

ORIGINAL ARTICLE

Effects of temperature on growth rate and gross growth efficiency of an Antarctic bacterivorous protist

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The effects of temperature on the growth rate and gross growth efficiency (GGE) of the heterotrophic nanoflagellate, *Paraphysomonas imperforata*, cultured from the Ross Sea, Antarctica were investigated using five experimental temperatures (range = 0–20 °C). This bacterivorous protist exhibited measurable growth over the temperature range examined, although temperature exerted a significant effect on its growth rate. There was no evidence for an effect of temperature on GGE. The growth rates and GGE of our Antarctic *P. imperforata* isolate were compared to values reported for other cultures of species from this genus. A wide range of growth efficiencies have been reported for different strains of *Paraphysomonas* spp., but our estimates were comparable to mean/median values reported in the literature. The growth rates of our Antarctic *P. imperforata* were similar to rates obtained for an Arctic conspecific at low temperatures (0–5 °C), among the highest reported rates for any *Paraphysomonas* species at intermediate temperatures (10–15 °C) and similar to rates reported for temperate congeners and conspecifics at 20 °C. Q_{10} values of 15, 2.2, 3.6 and 0.93 were calculated for growth rates at 5 °C intervals between 0 and 20 °C, respectively. Results indicated that our Antarctic *P. imperforata* grew at rates comparable to other polar isolates at ambient polar temperatures, but these low temperatures may be outside the physiological optimum for the isolate. *The ISME Journal* (2009) 3, 252–260; doi:10.1038/ismej.2008.96; published online 9 October 2008

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Introduction

Heterotrophic protists play key ecological roles in aquatic ecosystems. These organisms contribute significantly to the total living biomass within these systems, serve as the major top-down control on bacterial assemblages, and are an important source of mortality for microalgae and other heterotrophic protists (Sanders *et al.*, 1992; Sherr and Sherr, 1994). Heterotrophic protists also function as important remineralizers of organic matter and nutrients in aquatic systems (Azam *et al.*, 1983; Caron and Goldman, 1990). In accordance with these important ecological roles, heterotrophic protists have been the subject of considerable study both in the field and

laboratory. Cultures of these species have been examined to determine the effects of a wide range of physical and chemical parameters on growth rate and other physiological and biogeochemical processes.

Temperature is recognized as a fundamental determinant of physiological rates of heterotrophic protists including growth rates. In general, growth rates increase proportionally with temperature up to an optimum and then decrease beyond the physiological limits for the species (Lee and Fenchel, 1972; Martinez, 1980; Baldock and Berger, 1984; Caron *et al.*, 1986; Muller and Geller, 1993; Weisse *et al.*, 2001). This relationship is generally expressed using a Q_{10} value, which represents the ratio of change in growth rate with a 10 °C change in temperature. Q_{10} values are calculated using the Eq. $Q_{10} = (\mu_1/\mu_2)^{10/(t_1-t_2)}$, where μ_1 and μ_2 are growth rates determined at temperatures t_1 and t_2 , respectively.

Most laboratory studies involving cultured heterotrophic protists have focused on species isolated from temperate environments and have examined protistan physiology at relatively high water temperatures (> 15 °C) (Hansen *et al.*, 1997; Rose and

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Caron, 2007). Thus, the extrapolation of physiological rate information obtained from temperate cultures to infer rate processes of protists in permanently cold environments may be inappropriate. To date, three published studies have examined growth rates of cultured heterotrophic protists from permanently cold environments (Lee and Fenchel, 1972; Choi and Peters, 1992; Mayes *et al.*, 1997). All three studies reported low growth rates of polar isolates within the range of ambient polar temperatures (-1.8 to 2 °C). However, these studies reported conflicting conclusions when growth rates of polar isolates were compared to growth rates of temperate congeners at low environmental temperature.

Lee and Fenchel (1972) reported a growth rate of 0.16 day^{-1} for the Antarctic ciliate *Euplotes antarcticus* at -2 °C. The growth rate of this species increased exponentially with increasing temperature, although growth was not sustained above 10 °C. The Q_{10} values for the temperature ranges -1.8 to 2 °C and 2 – 5 °C were 4.8 and 4.6 , respectively. The growth rates of this Antarctic ciliate were comparable to those of temperate and tropical congeners when measured at similar temperatures (5 °C or 10 °C). In contrast, an Arctic strain of *P. imperforata* examined by Choi and Peters (1992) grew more rapidly at -1.5 °C and 6 °C than a subarctic conspecific examined in the same study, but more slowly than the subarctic conspecific at 15 °C. The authors interpreted these results as indicative of adaptation by the Arctic *P. imperforata* to low environmental temperature. Mayes *et al.* (1997) reported growth rates for five species of Antarctic naked marine amoebae over the temperature range -2 to 4 °C. The observed growth rates of these amoebae were significantly less than the rates predicted for their growth at ambient Antarctic temperatures by the extrapolation of available growth rate information for temperate amoebae. In contrast to the conclusion of Choi and Peters, Mayes *et al.* hypothesized that the disparity between the observed growth rates of the Antarctic amoebae and rates predicted from the extrapolation of growth from temperate amoebae was due to the energetic costs of inhabiting cold environments.

In general, the effects of temperature on protistan gross growth efficiency (GGE; defined as the amount of protistan carbon or biovolume produced relative to the amount of prey consumed) are not well understood. Some studies have reported increases in GGE with decreasing temperature (Rassoulzadegan, 1982; Verity, 1985; Choi and Peters, 1992), whereas others have reported decreases with decreasing temperature or very low GGE at extreme low temperature (Laybourn and Stewart, 1975; Rogerson, 1981; Mayes *et al.*, 1997). Still others have reported no effect of temperature on GGE (Caron *et al.*, 1986; Ishigaki and Sleight, 2001). Choi and Peters (1992) reported high biovolume-based GGEs (60% and 71%) for an Arctic and a subarctic *P. imperforata* measured at -1.5 °C. In contrast, Mayes *et al.* (1997)

reported exceptionally low GGEs for Antarctic amoebae over the temperature range -2 to 4 °C (GGE = 0.8 – 7.2%).

This study is the first to document growth characteristics of a cultured marine Antarctic heterotrophic nanoflagellate. *P. imperforata* was selected as a study organism because of its wide geographic distribution (Finlay and Clarke, 1999) and its potential importance as a source of bacterial mortality in aquatic ecosystems (Sherr and Sherr, 1994). Studies detailing the growth and grazing rates, growth efficiencies and nutrient remineralization efficiencies of this and closely related species isolated and cultured from a range of other environments have been published previously (Fenchel, 1982; Sherr *et al.*, 1983; Goldman and Caron, 1985; Caron *et al.*, 1986; Choi and Peters, 1992; Eccleston-Parry and Leadbeater, 1995). The purpose of this investigation was to study the effect of temperature on the growth rates and GGE of this Antarctic bacterivorous flagellate. Specifically, we sought to identify the responses of growth rate and GGE of this polar flagellate to low environmental temperature. Finally, we used the data published for congeners and conspecifics to place the rates and efficiencies observed in this study into a larger context and to investigate patterns of temperature effects at the genus level.

Materials and methods

Cultures

P. imperforata (clone RS-4-2) was isolated from an enriched water sample obtained from the Ross Sea, Antarctica ($76^{\circ}01.38'$ S, $165^{\circ}24.46'$ W). The natural bacterial flora in the sample was enriched with sterilized yeast extract, rice grains and the temperate marine bacterium *Halomonas marina* (*Halomonas halodurans*, *Pseudomonas halodurans*, obtained from R Cuhel, Great Lakes Water Institute, originally isolated by G Jones, University of New Hampshire). This bacterial strain has been used previously and shown to be a good source of food for a range of cultured temperate heterotrophic protists (Caron *et al.*, 1991; Rose *et al.*, 2004). Enrichment cultures were maintained at 0 – 2 °C at all times to prevent selection against heat-sensitive protists. *P. imperforata* was isolated by dilution extinction, and clonal (uniprotistan) cultures were identified based on the structure of surface scales according to Preisig and Hibberd (1982), using transmission electron microscopy (Figure 1).

H. marina was used as prey in the growth experiments. Two additional bacterial strains were isolated from the original flagellate enrichment culture approximately 1 year after establishment of the original enrichments to examine protistan growth rate and growth efficiency on prey of potentially different composition. Enriched water was plated onto marine agar (DIFCO Laboratories,

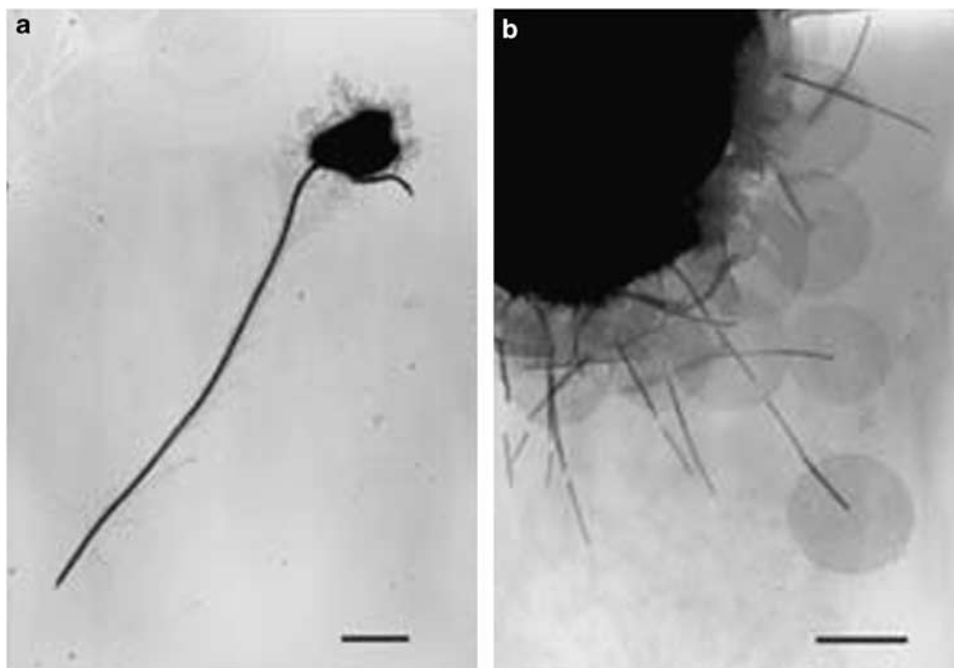


Figure 1 Transmission electron micrograph of *P. imperforata* strain RS-4-2 (negative stain using 1% uranyl acetate). **(a)** Whole cell view showing flagellation and surface scales, $\times 5000$. Marker bar = $2 \mu\text{m}$. **(b)** High magnification showing scale morphology, $\times 10000$. Marker bar = $0.5 \mu\text{m}$.

Sparks, MD, USA) and two colonies were picked and streaked repeatedly on marine agar plates to ensure purity. Partial sequences (~ 550 bp) of the small subunit ribosomal RNA genes (16S) from the isolated bacterial strains were obtained using traditional cloning and sequencing protocols (Countway *et al.*, 2005). Taxonomic identity of the two isolates was established from the NCBI sequence database using BLAST (Altschul *et al.*, 1997). Surprisingly, both isolates returned *H. marina* (a.k.a *Cobetia marina*, Arahall *et al.*, 2002) as the best match during BLAST analysis, that is, the same identity as our bacterial enrichment culture. This result was completely unexpected because of the amount of time between initiation of the enrichment cultures with *H. marina* and the time of isolation of the two clonal strains. Given that the bacteria had co-existed and dominated the cultivable bacteria within the flagellate culture through numerous transfers and subcultures at ambient Antarctic temperatures, we used the two strains in the growth experiments with the flagellate.

Growth experiments

Two experiments were conducted with the Antarctic *P. imperforata* to examine the effects of temperature on growth rate and GGE. The first experiment used all three of the strains of *H. marina* (that is, the previously cultured strain and our two isolates) to examine growth rates and GGEs of the *P. imperforata* at 0°C , 5°C and 10°C , while the second experiment examined growth rate and GGE of the Antarctic

P. imperforata acclimated to different temperatures (0°C , 5°C , 10°C , 15°C and 20°C). *P. imperforata* cultures in both experiments were acclimated to experimental temperatures above 2°C over a period of several months by gradually raising the culture temperature $3\text{--}5^\circ\text{C}$, and maintaining the culture at that temperature for a period of several weeks before further increasing the temperature.

H. marina strains A and B (that is, the two recent isolates) were grown to late stationary growth phase at 5°C on marine broth (DIFCO Laboratories), harvested by centrifugation ($3220 \times g$ for 20 min), rinsed, resuspended and recentrifuged three times with sterile seawater to remove residual organic medium. *H. marina* strain C (the previously cultured strain) was grown at 20°C on marine broth and harvested using the same method. *H. marina* strain A was used in both experiments, but strains B and C were used in experiment 1 only. Rinsed bacteria were inoculated into 600 ml sterile $0.2 \mu\text{m}$ filtered, autoclaved, seawater in 1.25 l polycarbonate bottles at a starting concentration of $\sim 10^7$ cells ml^{-1} and brought to experimental temperatures before beginning both experiments. Protists were grown to high abundance before the experiments and added to the bacterized seawater at $1\text{--}5 \times 10^3$ cells ml^{-1} . Protist-free bacterized seawater was used as a control for each temperature. Experimental treatments were performed in triplicate, and controls were performed in duplicate. Experiments were conducted in the dark in incubators set to the appropriate temperatures. Sampling was performed on ice in a cold room for cultures incubated at 0°C , in a 5°C

cold room for cultures incubated at 5 °C and 10 °C and in a 15 °C walk-in incubator for the cultures at 15 °C and 20 °C to avoid temperature shock during the experiments.

Measurements and calculations

Bacterial abundances were measured by flow cytometry on samples preserved in 1% formaldehyde and frozen at -20 °C (FACScalibur flow cytometer, BD Biosciences, San Jose, CA, USA). Samples were stained in the dark for 15 min with 2.5 µM SYTO 13 (Molecular Probes, Eugene, OR, USA) and analyzed by flow cytometry according to del Giorgio *et al.* (1996). Event counts were converted to cell abundances by weighing sample tubes on an analytical balance before and after each analysis on the flow cytometer to determine the volume analyzed.

Protistan abundances were measured by light microscopy of samples preserved in 2% acid Lugols solution (final concentration) and stored in the dark at room temperature. Protists were enumerated using a Palmer-Maloney counting chamber, and at least 200 cells were counted per sample. Protistan growth rates were calculated based on a regression of natural log cell abundance versus time, while cultures were in logarithmic growth phase (linear portion of the growth curve).

Protistan GGEs in the first experiment were calculated in two ways: based on changes in particulate carbon in the cultures, and based on bacterial biovolume consumed relative to protistan volume produced (Fenchel, 1982). In the second experiment, protistan GGEs were calculated based on biovolume only. For carbon-based estimates of GGE, 20 ml of sample was filtered onto a precombusted GF/F filter (Pall Corporation, East Hills, NY, USA) and frozen at -20 °C until analysis for particulate organic carbon (analysis conducted by the MSI Analytical Lab, Santa Barbara, CA, USA). GGE was calculated by comparing the particulate carbon concentration in each culture at the time of maximum *P. imperforata* abundance (end of log phase growth) to initial particulate carbon concentration. This procedure assumes the contribution of particulate carbon from *P. imperforata* at the time of inoculation was negligible.

Biovolume-based estimates of GGE were performed on samples 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 IRBE) at ×1000 magnification. Measurements of protistan diameter and bacterial length and width were performed on the digital photographs using the software program Openlab 3.5.1. Biovolumes of protists and bacteria 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 use in GGE calculations.

The initial total bacterial biovolume (abundance × cell volume) was compared to the total bacterial volume at the time of maximal *P. imperforata* abundance to estimate the volume of bacteria consumed. This value was compared to total protistan volume produced to calculate biovolume-based GGE.

Statistics

P. imperforata growth rates and GGEs were analyzed with a percentile bootstrap method for comparing means of multiple groups, using the statistical software package R (Liu and Singh, 1997; Wilcox, 2003). The percentile bootstrap method was selected over the commonly used analysis of variance because it has the advantage of no assumptions of equality of variance or normal distribution. The multiple comparison test consisted of a series of percentile bootstrap tests of each pair, in conjunction with Hochberg's sequentially rejective method to control familywise error rate (Hochberg, 1988; Wilcox, 2003).

Results

Experiment 1

The growth rates of *P. imperforata* increased significantly with increasing temperature (all *P*-values <0.01), (Figure 2). The growth rates were 0.58 day⁻¹, 1.6 day⁻¹ and 2.6 day⁻¹ at 0 °C, 5 °C and 10 °C. Average growth rates at 0 °C on the three strains of *H. marina* were 0.61 day⁻¹, 0.56 day⁻¹ and 0.56 day⁻¹ on strains 1, 2 and 3. *Q*₁₀ values determined from these growth rates were 7.6 between 0 and 5 °C, 2.6 between 5 and 10 °C and 4.5 between 0 and 10 °C.

Gross growth efficiency of the flagellate was relatively recalcitrant to different culture temperatures, although biovolume-based GGEs were consistently lower than those based on particulate carbon (means = 36% and 46%, respectively). GGE was not significantly affected by temperature in the first experiment, regardless of whether efficiencies were calculated based on estimates of carbon or biovolume (*P* = 0.30 and 0.92, respectively; range 36–37% for biovolume-based estimates versus 42–45% for carbon-based estimates), (Figure 3). GGE of *P. imperforata* on three strains of *H. marina* (the original and two strains isolated after 1 year of co-culture with the flagellate) ranged from 28 to 42% by volume and 42–54% by carbon.

Experiment 2

The growth rates of *P. imperforata* in experiment 2 were examined when the flagellate was fed Antarctic bacterial strain A over a wider temperature range than in experiment 1. Flagellate growth rates increased significantly with increasing temperature up to 15 °C, but did not increase further at 20 °C (*P* < 0.01 for all pairs except for 15 °C versus 20 °C,

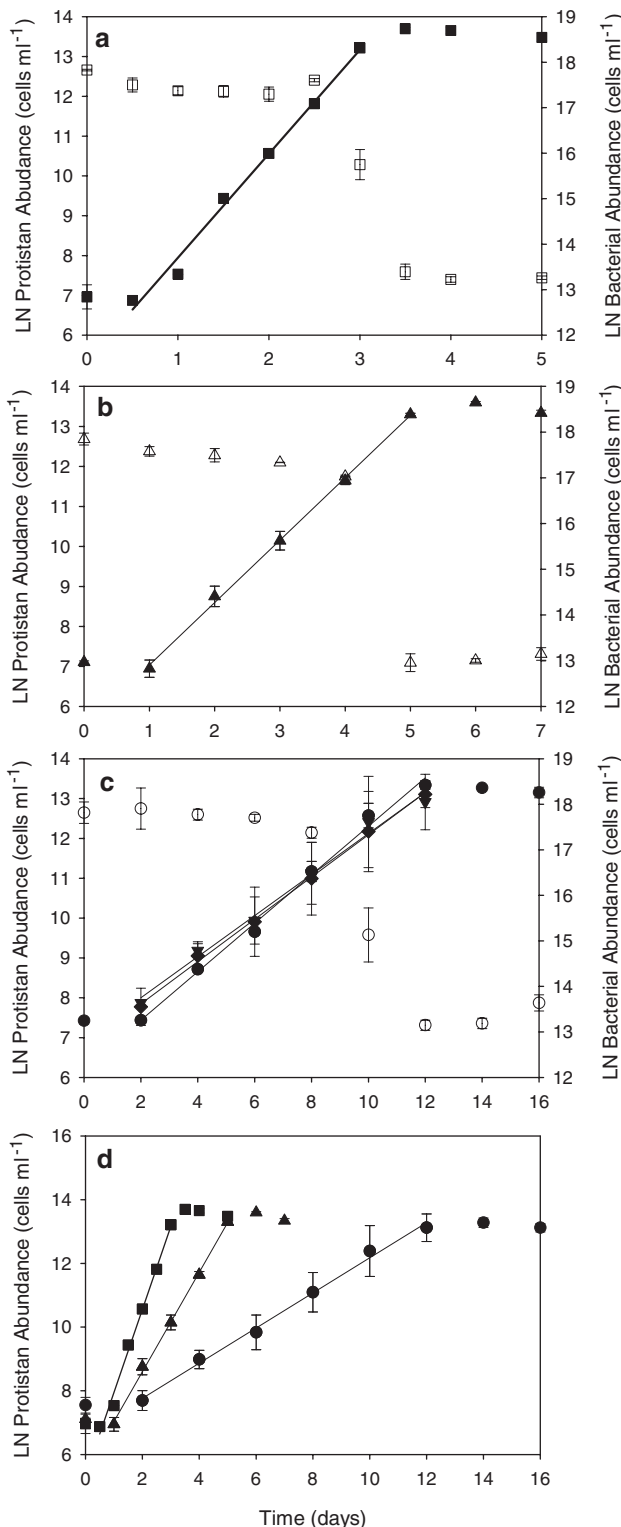


Figure 2 Growth dynamics of an Antarctic strain of *P. imperforata* (filled symbols) and removal of bacterial prey (open symbols) at three temperatures (a–c): 10 °C (a), 5 °C (b) and 0 °C (c). Different symbols in (c) indicate growth of the protist on three strains of *H. marina*. Composite of information from (a–c) comparing the growth of *P. imperforata* at 10 °C (■), 5 °C (▲) and 0 °C (●) (d) The 0 °C symbols in (d) represent the average growth on all *H. marina* strains.

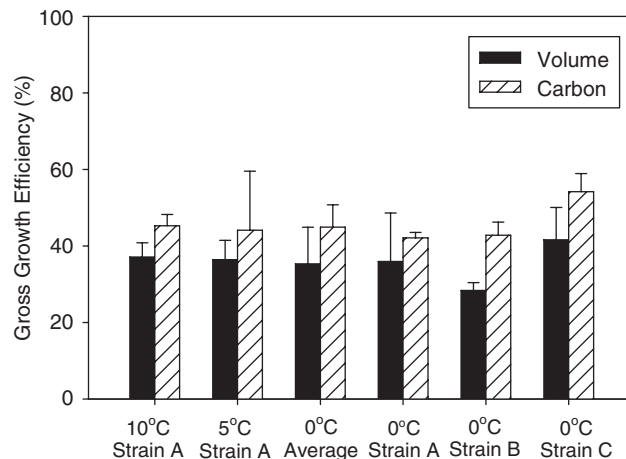


Figure 3 Gross growth efficiencies of the Antarctic strain of *P. imperforata* based on measurements of cell volume (solid) and carbon (hatched). Protists were grown at three temperatures and on three strains of *H. marina*, as indicated. Results are plotted at 0 °C for the three *H. marina* strains separately and averaged. Error bars represent one standard deviation, $n = 3$.

which were not significantly different; Figure 4a). The Q_{10} values for growth rates calculated at 5 °C intervals from 0° to 20 °C in experiment 2 were 15, 2.2, 3.6 and 0.93. GGE was not consistently affected by increasing temperature (Figure 4b). Only two pairs of GGEs were statistically different (0 °C versus 10 °C and 0 °C versus 15 °C, $P < 0.01$ for both; all other P -values were > 0.05). Thus, GGE observed at 0 °C was not significantly different from the GGE observed at 5 °C or 20 °C. The mean values of GGE at 5 °C and 10 °C were virtually identical (42% versus 44%), but the error term was much larger (standard deviations were 11 versus 4 for 5 °C versus 10 °C, respectively). It is possible that there were significant differences between 0 and 5 °C that were not detected because of the relatively large error term. Overall, GGEs in experiment 2 ranged from 31% at 0 °C to 53% at 15 °C based on volume.

Discussion

Studies detailing the effects of temperature on the growth rates of heterotrophic protists are numerous. A wide range of values for this parameter has been published, particularly for taxa that are believed to be of cosmopolitan distribution and considerable ecological significance such as species of the nanoflagellate genus *Paraphysomonas* (Figure 5a; Fenchel, 1982; Sherr *et al.*, 1983; Goldman and Caron, 1985; Caron *et al.*, 1986; Choi and Peters, 1992; Eccleston-Parry and Leadbeater, 1995; Finlay and Clarke, 1999). Collectively, these data indicate that temperature sets an upper limit on the growth rates of *Paraphysomonas* spp. Although it is probable that long-term exposure of conspecifics to different climatic regions has resulted in the evolution

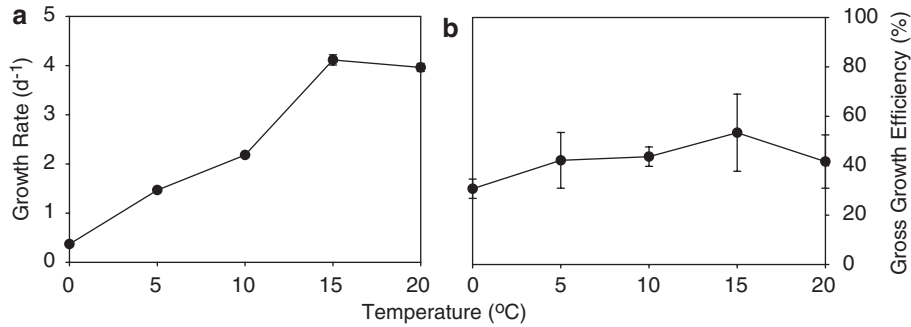


Figure 4 The relationship between temperature and growth rate (a) or gross growth efficiency (b) for an Antarctic strain of *P. imperforata* grown on *H. marina* strain A. Error bars represent one standard deviation, $n = 3$.

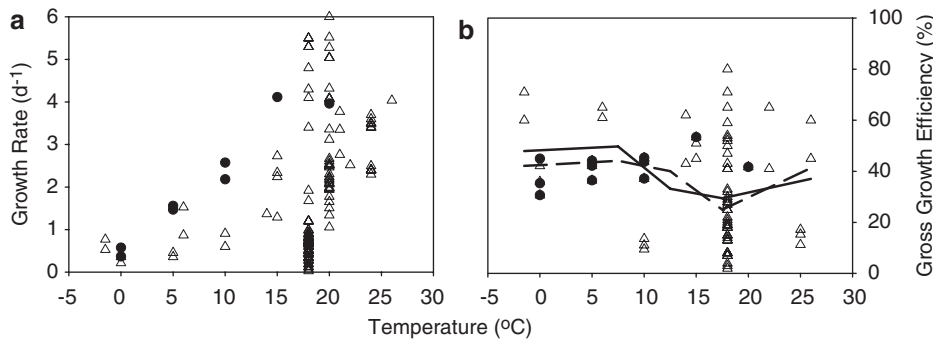


Figure 5 The relationship between temperature and growth rate (a) or gross growth efficiency (GGE) (b) for strains of *Paraphysomonas* spp. observed in this study (● growth rates and GGE from this study), and in published reports for temperate and polar strains (△ growth rates and GGE from other studies) (Fenchel, 1982; Caron *et al.*, 1985, 1986, 1991; Goldman and Caron, 1985; Goldman *et al.*, 1987; Choi and Peters, 1992; Eccleston-Parry and Leadbeater, 1994, 1995; Ishigaki and Sleigh, 2001; John and Davidson, 2001; Delaney, 2003; Fu *et al.*, 2003; Selph *et al.*, 2003). Solid lines in (b) indicate mean values and dashed lines indicate median values for each 5 °C temperature interval. There was no distinction made between carbon- and volume-based GGEs in (b).

of unique biochemical and physiological adaptations to allow existence in different temperature regimes, there is little direct evidence that these adaptations can result in rapid growth of protists at low temperature (Rose and Caron, 2007).

The Antarctic *P. imperforata* examined in this study was capable of growth at all temperatures examined (Figure 5a). Q_{10} values were relatively high at low temperatures and decreased as temperature increased within the range examined. Q_{10} values considerably higher than 2 are typically observed at temperatures below optimal, and may be indicative of increased energy barriers to the biochemical processes involved in determining growth rates (Hochachka and Somero, 2002). The Q_{10} values obtained in this study between 0 and 5 °C (6.9, experiment 1; 15, experiment 2) were greater than all other values observed between 5 and 20 °C (0.93–3.6), implying that our Antarctic *P. imperforata* was growing outside its optimal physiological range at ambient Antarctic temperatures.

A literature review was compiled of growth rates and GGEs for *Paraphysomonas* spp. for comparison to the information obtained in this study. A total of 131 growth rates from 14 studies (Figure 5a, open symbols) and 55 GGEs from four studies (Figure 5b, open symbols) were compared to data reported in

this study (Figure 5a and b, closed symbols). Temperature had a strong effect on the maximal growth rates reported for *Paraphysomonas* spp. The growth rates obtained in this study were similar to rates reported for an Arctic strain of *P. imperforata* at low temperatures (≤ 6 °C). The growth rates obtained at 5 °C, 10 °C and 15 °C in this study were higher than those reported for temperate *Paraphysomonas* spp. at the same temperatures, while growth rates obtained in this study at the highest temperature (20 °C) were less than maximal rates observed for *Paraphysomonas* species in other studies but within the range of rates reported for temperate species.

We believe that the response of the growth rate of our isolate of *P. imperforata* to temperature is representative of this species in Antarctic marine ecosystems. The isolation of the strain of *P. imperforata* used in this study was performed without ever increasing the temperature above 2 °C to prevent selection against heat-sensitive microorganisms. In addition, the temperature acclimation was carried out slowly to prevent heat shock of the protist. The growth rates observed in this study were among the highest rates reported over the temperature range –1.5 to 15 °C. However, our isolate has a lower maximal growth rate at 20 °C relative to the

maximal growth rates reported for other *Paraphysomonas* spp. (solid circles versus open triangles in Figure 5a). We speculate that the inability of our Antarctic strain of *P. imperforata* to grow as rapidly as some conspecific and congeneric strains at 20 °C may reflect a physiological compromise that enabled robust growth at extremely low seawater temperature.

Although temperature exerts a significant effect on maximal growth rates of protists, its effect on GGE is less clear. In theory, a significant relationship between GGE and growth rates of protists would not be expected because, unlike large, multicellular organisms, protists do not have significant basal respiration (Fenchel, 1986). Published data indicate that GGE of protists may increase, decrease or remain unchanged with changes in temperature (Laybourn and Stewart, 1975; Rogerson, 1981; Rassoulzadegan, 1982; Verity, 1985; Caron *et al.*, 1986; Choi and Peters, 1992; Mayes *et al.*, 1997; Ishigaki and Sleigh, 2001). GGEs measured in this study yielded a relatively narrow range of values, which did not vary consistently with temperature. Overall averages of GGE across both experiments were 39% based on biovolume and 46% based on carbon. Differences of approximately 10% between these methods for calculating GGE have been noted previously and are a consequence of the different assumptions inherent in the two approaches (Caron *et al.*, 1986).

Gross growth efficiencies summarized for the two *Paraphysomonas* species for which published data are available (*P. bandaiensis* and *P. imperforata*) spanned a very wide range (Figure 5b; 2–80%). The values obtained for *P. imperforata* in this study were considerably more constrained (28–54%) and in general agreement with the overall mean and/or median values for GGEs across the temperature range –1.5 to 26 °C (overall mean = 33%, solid line in Figure 5b; overall median = 31%, dashed line in Figure 5b). Averaged over the temperature intervals <5 °C, ≥5° to <10 °C, ≥10 to <15 °C, ≥15 to <20 °C, ≥20 °C, the range of mean and median GGEs (29–50% for mean and 25–44% for median GGE) were quite similar to the narrow range of GGEs obtained in this study.

Although the range of reported values of GGE for two species of *Paraphysomonas* was quite large from a single study (2%–80%; Selph *et al.*, 2003), the mean and median values for each 5 °C increment of temperature were constrained to a relatively narrow range (mean = 29–50%, median = 25–44%; solid and dashed lines, respectively in Figure 5b). Selph *et al.* (2003) reported extremely low GGEs at 18 °C, which resulted in mean and median estimates in the ≥15 to 20 °C interval, otherwise the range of mean and median values would have been even narrower: 33–50% and 40–51%, respectively. The mean and median values of GGE thus show no apparent relationship with temperature.

Given the narrow range of GGEs measured in this study, and the agreement of our values with mean GGEs reported for two species of *Paraphysomonas*, it is not clear why the range of GGEs in the literature is so wide (2–80%, Figure 5b). Factors such as poor food quality or rapid acclimation of cultures to experimental temperatures might explain reports of low values, but do not satisfactorily explain some high GGEs that have been reported. Some differences in GGE are to be expected due to inherent differences in the calculation of efficiencies. Carbon-based GGE calculations assume that protists make an insignificant contribution to particulate carbon at the beginning of an experiment (because of relatively high bacterial abundances). This assumption allows the calculation of carbon content in a single bacterium, which is then used as a constant in the calculation of GGE. The use of a constant assumes that average carbon content per bacterium does not change over the course of an experiment. This method also assumes that all particulate carbon can be attributed to either protistan or bacterial cells, and that egested particulate carbon does not contribute significantly to total carbon. If the average carbon content of bacteria decreases over the course of an experiment (due to starvation), carbon-based GGE could underestimate actual GGE. On the other hand, if egested particulate carbon comprises a significant portion of the total, carbon-based GGE could overestimate actual protistan GGE.

Biovolume-based GGE takes into account the changes in bacterial volume at the beginning and end of an experiment and excludes egested particulate carbon from the calculation. Biovolume-based GGEs can underestimate actual GGE, although, due to greater loss of protistan cell volume than bacterial cell volume from cell shrinkage upon preservation and preparation for microscopy. Shrinkage due to preservation can be prevented through the analysis of live samples. Estimates of cell volume (and GGEs) have been reported based on measurements of live cultures of protists and bacteria by flow cytometry (Choi and Peters, 1992), but the accurate conversion of measurements of forward scatter to cell size using reference beads is difficult, as the surface of the reference beads will scatter light differently than the surface of a cell. Neither carbon nor volume-based GGE take into account cryptic growth of the bacteria during the grazing phase of the protist.

Conclusions

This study provides some of the first information on growth of a cold-adapted nanoflagellate at extreme low temperature (0 °C). Our Antarctic strain of *P. imperforata* grew slowly but with high efficiency at this temperature, while growth was much more rapid (with a similar efficiency) at higher temperatures. The relationship between temperature and GGE of heterotrophic protists has been debated in

the past. GGE values compiled here indicate that if a relationship exists between temperature and GGE for two species of *Paraphysomonas*, it was more minor than that has been suggested previously. In addition, the wide variance in GGE reported in the literature demonstrates that the introduction of artifact into calculations of GGE due to methodological issues can be significant.

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