**), 5 °C (**b**), and 10 °C (**c**), and at 0 °C on Antarctic bacterial strain b (**d**) and *Halomonas halodurans* (**e**). Changes in total dissolved phosphorus concentration in all treatments (**f**). Note different ranges for *x*- and *y*-axes in (**a**–**f**). *Error bars* represent one standard deviation

increase of 0.22 μM SRP was observed in the five control treatments between the initial and final time points, as opposed to an average decrease of 4.0 μM PP and increase of 3.4 μM SRP in the five experimental treatments.

Absolute changes in the dissolved and particulate fractions of nitrogen and phosphorus were compared at the time the cultures were inoculated to the time of maximal protistan abundance (Table 1). Initial PN concentrations ranged from 208 to 247 μM (average, 218 μM) and decreased to 43–123 μM (average 88.1 μM) by the time of maximal protistan abundance. Ammonium concentrations during this period increased from 2.2–6.0 μM (average, 4.5 μM) to 82.5–101 μM (average, 88.5 μM). By mass balance, this implies that DON (not measured in this study) averaged approximately 45 μM at the onset of the stationary growth phase of the flagellate (approximately 20% of the initial PN concentration). Initial PP concentrations ranged from 8.6 to 15.8 μM (average, 10.7 μM) and decreased to 3.5–8.9 μM (average, 6.7 μM) by the time of maximal protistan abundance. SRP concentrations increased from 0.1–1.9 μM (average, 0.6 μM) to 2.1–7.8 μM (average, 3.9 μM). Thus, the average decrease in the concentration of PP and the increase in SRP were virtually in balance. This is consistent with our finding that SRP constituted most of the TDP appearing in the cultures.

The rates of nutrient remineralization were clearly affected by temperature but not prey type (Figs. 2 and 3), whereas remineralization efficiencies were unaffected by either temperature or prey type. This result was apparent by normalizing the concentrations of regenerated nutrients against natural log cell abundances of the protist in each treatment (Fig. 4). The pattern of ammonium regeneration vs natural log cell abundance was strongly linear for both constituents, indicating a strong direct relationship between protistan growth and the appearance of remineralized nitrogen in the medium. A similar pattern was apparent for phosphorus. In addition, there was no apparent effect of temperature or prey type on the amount of nutrient present in the medium at a given protistan abundance.

Changes in the percentages of dissolved and particulate fractions of nitrogen and phosphorus in the cultures were compared at the time of inoculation with the flagellate and at the time of maximal protistan abundance to examine the effect of temperature and prey type on the efficiency of protistan nutrient incorporation and remineralization (Fig. 5). Percentages were obtained by dividing concentrations of each constituent at the beginning and end of the grazing phase of the flagellate by the value at the beginning. We chose to divide by the initial particulate nutrient concentrations for both the initial and final time points, as we did not directly quantify DON produced during the experiment. Initially, an average of ≥98% of the nitrogen and 89–99% of the phosphorus was present in

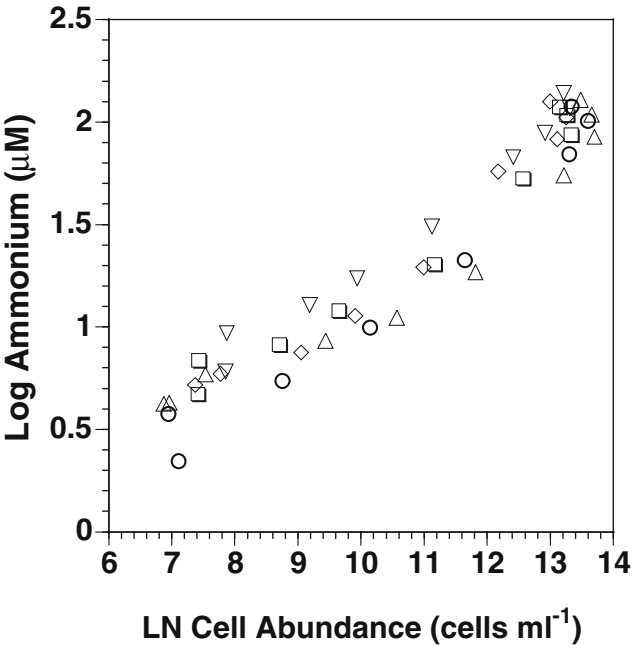

Figure 4 Comparison of ammonium concentrations in grazed cultures of bacteria normalized to the natural log abundance of *Paraphysomonas imperforata* in the five treatments. 0 °C, Antarctic bacterial strain a (*open square*); 5 °C, Antarctic bacterial strain a (*open circle*); 10 °C, Antarctic bacterial strain A (*open triangle*); 0 °C, Antarctic bacterial strain b (*open diamond*); 0 °C, *Halomonas halodurans* (*open inverted triangle*). A regression of the combined data set yielded the equation $y=0.52x-2.3$ with $r^2=0.93$

particulate form across all treatments. An average of 40% of the initial total nitrogen was present as ammonium across all treatments at the time of maximal protistan abundance (range=35–48%), whereas a similar proportion (39%) remained in the particulate fraction. There was no significant difference in the efficiency with which initial PN was regenerated as ammonium among the three bacterial food types (all $p>0.05$). There was no significant difference in regeneration efficiency between the 0 and 10 °C treatments ($p>0.05$). There were significant differences between the 5 °C and other two temperature treatments ($p<0.05$). These values do not account for DON, which, by mass balance, may have constituted approximately 20% of the total nitrogen pool at the time of maximal protistan abundance.

An average of 33% of the initial total phosphorus was present in the soluble reactive form across all treatments at the time of maximal protistan abundance. This average is similar to the percentage of nitrogen appearing as ammonium, although a wider range of SRP concentrations was observed (24–47%). There were no significant differences in the percentage of initial PP regenerated as SRP among the three bacterial prey types (all $p>0.05$). There was also no significant difference in phosphorus regeneration efficiency between the 0 and 10 °C treatments ($p>0.05$). There were significant differences observed between the 5 °C and other two temperature treatments ($p<0.05$). The percentage of

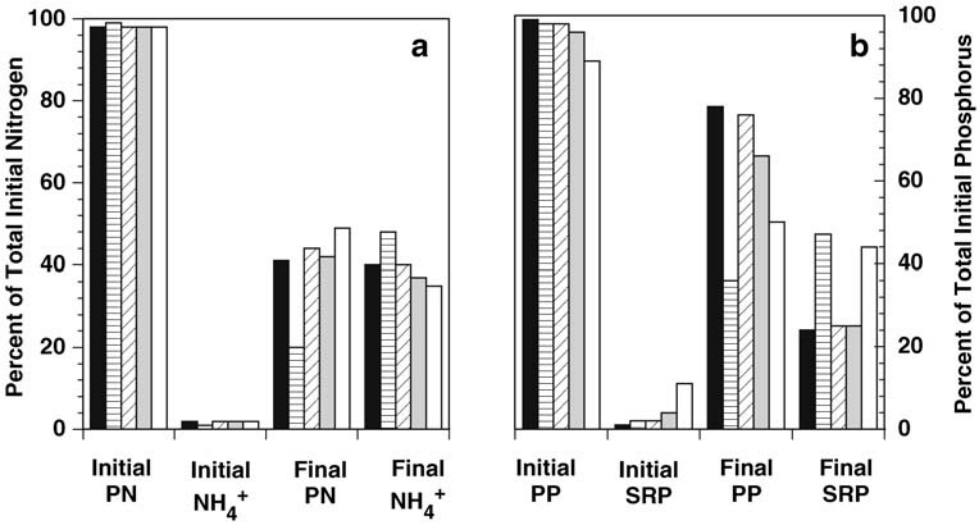

Figure 5 Changes in particulate and dissolved fractions of nitrogen (**a**) and phosphorus (**b**) in bacterial cultures grazed by *P. imperforata*. *Bars* correspond to particulate or dissolved nutrient concentrations as a percentage of the initial total nutrient concentration (particulate plus dissolved). 0 °C, Antarctic bacterial strain a (*solid bars*); 5 °C, Antarctic bacterial strain a (*horizontal stripes*); 10 °C, Antarctic bacterial strain a (*diagonal stripes*); 0 °C, Antarctic bacterial strain b (*dotted bars*); 0 °C, *Halomonas halodurans* (*white bars*)

phosphorus remaining in the particulate fraction at the time of maximal flagellate abundance (61%) was considerably higher than the percentages observed for either nitrogen or carbon (40 and 42%, respectively). Unlike DON, DOP constituted a minor component of the total initial phosphorus at the time of maximal abundances of *P. imperforata* (<5%).

The percentages of initial PN and PP that were converted to ammonium or TDP (i.e., nutrient regeneration efficiency) at the time of maximal protistan abundance were compared to results published for temperate conspecifics (Table 2). These values were calculated by subtracting the initial ammonium or SRP concentrations from final concentrations, then dividing by the initial PN or PP concentrations. Values ranged from 33 to 47% of PN regenerated as ammonium, with an average of 39%. The percent of PP regenerated as SRP ranged from 22 to 46% with an average of 30%. There were no apparent trends in percent regeneration of nutrients with either temperature or prey type. The values obtained in this study were compared to published values for a temperate strain of *P. imperforata* [1, 4, 14]. The percentage of PN regenerated as ammonium ranged from 27 to 51% for the entire data set (including published values and ones presented in this article), with an average of 40%. There was no significant difference between the range of nitrogen regeneration efficiencies reported for the temperate strain of *P. imperforata* and the range observed in this article ($p>0.05$). Only one additional value for percentage of PP regenerated as SRP was available, and it was similar to the average observed in this study (36 vs 30% in this study).

Discussion

Heterotrophic protists play an important role as remineralizers of major nutrients in aquatic ecosystems [3, 11]. However, data for rates and efficiencies of remineralization by heterotrophic protists are limited, and observations have been conducted primarily on cultures isolated from temperate regions. Very few studies have compared the growth rates and growth efficiencies of heterotrophic protists from permanently cold regions to those of temperate heterotrophic protists [8, 20, 21]. In general, it is recognized that protists from all environments grow slowly at low temperature (<5 °C) relative to growth rates of protists at higher temperatures [29]. However, there is conflicting information regarding the relationship between growth efficiency and temperature. Studies have reported increases in gross growth efficiency with increases in temperature [8, 27, 37], decreases in gross growth efficiency with increases in temperature, very low gross growth efficiency at low temperature [18, 21, 28], and no change in gross growth efficiency with changes in

Table 2 Comparison of percent of initial particulate nutrients regenerated as ammonium or soluble phosphorus at the time of peak protistan abundance for strains of bacterivorous *Paraphysomonas imperforata* examined at different temperatures

Source	Temp. (°C)	% PN regenerated as NH_4^+	% PP regenerated as SRP
Caron et al (1986)	14	27	
	18	44	
	22	51	
	26	47	
Goldman et al (1985)	20	35	
Andersen et al (1986)	20		36
This study	0	38	23
	5	47	46
	10	39	24
	0	36	22
	0	33	37
Average (SD)		40 (7)	31 (10)

temperature [4, 17]. Whereas some degree of variability between species might be expected, it is probable that experimental artifact has contributed to these disparate values. These conflicting reports on the interplay between temperature and growth efficiency confound our understanding of the effect of temperature on protistan nutrient remineralization because one would anticipate that the fates of ingested nutrients (N, P) are closely related to the fate of ingested carbon (i.e., growth efficiency).

Very few data are available on the effects of temperature on the rates and efficiencies of nutrient regeneration. One study examining the effect of temperature on nutrient remineralization found increased rates of ammonium regeneration at higher temperatures for a temperate strain of the heterotrophic protist *P. imperforata*, but the total amount of ammonium regenerated (remineralization efficiency) was unaffected by culture temperature [4]. Another study reported increased excretion rates when the heterotrophic protist *Monas* sp. was grown above or below its optimal temperature range for growth [30]. These latter results may be more indicative of nutrient release under stressful growth conditions rather than a direct effect of temperature per se. We are unaware of any studies reporting baseline nutrient regeneration information for heterotrophic protists from permanently cold environments at appropriate environmental temperatures.

Rates and patterns of change in protistan abundance and PC as a function of protistan grazing indicated a strong effect of temperature on growth rates of *P. imperforata* but no discernable effect on the overall efficiencies of its metabolic processes (Fig. 1, Table 1). The rate of increase in cell numbers was significantly higher as temperatures were increased above 0 °C, but abundances reached similar levels at all temperatures. Similarly, the rates of loss of PC from the cultures (due to respiratory losses and release of DOC) were significantly higher, as temperatures were increased above 0 °C, but the overall decreases in PC were similar at different temperatures and with different bacterial strains as prey. One exception appeared to be the change in PC in the culture fed Antarctic bacterial strain A at 5 °C. This culture yielded a lower PC than all other treatments. However, this difference was probably a consequence of the timing of sample collection for PC analysis in the experiment. If samples are not collected precisely at the peak of flagellate abundance, the estimate of PC remaining in the culture can be affected [6]. Overall, the changes in PC and cell abundances imply that growth at low temperature, while significantly affecting growth rate, did not create a metabolic demand (or savings) that was significantly different than growth at much higher temperatures (Table 1).

Changes in the dissolved and particulate nitrogen and phosphorus pools in the cultures mirrored rates and trends observed for protistan abundances and carbon. PN and PP decreased steadily in all treatments from the initial time point, and loss rates were faster in the 5 and 10 °C treatment (Figs. 2a–e and 3a–e, filled symbols). The average amount of PN remaining in the cultures at the end of the exponential growth phase of the flagellate was quite similar to the average PC remaining in the cultures across all treatments (40 and 42%, respectively). Given that the biomass of the cultures at the end of exponential growth was dominated by living flagellates, these values strongly indicate that the incorporation of nitrogen from prey biomass into flagellate biomass was similar stoichimetrically to carbon incorporation.

In contrast, although the general pattern of decrease in PP remaining in the cultures was similar to PC and PN, a substantially higher percentage of PP remained at the end of the exponential growth phase of the flagellate than for carbon or nitrogen (PP=61%). This result indicates either that phosphorus was preferentially retained as flagellate biomass during growth or that excretion of this element was temporally shifted from the release of nitrogen or carbon and did not take place before the termination of the experiment. We found no evidence to support the latter hypothesis. Goldman et al. [13] noted the ability of a temperate species of *P. imperforata* to efficiently retain phosphorus from prey when the prey has a low phosphorus content relative to carbon and nitrogen. Interestingly, the N/P ratio of the initial prey biomass was higher than the Redfield Ratio (>20 compared to a Redfield Ratio of 16:1) in all treatments except the *H. halodurans* treatment, indicating an excess of nitrogen in the prey biomass (relative to phosphorus). We also found significantly lower initial C/P and N/P ratios when the *H. halodurans* treatment was compared to the other two bacterial treatments ($p<0.05$). We speculate that phosphorus was preferentially retained from ingested bacterial biomass by the flagellate to achieve an optimal elemental ratio in the biomass of the predator.

SRP concentrations in the *P. imperforata* culture fed *H. halodurans* increased at lower abundances of the protist than observed in the other treatments (Fig. 3a–e); that is, SRP release was elevated in that treatment relative to the other four treatments for a similar amount of protistan biomass. Initial PP concentrations were higher in the treatment with *H. halodurans* relative to PC and PN concentrations (compared to other treatments), indicating that *H. halodurans* was phosphorus-rich relative to the two Antarctic bacterial strains used in this experiment. The initial particulate N/P ratio in this treatment was approximately the Redfield Ratio of 16:1 (15.6:1). It is probable that the higher particulate content of *H. halodurans* led to a greater remineralization of SRP in this treatment because the flagellate could not use the excess phosphorus for growth (7.8 μM final SRP vs average 3.0 μM final SRP for the other treatments, Table 1). This explanation is consistent

with the contention that *P. imperforata* is able to decouple C, N, and P incorporation from prey biomass to achieve an optimal elemental stoichiometry [13].

Ammonium and SRP concentrations increased in all treatments after an initial lag and continued to increase into the stationary growth phase of the protist (Figs. 2a–e and 3a–e, open symbols). Ammonium and SRP regeneration occurred more rapidly at higher temperatures, but rates were not significantly affected by prey type (Figs. 2f and 3f). The two strains of Antarctic bacteria grown at 0 °C did not affect the rates or magnitude of nutrient regeneration by the Antarctic *P. imperforata* relative to growth of the flagellate on *H. halodurans* grown at room temperature and grazed by the flagellate at 0 °C. Thus, we found no evidence that the use of 'indigenous' vs 'nonindigeous' bacteria as prey affected nutrient remineralization for this flagellate.

The total average amounts of initial nitrogen and phosphorus appearing as ammonium or SRP, respectively, by the end of the growth phase of the flagellate were similar in the experiment (39 and 30%, respectively). On average, PP and SRP at the end of protistan growth constituted more than 95% of the initial phosphorus present. The inclusion of DOP yielded a reasonable mass balance for this element. This same exercise for nitrogen indicated that approximately 20% of this element was not accounted for by measurements of PN and ammonium at the end of flagellate growth. The remaining fraction of the nitrogen pool was presumably released as DON, which was not measured in the experiment. This percentage of DON (approximately 20% of the initial concentration of PN) is consistent with results obtained for nitrogen release by a temperate isolate of *P. imperforata* [14].

As noted above, the overall patterns of carbon, nitrogen, and phosphorus incorporation and remineralization were similar for all treatments in this study (Table 1). However, slight differences in these values were observed due to the inability to sample at precisely the same point in the growth phase of the flagellate across all treatments because the cultures at different temperatures progressed at different rates. For example, the treatment at 5 °C containing Antarctic bacterial strain A had higher dissolved nutrients and lower particulate nutrients (both nitrogen and phosphorus) than the other treatments. The sampling times for this treatment were just before and just after the protistan culture entered stationary growth phase, whereas the other treatments had sampling times that were almost exactly at the beginning of the stationary growth phase. The sampling time immediately after the stationary growth phase of the 5 °C culture was closer to the projected time of peak protistan abundance, so that time point was used for nutrient calculations (Table 1). Therefore, the protists in this treatment had slightly more time to release ingested nutrients. To assess the effect of this difference, the values of Table 1 were recalculated using the earlier time point for that treatment. They yielded values of 113 μM for final PN, 8.7 μM for final PP, 70 μM for final NH_4^+, and 0.6 μM for final SRP. The values obtained at these two sampling times for the 5 °C treatment bracket the values for other treatments obtained at precisely the end of exponential growth (Table 1). Thus, it is unlikely that the small differences in particulate and regenerated nutrients in the 5 °C treatment relative to the other treatments were due to effects of temperature or bacterial type.

The similarity in the patterns of nutrient remineralization among the treatments became obvious when the effects of temperature on rate processes were eliminated. Rates of nitrogen remineralization normalized by plotting the log ammonium concentration against the natural log of cell abundance for each treatment (Fig. 4) showed similar patterns to protistan abundance. Differences in absolute concentrations across treatments were also normalized by plotting regeneration of dissolved inorganic nutrients as a percentage of total initial nitrogen or phosphorus concentration (Fig. 5). This normalization removed the effect of higher initial nutrient concentrations in the 0 °C *H. halodurans* treatment to demonstrate that the amount of N and P remineralized was similar in this treatment to the other bacterial types and the higher temperatures. As observed in Table 1, total regeneration of nutrients was similar across all treatments with the exception of P in the 5 °C bacterial strain A as discussed above.

Total regeneration of nutrients by the Antarctic strain of *P. imperforata* observed in this study was similar to nutrient regeneration values published for temperate conspecifics [1, 4, 14] (Table 2). These studies and the present study include observations measured across a temperature range of 0–26 °C. The total average percent ammonium regenerated across all studies was 40% and remarkably consistent across these studies. The average for this study was 39%, and the average for the studies examining temperate *P. imperforata* was 41%. Only one study examined regeneration of SRP; however, the percent regenerated phosphorus observed in that study (36%) was similar to the average observed in this study (30%).

Conclusions

Temperature affected the rate of nutrient regeneration by an Antarctic strain of *P. imperforata* but did not affect the total quantity of nutrients remineralized. Different prey species fed to the flagellate at a single temperature had no effect on its rates of nutrient regeneration or total quantity of nutrients regenerated. Total nutrient regeneration by the Antarctic *P. imperforata* was also similar to values published for a temperate conspecific examined at much higher temperatures. Rates of nutrient regeneration were greatly reduced at low temperature, suggesting that in

permanently cold systems, nutrient recycling by heterotrophic protists occurs slowly. However, as total regeneration of nutrients was similar at all temperatures examined in this study, it is probable that the efficiency with which heterotrophic protists convert nutrients contained in particulate prey to their remineralized forms is similar across ecosystems with extremely different temperature regimes.

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