

## Effects of Temperature on Two Psychrophilic Ecotypes of a Heterotrophic Nanoflagellate, *Paraphysomonas imperforata*

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Two different psychrophilic types of the heterotrophic nanoflagellate *Paraphysomonas imperforata* were isolated from Newfoundland coastal waters and the Arctic Ocean. When fed bacteria without food limitation, both isolates were able to grow at temperatures from  $-1.8$  to  $20^{\circ}\text{C}$ , with maximum growth rates of  $3.28\text{ day}^{-1}$  at  $15^{\circ}\text{C}$  and  $2.28\text{ day}^{-1}$  at  $12.3^{\circ}\text{C}$  for the Newfoundland and the Arctic isolates, respectively. Ingestion rates increased with temperature from 14 to 62 bacteria flagellate $^{-1}\text{ h}^{-1}$  for the Newfoundland isolate and from 30 to 99 bacteria flagellate $^{-1}\text{ h}^{-1}$  for the Arctic isolate. While temperature did not affect cell yields (number of protozoa produced divided by number of bacteria consumed), it affected flagellate sizes. This differential effect of temperature on cell yield and cell size resulted in a changing gross growth efficiency (GGE) in terms of biovolume; colder temperatures favored higher GGEs. The comparison of  $Q_{10}$  values for growth rates and ingestion rates between the isolates shows that the Arctic isolate is better adapted to extremely cold temperature than the Newfoundland isolate. At seawater-freezing temperature ( $-1.8^{\circ}\text{C}$ ), the estimated maximum growth rates and maximum ingestion rates are  $0.81\text{ day}^{-1}$  and 30 bacteria flagellate $^{-1}\text{ h}^{-1}$  for the Arctic isolate and  $0.54\text{ day}^{-1}$  and 12 bacteria flagellate $^{-1}\text{ h}^{-1}$  for the Newfoundland isolate. Our findings about psychrophilic nanoflagellates fit the general characteristics of cold-water-dwelling organisms: reduced physiological rates and higher GGEs at lower temperatures. Because of the large and persistent differences between the isolates, we conclude that they are ecotypes adapted to specific environmental conditions.

Since microorganisms were incorporated as key components in marine food webs (43), heterotrophic nanoflagellates have been identified as major consumers of bacteria (51) as well as important remineralizers of nutrients in aquatic ecosystems (24). Heterotrophic nanoflagellates are also a trophic link between picoplankton and microzooplankton (1). The study of the energetics of these organisms is crucial to understanding the so-called microbial loop as well as aquatic ecosystems as a whole.

Despite the obvious need for energetic studies of bacterivorous nanoflagellates, data are very limited and confined to temperate species (11, 16, 23, 52, 54). To our knowledge, no ecophysiological data about heterotrophic protozoa isolated from cold waters are available except for a comparative study of temperature tolerances for a psychrophilic ciliate (35). The definition of psychrophilic organisms originally given for bacteria by Morita (38) requires that the organisms grow well at  $-1.5^{\circ}\text{C}$ , have an optimum growth temperature at or below  $15^{\circ}\text{C}$ , and do not grow above  $20^{\circ}\text{C}$ . We report here on the physiological parameters of two psychrophilic nanoflagellates, including growth and grazing rates and gross growth efficiencies (GGEs), and the effects of temperature on these parameters. Since our isolates are two ecotypes of *Paraphysomonas imperforata* collected at different latitudes, it is also possible to investigate genetically fixed responses among ecotypes by comparing our isolates with their temperate counterparts.

### MATERIALS AND METHODS

**Protozoa and bacteria.** *P. imperforata*, identified by scanning electron microscopy with the keys of Preisig and Hibberd (46), was isolated from a water sample at  $79^{\circ}37.3'\text{ N}$ ,  $108^{\circ}51.2'\text{ W}$ , in the Arctic Ocean (44), where the seawater

temperature is below  $2^{\circ}\text{C}$  year-round. The same chrysoomonad species was isolated from a water sample from Conception Bay, Newfoundland, Canada, where surface temperatures rise above  $5^{\circ}\text{C}$  by the end of spring. One species of rod-shaped bacterium (ca.  $1.5$  to  $2$  by  $1\text{ }\mu\text{m}$  when grown in organic rich medium), designated T1, was also isolated from the Newfoundland water sample and was used as a food source for the protozoa. Bacteria were grown axenically at  $4^{\circ}\text{C}$  in h/2 medium (25) without silicate but enriched with 0.1% yeast extract. Bacterial stock cultures were maintained in the same medium at  $4^{\circ}\text{C}$  by transferring 1 or 2 drops of the culture into fresh medium once a month. Protozoan stock cultures were also maintained at  $4^{\circ}\text{C}$  and transferred once a month by inoculating 1 or 2 drops of the culture into a T1 bacterial culture of  $10^8$  to  $10^9$  cells  $\text{ml}^{-1}$ . Sterile techniques were used throughout.

**Temperature range for growth.** Each nanoflagellate isolate was inoculated in 125-ml Erlenmeyer flasks with 50 ml of bacterial culture, with a final concentration of ca.  $10^3$  flagellates  $\text{ml}^{-1}$ . Three replicate flasks were incubated in a temperature-controlled water bath ( $\pm 0.1^{\circ}\text{C}$  accuracy) at specific temperatures. For each replicate, single samples of 2 ml each were taken every 6 to 12 h depending on the temperature and fixed with  $15\text{ }\mu\text{l}$  of Lugol's solution, and the protozoa were counted at least until the stationary phase was reached. The growth rate was determined from the exponential part of the curve. This procedure was repeated for both isolates at temperatures ranging from  $-1.8$  to  $20^{\circ}\text{C}$ .

**Grazing experiments.** Grazing experiments were performed with both nanoflagellate isolates preying upon heat-killed bacteria at three different temperatures ( $-1.5$ , 6, and  $15^{\circ}\text{C}$ ). The increase in nanoflagellate numbers and the disappearance of bacteria were monitored over time. Heat-killed bacteria were used as prey to avoid growth of bacteria in the grazing vessel, which would have complicated the estimation of prey disappearance. Bacteria were grown as

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described above at 4°C (200-ml cultures in 500-ml Erlenmeyer flasks) and harvested while in the exponential phase of growth with a concentration of ca.  $5 \times 10^8$  bacteria  $\text{ml}^{-1}$ . They were then heat killed at 60°C for 30 min (11). The effectiveness of heat treatment was tested by inoculating heat-killed bacteria into enriched h/2 medium; growth was never observed. Heat-killed bacteria were spun down at  $1,750 \times g$  for 15 min in sterile 50-ml polypropylene centrifugation tubes and resuspended in sterile filtered (0.2- $\mu\text{m}$ -pore-size filter) seawater. The size of heat-killed bacteria was measured by flow cytometry for later calculation of the GGEs of the nanoflagellates.

The nanoflagellates were grown on bacteria at the same temperature as in the actual grazing experiment to reduce the possible lag period of temperature acclimatization. The nanoflagellates were grown to the end of the exponential phase, usually about  $5 \times 10^5$  cells  $\text{ml}^{-1}$ , to maximize the density of nanoflagellates and minimize the density of live bacteria. The nanoflagellates were then separated from the remaining live bacteria by two rounds of centrifugation, one at  $215 \times g$  for 10 min and the other at  $70 \times g$  for 10 min. The resulting pellets were resuspended in sterile filtered (0.2- $\mu\text{m}$ -pore-size filter) seawater. This procedure reclaimed ca. 50% of the protozoa and eliminated ca. 99% of the live bacteria (from  $10^8$  to  $10^6$  bacteria  $\text{ml}^{-1}$ ). Nanoflagellates and heat-killed bacteria were then counted and diluted with sterile filtered (0.2- $\mu\text{m}$ -pore-size filter) seawater to the desired concentrations. The conditions for starting concentrations of bacteria and nanoflagellates were as follows: (i) the initial bacterial concentration was high enough for nanoflagellates to grow exponentially for a reasonable length of time in order to calculate their growth rates, and (ii) the initial bacterial concentration was low enough for nanoflagellates to graze a significant portion of bacteria so that ingestion rates for nanoflagellates could be calculated. Initial bacterial concentrations ranged from  $3 \times 10^8$  to  $6.7 \times 10^8$  cells  $\text{ml}^{-1}$  and initial nanoflagellate concentrations ranged from  $9.4 \times 10^4$  to  $2.5 \times 10^5$  cells  $\text{ml}^{-1}$ , depending on the growth rates of the nanoflagellates. For each nanoflagellate isolate at each temperature, three replicates of 45 ml each in 125-ml Erlenmeyer flasks were incubated in a temperature-controlled water bath bubbled with filtered air to avoid settling of bacteria and oxygen limitation. Samples were taken at 2- to 6-h intervals for 18 to 36 h, depending on the growth rates of the nanoflagellates, and fixed with Lugol's solution. Live bacteria carried over with the flagellates still had the potential to grow. However, this growth was probably far from maximal because the bacteria were in filtered (0.2- $\mu\text{m}$ -pore-size filter) seawater rather than enriched medium. The initial concentrations of live bacteria were calculated conservatively so that even assuming maximal bacterial growth, the number of live bacteria would not exceed 10% of the number of heat-killed bacteria by the end of the experiment. Because live and heat-killed bacteria had somewhat different morphologies (rod shaped versus spherical, respectively) they could be distinguished microscopically. Live bacteria were observed only occasionally during the grazing experiments.

**Counting and sizing.** Nanoflagellates were counted in a hemocytometer at a magnification of  $\times 100$  by bright-field microscopy. Bacteria were counted by the acridine orange direct count technique (30) on 0.2- $\mu\text{m}$ -pore-size black Nuclepore filters at a magnification of  $\times 1,250$  by epifluorescence microscopy. For later calculation of GGEs, the sizes of the flagellates were measured. It is well known that fixation has an effect on the size and morphology of organisms. In the case of protozoan fixation, shrinkage depends

on the fixation method and on the protozoan species (4, 12, 55). Even though the same fixation method and the same species were used, we sized live flagellates rather than fixed ones to avoid the possible differential effects of fixation on cell volumes of different ecotypes. Live nanoflagellates and heat-killed bacteria were sized with an EPICS 753 (Coulter) flow cytometer. Size measurements of live flagellates were done as quickly as possible, usually within 10 min after sampling. To increase sensitivity, a confocal lens with a beam spot of 16 by 40  $\mu\text{m}$  was used instead of the standard beam-shaping lens of 16 by 160  $\mu\text{m}$ . Forward angle light scatter was collected with a 325-nm band-pass filter, with an excitation wavelength at 320 nm. Forward angle light scatter signals were collected along different channels depending on particle diameter. Channel numbers were converted into lengths by an empirically derived relationship by using reference beads of known diameter. About  $2 \times 10^3$  to  $4 \times 10^3$  cells were sized with 4.4-, 6.0-, and 9.0- $\mu\text{m}$  beads for the flagellates and 1.5- and 4.4- $\mu\text{m}$  beads for the bacteria. Volumes were calculated by assuming spherical shapes for both nanoflagellates and heat-killed bacteria.

**Calculations and statistics.** From the exponential part of the protozoan growth curve, physiological parameters of the nanoflagellate isolates were calculated. The growth rate was obtained as the slope of the linear portion of a plot of the logarithm of protozoan concentration versus time. Ingestion rates and clearance rates were calculated by the equations of Frost (20), as modified by Heinbokel (28), to account for the increase in protozoan numbers. Mean concentrations of flagellates ( $\langle P \rangle$ ) and bacteria ( $\langle B \rangle$ ) were calculated as follows:  $\langle P \rangle = (P_2 - P_1)/(\ln P_2 - \ln P_1)$  and  $\langle B \rangle = (B_1 - B_2)/(\ln B_1 - \ln B_2)$ , where  $P_i$  and  $B_i$  are concentrations of protozoa and bacteria, respectively, at time  $t$  (time 2 being later than time 1). An instantaneous ingestion rate ( $i$ ) can be viewed at a rate of disappearance of bacteria when ingestion is the only loss factor and is calculated as  $i = (\ln B_2 - \ln B_1)/\Delta t$ , where  $\Delta t$  is time 2 minus time 1. The ingestion rate,  $I$ , defined as the number of bacteria consumed per protozoan per unit time, was then calculated as the instantaneous ingestion rate times the ratio of the mean concentration of bacteria to the mean concentration of protozoa. The clearance rate,  $C$ , defined as the volume of water cleared of bacteria by a protozoan cell per unit time, was computed as the ingestion rate divided by the mean concentration of bacteria. A cell yield ( $Y$ ), defined as the number of protozoa produced divided by the number of bacteria consumed, was also computed, by using the formula  $Y = (P_2 - P_1)/(B_1 - B_2)$ .

The GGE was calculated in terms of biovolume as the efficiency of conversion of bacterial volume into flagellate volume, by using the formula

$$\text{GGE} = \frac{(P_2 - P_1) \times V_p}{(B_1 - B_2) \times V_b} \times 100$$

where  $V_p$  and  $V_b$  are the cell volumes of live flagellates and heat-killed bacteria, respectively.

$Q_{10}$  values were estimated for growth rates and ingestion rates by nonlinear regression. Exponential regressions were chosen on the basis of highest  $r^2$  among the tested models. Thus,  $\text{Rate} = A \times 10^{B \times T_{\text{temp}}}$ , and therefore  $Q_{10} = 10^{10 \times B}$ , where Rate is the growth rate or ingestion rate,  $A$  and  $B$  are constants, and Temp is the temperature at which Rate is measured.

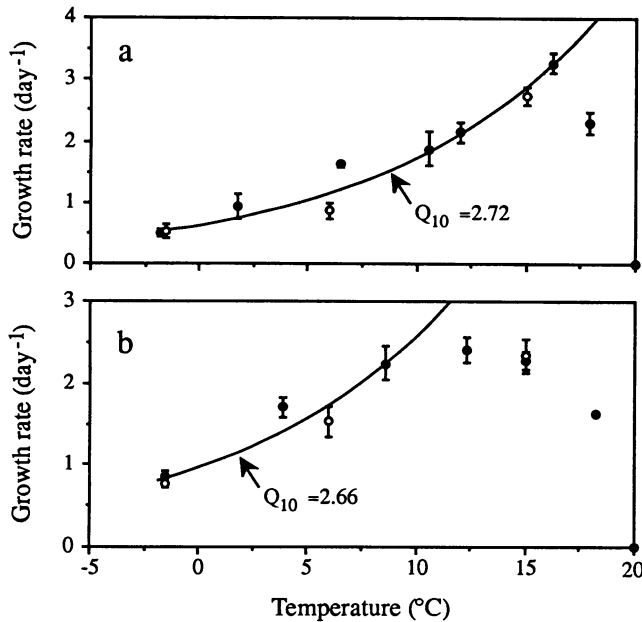


FIG. 1. Growth rates (at different temperatures) of *P. imperforata* Newfoundland (a) and Arctic (b) isolates. Symbols represent the mean of three replicates, and bars indicate  $\pm 1$  standard error.  $\bullet$ , growth experiments;  $\circ$ , grazing experiments. The curves are exponential regressions of points below the maximum growth rate. The  $Q_{10}$  values are calculated from the regression lines.

## RESULTS

The growth rates of the nanoflagellates in the temperature interval from  $-1.8$  to  $20^\circ\text{C}$  ranged between  $0$  and  $3.28 \text{ day}^{-1}$  for the Newfoundland isolate and between  $0$  and  $2.28 \text{ day}^{-1}$  for the Arctic isolate (Fig. 1). The growth rates of the two isolates showed similar patterns in response to temperature change: (i) they decreased above and below the optimal temperature, which was about  $15^\circ\text{C}$  for the Newfoundland isolate and  $12.3^\circ\text{C}$  for the Arctic isolate; (ii) the change in growth rates was slower below than above the optimal temperature; and (iii) neither isolate survived at  $20^\circ\text{C}$ . In most grazing experiments, the nanoflagellates started to grow exponentially at their expected rates immediately after being introduced into grazing vessels (Fig. 2a). Occasionally, the nanoflagellates showed a lag period (up to 6 h in an extreme case) before entering an exponential phase (Fig. 2b). Exponential growth lasted 11 to 25 h, depending on initial bacterial concentrations and nanoflagellate growth rates (two examples in Fig. 2). The growth rates during the grazing experiments were consistent with those obtained previously (Fig. 1).

For both nanoflagellates at all three temperatures, ingestion rates increased with increasing growth rates, from 14 to 62 bacteria flagellate $^{-1} \text{ h}^{-1}$  for the Newfoundland isolate and from 30 to 99 bacteria flagellate $^{-1} \text{ h}^{-1}$  for the Arctic isolate, showing significant differences ( $P < 0.01$ ) between temperatures (Fig. 3; Table 1). The Arctic isolate always had a higher ingestion rate than the Newfoundland isolate at any given temperature. Even though clearance rates were significantly different at the different temperatures, no pattern emerged (Table 1). Clearance rates depend on prey concentrations when concentrations are not limiting (42); the higher the prey concentration, the lower the clearance rate. Since all of the grazing experiments were performed without prey

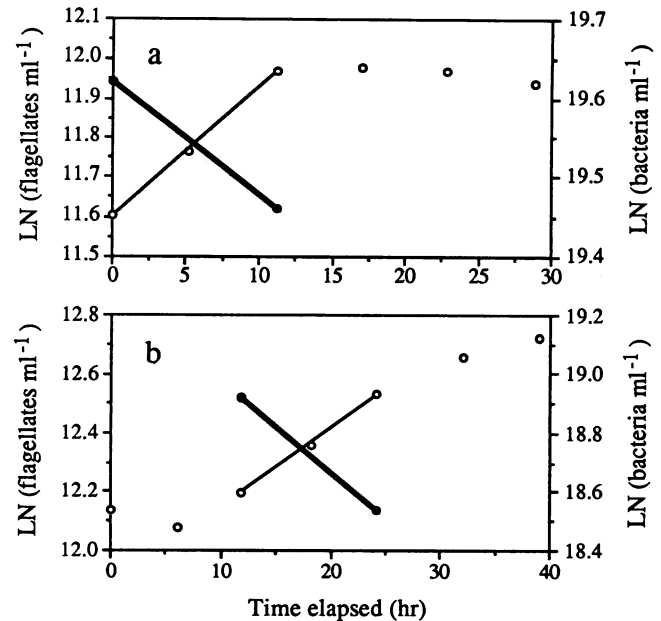


FIG. 2. Concentrations of *P. imperforata* and bacteria from grazing experiments with the Arctic isolate at  $-1.5^\circ\text{C}$  (replicate B) (a) and the Newfoundland isolate at  $-1.5^\circ\text{C}$  (replicate B) (b). Symbols:  $\circ$ , protozoa;  $\bullet$ , bacteria. Lines represent the intervals used to calculate rates.

limitation, temperature effects on clearance rates were obscured because of differences in prey concentrations. Cell yields (Table 1) showed no statistical differences between temperatures; therefore, means based on all three temperatures were computed, resulting in  $1.56 \times 10^{-3}$  flagellates bacterium $^{-1}$  for the Newfoundland isolate and  $1.12 \times 10^{-3}$  flagellates bacterium $^{-1}$  for the Arctic isolate.

Cell sizes of nanoflagellates were significantly different at different temperatures (Table 1). The Newfoundland isolate was significantly larger at  $-1.5$  than at  $6$  and  $15^\circ\text{C}$ . The Arctic isolate was largest at  $-1.5^\circ\text{C}$ , but the only significant difference at an  $\alpha$  level of  $0.05$  was between the extremes of temperature. Because of the characteristics of flow cytometry, with a large number of cells (ca. 2,000 to 4,000) sized for each replicate, it was possible to detect slight differences in the cell size of nanoflagellates (down to  $0.01 \mu\text{m}$ ) at different temperatures which would have been missed had regular microscopy been used. Even though the smallest diameter was 93 and 96% of the largest for the Newfoundland isolate and the Arctic isolate, respectively, the smallest cell volume was 80 and 90% of the largest for the Newfoundland isolate and the Arctic isolate, respectively. Heat-killed bacteria had an average diameter of  $0.99 \pm 0.14 \mu\text{m}$  and a corresponding volume of  $0.53 \pm 0.22 \mu\text{m}^3$  (mean  $\pm$  standard deviation) (Fig. 4).

At all three temperatures, GGE decreased from 71 to 45% with increasing temperature for the Newfoundland isolate. The Arctic isolate showed similar values, but with a highest efficiency of 61% at  $6^\circ\text{C}$  and a lowest efficiency of 51% at  $15^\circ\text{C}$  (Table 1). Since GGE was defined in terms of biovolume in this study and a constant biovolume of heat-killed bacteria was used, the calculation of GGE becomes  $\text{GGE} = \text{cell yield} \times \text{flagellate cell volume} \times \text{constant} \times 100$ , where the constant is  $1/\text{bacterial cell volume}$ . Because the statistical analysis showed that temperature had no measurable

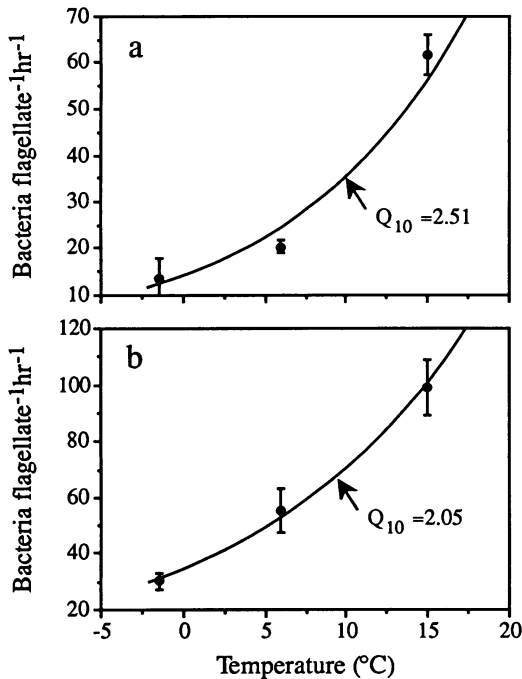


FIG. 3. Ingestion rates versus temperature for *P. imperforata* Newfoundland (a) and Arctic (b) isolates. Datum points represent the mean of three replicates, and bars indicate  $\pm 1$  standard error. The curves are exponential regressions. The  $Q_{10}$  values are calculated from the regression lines.

effect on the cell yields, differences in GGEs between temperature treatments depend completely on nanoflagellate cell volumes. GGEs calculated with an average cell yield for each isolate (Table 1) were highest at  $-1.5^{\circ}\text{C}$ : 68% for the Newfoundland isolate and 61% for the Arctic isolate.

## DISCUSSION

**Growth and grazing rates.** By extrapolation of the regression lines in Fig. 1, the estimated growth rates at  $-1.8^{\circ}\text{C}$  (freezing point of seawater) are  $0.54\text{ day}^{-1}$  for the Newfoundland isolate and  $0.81\text{ day}^{-1}$  for the Arctic isolate. Extrapolating in the same way to obtain ingestion rates from

the data in Fig. 2, we obtain rates of 12 bacteria flagellate $^{-1}\text{ h}^{-1}$  for the Newfoundland isolate and 30 bacteria flagellate $^{-1}\text{ h}^{-1}$  for the Arctic isolate. These growth and ingestion rates are relatively high, and in situ rates are expected to be somewhat lower because of reduced bacterial size and concentration. In this study, bacterial concentrations were 2 to 3 orders of magnitude higher than the naturally occurring concentrations of  $10^4$  to  $10^5$  bacteria  $\text{ml}^{-1}$  (44, 45). Preliminary grazing experiments using fluorescence-labeled bacteria (53) suggest that our nanoflagellate isolates cannot graze effectively below a bacterial prey density of  $10^5$  cells  $\text{ml}^{-1}$  (data not shown). Moreover, the biovolume of naturally occurring bacteria is about 2 orders of magnitude smaller than that of laboratory-cultured T1, suggesting that *P. imperforata* would need higher concentrations of natural bacteria to achieve the same growth rates as with cultured bacteria. Nevertheless, even at  $-1.5^{\circ}\text{C}$ , the nanoflagellate isolates have the potential to exert a significant grazing pressure on bacteria under high prey density conditions, such as on marine aggregates or in blooms associated with sea ice.

The direct relationship of protozoan growth rates to temperature has been repeatedly documented (2, 10, 19, 47, 48, 52). The  $Q_{10}$  values of *P. imperforata* growth rates are somewhat higher than the theoretical value of 2, but they are in good agreement with those of other studies (2.48 for *P. imperforata* between 14 and  $26^{\circ}\text{C}$  [10] and 2.49 for a *Monas* sp. between 3 and  $18^{\circ}\text{C}$  [52]). For ingestion, the Newfoundland isolate and the Arctic isolate have  $Q_{10}$  values of 2.51 and 2.05, respectively, which are lower than other reported values (3.73 for *P. imperforata* between 14 and  $26^{\circ}\text{C}$  [10] and 3.12 for mixed 3- to  $5\text{-}\mu\text{m}$  flagellates between 3 and  $16^{\circ}\text{C}$  [54]).

When our isolates are compared with a *P. imperforata* isolate from a tidal channel near Vineyard Sound, Mass. (10), general trends of adaptation in response to temperature are suggested. The following trends may be inferred from the growth rates shown in Fig. 5. (i) The optimum temperatures increase with decreasing latitude of collection sites. (ii) The maximum growth rates at optimal temperatures also increase with decreasing latitude. (iii) The slopes of growth rates versus temperature below the optimal temperature decrease more slowly for isolates from cold water, indicating that isolates from colder water grow faster below a certain temperature; e.g., the Arctic isolate grows faster than the Newfoundland isolate below  $12.7^{\circ}\text{C}$  (refer to the intersection

TABLE 1. Summary of effects of temperature on different physiological parameters of two marine cold-water isolates of *P. imperforata*<sup>a</sup>

Isolate	Temp ( $^{\circ}\text{C}$ )	Growth rate ( $\text{day}^{-1}$ )	Ingestion rate (bacteria flagellate $^{-1}\text{ h}^{-1}$ )	Clearance rate (pl flagellate $^{-1}\text{ h}^{-1}$ )	Cell yield (flagellates bacterium $^{-1}$ )	Diam ( $\mu\text{m}$ )	Vol ( $\mu\text{m}^3$ )	GGE (%) <sup>b</sup>
Newfoundland	-1.5	0.53 (0.07)	14 (4.1)	107 (23.6)	$1.63 \times 10^{-3}$ ( $0.11 \times 10^{-3}$ )	7.62 (0.02)	232 (1.6)	71 (68)
	6.0	0.87 (0.07)	20 (1.5)	233 (19.8)	$1.83 \times 10^{-3}$ ( $0.32 \times 10^{-3}$ )	7.09 (0.06)	187 (5.0)	65 (55)
	15.0	2.73 (0.08)	62 (4.9)	104 (10.0)	$1.25 \times 10^{-3}$ ( $0.05 \times 10^{-3}$ )	7.16 (0.04)	192 (2.9)	45 (57)
Significance <sup>c</sup>		**	**	**	NS	**	**	
Arctic	-1.5	0.77 (0.03)	30 (3.0)	99 (10.7)	$1.10 \times 10^{-3}$ ( $0.15 \times 10^{-3}$ )	8.20 (0.03)	288 (3.3)	60 (61)
	6.0	1.53 (0.11)	55 (8.1)	169 (25.0)	$1.18 \times 10^{-3}$ ( $0.12 \times 10^{-3}$ )	8.07 (0.02)	275 (1.8)	61 (58)
	15.0	2.34 (0.12)	99 (9.9)	316 (54.4)	$1.04 \times 10^{-3}$ ( $0.02 \times 10^{-3}$ )	7.89 (0.09)	258 (8.6)	51 (54)
Significance		**	**	**	NS	*	*	

<sup>a</sup> Except for GGEs, data are means of three replicates with standard error in parentheses.

<sup>b</sup> Values in parentheses are GGEs calculated with an overall mean cell yield for each isolate. See text.

<sup>c</sup> Statistical result from one-way analysis of variance tests using temperature as treatment. \*\*, significant at  $\alpha = 0.01$ ; \*, significant at  $\alpha = 0.05$ ; NS, not significant. Where significant differences were found, comparisons of means were conducted by Tukey's test.

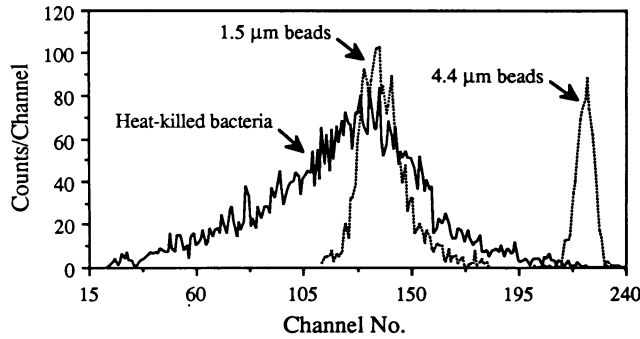


FIG. 4. Forward angle light scattering distribution of a population of heat-killed bacteria (isolate T1) obtained by flow cytometry. Channel number can be related to size with reference beads of known diameter.

points in Fig. 5). Since the ingestion rate data available for the temperate isolate are based on herbivory (10), it is not possible to compare the absolute values of ingestion rates. However, like the slopes of growth rates, the slopes of ingestion rates versus temperature are lower for colder-water isolates; that is,  $Q_{10}$  values increase with decreasing latitude of collection sites. Psychrophilic bacterial have also been reported to have a similar response to low temperature. As the temperature decreases below the optimum, growth rates (27) and metabolic rates (31) of psychrophiles decrease more slowly than those of mesophiles. For microorganisms, it appears to be a general phenomenon that psychrophiles have higher physiological rates at cold temperatures but have lower maximum rates at optimum temperatures than mesophiles. This suggests that there is a trade-off between maintaining high rates at low temperatures and having high maximum rates at optimum temperatures, although the physiological and biochemical basis for this hypothesis is inadequately understood. Spatial and temporal changes of temperature in aquatic ecosystems could lead to changes in microbial communities by giving a competitive advantage to one or another group of microorganisms.

**Size and GGE.** Isolates of *P. imperforata* are larger at higher-latitude collection sites than at lower latitudes. In this study, the diameter range over the three tested temperatures was 7.89 to 8.20  $\mu\text{m}$  for the Arctic isolate and 7.16 to 7.62  $\mu\text{m}$  for the Newfoundland isolate, while a diameter of 7  $\mu\text{m}$  was reported for the temperate isolate (23). For our isolates, cell

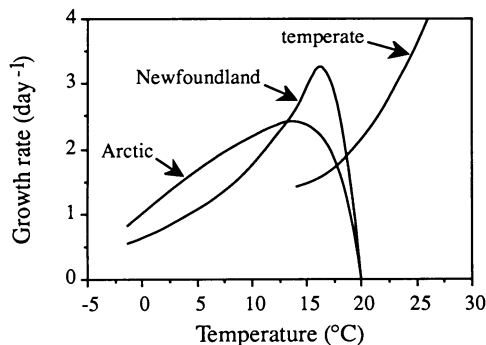


FIG. 5. Growth rate-versus-temperature curves for different isolates of *P. imperforata*. The curves were fitted visually from observed data.

size changed with temperature, while no change was observed for the temperate isolate (10). Since these changes are quite small (less than 1  $\mu\text{m}$ ), they are not easily detected by light microscopy and might have been overlooked previously. Our nanoflagellates had their largest volumes at the coldest growth temperature ( $-1.5^{\circ}\text{C}$ ). Other protozoans such as ciliates (19, 32, 35) and amoebae (2, 49), which are much larger than nanoflagellates, show the same trend of increased sizes when grown at low temperatures.

Even though carbon has been most widely used as a currency for GGE, biomass production of protozoa in terms of carbon usually has not been measured directly but rather has been estimated by multiplying the number of newly produced protozoa by a fixed value of carbon content per protozoan cell, without considering possible changes in carbon content with different culture conditions (15, 52). This automatically guarantees constancy of GGE (in terms of carbon) once cell yields are constant. Even when biomass carbon production was measured directly, accurate estimates were hindered by the inability to separate completely protozoa from prey particles and egested particles (9, 10, 55). Underestimation of GGE was also suggested, however, when it was based on cell volume of fixed protozoa, because of shrinkage caused by fixation (10). Release of dissolved carbon during fixation and/or filtration could also contribute to underestimations of GGE. Considering the artifacts discussed above, we believe that GGE based on live-cell volume gives a more accurate estimate, especially when protozoan cells are sized by flow cytometry.

The GGE of heterotrophic nanoflagellates can vary depending on (i) prey condition (density, type, and chemical composition), (ii) flagellate condition (physiological status), and (iii) physical conditions (temperature, pH, and shaken versus nonshaken, etc.). When other growth conditions are held constant, prey density does not affect the GGE of protozoa during balanced growth (15, 16). Fenchel (16) and Fenchel and Finlay (17) reasoned that this is because almost all of the energy of growing flagellates is invested in biomass production, while maintenance energy is minimal. The effect of temperature on GGE of protozoa appears to be more complex. Usually, a decrease in temperature is accompanied by an increase in GGE (references 47 and 59 and this study), but there are reports of decreased GGE (34, 49) and temperature-independent GGE (10). However, some of these results may be due to the methods used because, as mentioned earlier, GGE will always appear constant once cell yields have been found constant and a fixed conversion factor such as a fixed value of carbon content per protozoan cell (15, 16) or a fixed value of protozoan cell volume (16) is used. Increased sizes of protozoa at lower temperatures (references 19 and 32 and this study) strongly suggest that the GGE of protozoa generally increases with decreasing temperature under balanced growth while cell yields are constant.

Our volume-based GGE estimates are higher than the GGE values found in the literature (see, for example, reference 8), which were generally calculated on the basis of carbon. The fact that the carbon/biovolume ratio is higher for bacteria (ca. 200 fg of C  $\mu\text{m}^{-3}$  for our bacteria [results of an unpublished study]) than for flagellates (100 fg of C  $\mu\text{m}^{-3}$  for a living *Monas* sp. [5]) can account for this difference.

**Ecological implications.** The high growth and grazing rates of our flagellate isolates may be unrealistic for cold marine pelagic environments, mainly because of the difference in prey size and concentration. However, bacteria have been found at concentrations as high as  $2 \times 10^7$  cells  $\text{ml}^{-1}$  with an

average cell volume of  $0.47 \mu\text{m}^3$  in and near sea ice (57). There is increasing evidence that in this type of environment, a complex trophic web with high concentrations of protozoa develops (18, 22, 33, 36, 56). The increased GGE of these organisms at low temperatures would accentuate their role as a trophic link to the thriving higher trophic levels near sea ice. *P. imperforata* can grow not only on bacteria but also on nanoautotrophs (10, 23). This would probably allow it to grow well in open water, especially during blooms.

*P. imperforata* has a widespread distribution (46), but it remains unknown how representative it is of the entire flagellate community at the sampling sites. However, the ecophysiological trends of increased size and GGE and decreased growth rates at low temperatures are found in most protozoa. Over a broad taxonomic range, from bacteria to fishes, these trends are repeatedly observed. For psychrophilic isolates of bacteria of the genus *Vibrio*, higher efficiencies and lower growth rates were found at low temperatures (13, 29, 39). Cells of bacteria such as *Salmonella typhimurium* (50) and a *Pseudomonas* sp. (14) are larger when grown at lower temperatures. Hagström and Larsson (26), working with pelagic bacteria, reported decreased growth rates and increased cell sizes at low temperatures. Chrzanowski et al. (14) reported a seasonal variation in cell volume of epilimnetic bacteria with an inverse relationship between cell volume and temperature. In the case of yeasts, a psychrophilic *Candida* strain had much higher growth rates and was much more active metabolically than the mesophile *Candida lipolytica* below  $10^\circ\text{C}$  (3). Brown and Rose (7), working with *Candida utilis*, reported an increase in cell volume at low temperatures. Planktonic copepods in cold water increase both body size and fecundity and have a longer development time (37). The arctic ctenophore *Mertensia ovum* reduces its metabolic rates and increases its size in winter (41). In addition, the sockeye salmon, *Oncorhynchus nerka*, has its maximum GGE at a temperature lower than that at which it has its maximum growth rate (6), and low temperature has also been shown to increase the size of zooplanktivores living in deep waters (58). Thus, there appears to be a general phenomenon of an increase in GGE when ectotherm heterotrophic organisms grow below the optimal temperature for growth. This means that even though growth rates decrease, relatively more energy is put into biomass production and less is lost via respiration, defecation, and excretion, etc.

With respect to the autoecology of *P. imperforata*, the isolates differ significantly in characteristics such as cell size, growth traits (the Newfoundland isolate tended to aggregate much more heavily in the stationary phase), and growth responses to temperature. In the limited studies of population genetics of protists, genetic differences between clones of diatoms have been recognized for *Thalassiosira* spp. (40), and the physiological differences among clones of *Skeletonema costatum* are actually related to genetic differences, as determined by electrophoretic banding patterns (21). In the case of *P. imperforata* isolates, large differences in physiological characteristics suggest that the isolates have adapted to temperature not only physiologically but also genetically. The importance of these differences is strengthened by the fact that the isolates have remained unchanged for over 2 years of laboratory culturing. Thus, we believe that the isolates belong to different ecotypes.

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#### REFERENCES

1. Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.* **10**:257-263.
2. Baldock, B. M., J. H. Baker, and M. A. Sleight. 1980. Laboratory growth rates of six species of freshwater *Gymnamoebia*. *Oecologia* **47**:156-159.
3. Baxter, R. M., and N. E. Gibbons. 1962. Observations on the physiology of psychrophilism in a yeast. *Can. J. Microbiol.* **8**:511-517.
4. Booth, B. C. 1987. The use of autofluorescence for analyzing oceanic phytoplankton communities. *Bot. Mar.* **30**:101-108.
5. Børsheim, K. Y., and G. Bratbak. 1987. Cell volume to cell carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. *Mar. Ecol. Prog. Ser.* **36**:171-175.
6. Brett, J. R., J. E. Shelbourn, and C. T. Shoop. 1969. Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size. *J. Fish. Res. Board Can.* **26**:2363-2394.
7. Brown, C. M., and A. H. Rose. 1969. Effects of temperature on composition and cell volume of *Candida utilis*. *J. Bacteriol.* **97**:261-272.
8. Caron, D. A., and J. C. Goldman. 1990. Protozoan nutrient regeneration, p. 283-306. In G. M. Capriulo (ed.), *Ecology of marine protozoa*. Oxford University Press, New York.
9. Caron, D. A., J. C. Goldman, O. K. Andersen, and M. R. Dennett. 1985. Nutrient cycling in a microflagellate food chain: II. Population dynamics and carbon cycling. *Mar. Ecol. Prog. Ser.* **24**:243-254.
10. Caron, D. A., J. C. Goldman, and M. R. Dennett. 1986. Effect of temperature on growth, respiration, and nutrient regeneration by an omnivorous microflagellate. *Appl. Environ. Microbiol.* **52**:1340-1347.
11. Caron, D. A., K. G. Porter, and R. W. Sanders. 1990. Carbon, nitrogen, and phosphorus budgets for the mixotrophic phytoflagellate *Poteroochromonas malhamensis* (Chrysophyceae) during bacterial ingestion. *Limnol. Oceanogr.* **35**:433-443.
12. Choi, J. W., and D. K. Stoecker. 1989. Effects of fixation on cell volume of marine planktonic protozoa. *Appl. Environ. Microbiol.* **55**:1761-1765.
13. Christian, R. R., and W. J. Wiebe. 1974. The effects of temperature upon the reproduction and respiration of a marine obligate psychrophile. *Can. J. Microbiol.* **20**:1341-1345.
14. Chrzanowski, T. H., R. D. Crotty, and G. J. Hubbard. 1988. Seasonal variation in cell volume of epilimnetic bacteria. *Microb. Ecol.* **16**:155-163.
15. Curds, C. R., and A. Cockburn. 1968. Studies on the growth and feeding of *Tetrahymena pyriformis* in axenic and monoxenic culture. *J. Gen. Microbiol.* **54**:343-358.
16. Fenchel, T. 1982. Ecology of heterotrophic microflagellates. II. Bioenergetics and growth. *Mar. Ecol. Prog. Ser.* **8**:225-231.
17. Fenchel, T., and B. J. Finlay. 1983. Respiration rates in heterotrophic, free-living protozoa. *Microb. Ecol.* **9**:99-122.
18. Fenchel, T., and C. C. Lee. 1972. Studies on ciliates associated with sea ice from Antarctica. *Arch. Protistenk. Bd.* **114**:231-236.
19. Finlay, B. J. 1977. The dependence of reproductive rate on cell size and temperature in freshwater ciliated protozoa. *Oecologia* **30**:75-81.
20. Frost, B. W. 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. *Limnol. Oceanogr.* **17**:805-815.
21. Gallagher, J. C. 1982. Physiological variation and electrophoretic banding patterns of genetically different seasonal populations of *Skeletonema costatum* (Bacillariophyceae). *J. Phycol.* **18**:148-162.
22. Garrison, D. L., K. R. Buck, and M. W. Silver. 1984. Microhet-

- erotrophs in the ice-edge zone. *Antarct. J. U.S.* **19**:109–111.
23. Goldman, J. C., and D. A. Caron. 1985. Experimental studies on an omnivorous microflagellate: implications for grazing and nutrient regeneration in the marine microbial food chain. *Deep Sea Res.* **32**:899–915.
  24. Goldman, J. C., D. A. Caron, and M. R. Dennett. 1987. Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. *Limnol. Oceanogr.* **32**:1239–1252.
  25. Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates, p. 29–70. *In* W. L. Smith and M. H. Chanley (ed.), *Culture of marine invertebrate animals*. Plenum Press, New York.
  26. Hagström, A., and U. Larsson. 1984. Diel and seasonal variation in growth rates of pelagic bacteria, p. 249–262. *In* J. E. Hobbie and P. J. L. Williams (ed.), *Heterotrophic activity in the sea*. Plenum Press, New York.
  27. Harder, W., and H. Veldkamp. 1971. Competition of marine psychrophilic bacteria at low temperatures. *Antonie van Leeuwenhoek* **37**:51–63.
  28. Heinbokel, J. F. 1978. Studies on the functional role of tintinnids in the Southern California Bight. I. Grazing and growth rates in laboratory cultures. *Mar. Biol. (Berlin)* **47**:177–189.
  29. Herbert, R. A., and C. R. Bell. 1977. Growth characteristics of an obligately psychrophilic *Vibrio* sp. *Arch. Microbiol.* **113**:215–220.
  30. Hobbie, J. E., R. J. Daley, and S. Jasper. 1977. Use of Nucleopore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* **33**:1225–1228.
  31. Ingraham, J. L., and G. F. Bailey. 1959. Comparative study of effect of temperature on metabolism of psychrophilic and mesophilic bacteria. *J. Bacteriol.* **77**:609–613.
  32. James, T. W., and C. P. Read. 1957. The effect of incubation temperature on the cell size of *Tetrahymena pyriformis*. *Exp. Cell Res.* **13**:510–516.
  33. Kottmeier, S. T., and C. W. Sullivan. 1988. Sea ice microbial communities (SIMCO). 9. Effects of temperature and salinity on rates of metabolism and growth of autotrophs and heterotrophs. *Polar Biol.* **8**:293–304.
  34. Laybourn, J. E. M., and J. M. Stewart. 1975. Studies on consumption and growth in the ciliate *Colpidium campylum* Stokes. *J. Anim. Ecol.* **44**:165–174.
  35. Lee, C. C., and T. Fenchel. 1972. Studies on ciliates associated with sea ice from Antarctica. II. Temperature responses and tolerances in ciliates from Antarctic, temperate, and tropical habitats. *Arch. Protistenk. Bd.* **114**:237–244.
  36. Marchant, H. J. 1985. Choanoflagellates in the Antarctic marine food chain, p. 271–276. *In* W. R. Siegfried and R. M. Laws (ed.), *Antarctic nutrient cycles and food webs*. Springer-Verlag KG, Berlin.
  37. McLaren, I. A. 1974. Demographic strategy of vertical migration by a marine copepod. *Am. Nat.* **108**:91–102.
  38. Morita, R. Y. 1975. Psychrophilic bacteria. *Bacteriol. Rev.* **39**:144–167.
  39. Morita, R. Y., and L. J. Albright. 1965. Cell yields of *Vibrio marinus*, an obligate psychrophile, at low temperature. *Can. J. Microbiol.* **11**:221–227.
  40. Murphy, L. S., and R. R. L. Guillard. 1976. Biochemical taxonomy of marine phytoplankton by electrophoresis of enzymes. I. The centric diatoms *Thalassiosira pseudonana* and *T. fluviatilis*. *J. Phycol.* **12**:9–13.
  41. Percy, J. A. 1988. Influence of season, size, and temperature on the metabolism of an arctic cydippid ctenophore, *Mertensia ovum* (Fabricius). *Sarsia* **73**:61–70.
  42. Peters, R. H. 1984. Methods for the study of feeding, filtering and assimilation by zooplankton, p. 336–412. *In* J. A. Downing and F. H. Rigler (ed.), *A manual for the assessment of secondary productivity in fresh waters*. Blackwell Scientific Publications Ltd., Oxford.
  43. Pomeroy, L. R. 1974. The ocean's food web, a changing paradigm. *BioScience* **24**:499–504.
  44. Pomeroy, L. R., S. A. Macko, P. H. Ostrom, and J. Dunphy. 1990. The microbial food web in Arctic seawater: concentration of dissolved free amino acids and bacterial abundance and activity in the Arctic Ocean and in Resolute Passage. *Mar. Ecol. Prog. Ser.* **61**:31–40.
  45. Pomeroy, L. R., W. J. Wiebe, W. J. Deibel, R. J. Thompson, G. T. Rowe, and J. D. Pakulski. 1991. Bacterial responses to temperature and substrate limitation during the Newfoundland spring bloom. *Mar. Ecol. Prog. Ser.* **75**:143–157.
  46. Preisig, H. R., and D. J. Hibberd. 1982. Ultrastructure and taxonomy of *Paraphysomonas* (Chrysophyceae) and related genera 1. *Nord. J. Bot.* **2**:397–420.
  47. Rassoulzadegan, F. 1982. Dependence of grazing rate, gross growth efficiency and food size range on temperature in a pelagic oligotrichous ciliate *Lohmanniella spiralis* Leeg., fed on naturally occurring particulate matter. *Ann. Inst. Oceanogr.* **58**:177–184.
  48. Rogerson, A. 1980. Generation times and reproductive rates of *Amoeba proteus* (Leidy) as influenced by temperature and food concentration. *Can. J. Zool.* **58**:543–548.
  49. Rogerson, A. 1981. The ecological energetics of *Amoeba proteus* (Protozoa). *Hydrobiologia* **85**:117–128.
  50. Schaechter, M., O. Maaløe, and N. O. Kjeldgaard. 1958. Dependence on medium and temperature of cell size and chemical composition during balanced growth of *Salmonella typhimurium*. *J. Gen. Microbiol.* **19**:592–606.
  51. Sherr, B. F., and E. B. Sherr. 1984. Role of heterotrophic protozoa in carbon and energy flow in aquatic ecosystems, p. 412–423. *In* M. J. Klug and C. A. Reddy (ed.), *Current perspectives in microbial ecology*. American Society for Microbiology, Washington, D.C.
  52. Sherr, B. F., E. B. Sherr, and T. Berman. 1983. Grazing, growth, and ammonium excretion rates of a heterotrophic microflagellate fed with four different species of bacteria. *Appl. Environ. Microbiol.* **45**:1196–1201.
  53. Sherr, B. F., E. B. Sherr, and R. D. Fallon. 1987. Use of monodispersed, fluorescently labeled bacteria to estimate in situ protozoan bacterivory. *Appl. Environ. Microbiol.* **53**:958–965.
  54. Sherr, B. F., E. B. Sherr, and F. Rassoulzadegan. 1988. Rates of digestion of bacteria by marine phagotrophic protozoa: temperature dependence. *Appl. Environ. Microbiol.* **54**:1091–1095.
  55. Sieracki, M. E., L. W. Hass, D. A. Caron, and E. J. Lessard. 1987. Effect of fixation on particle retention by microflagellates: underestimation of grazing rates. *Mar. Ecol. Prog. Ser.* **38**:251–258.
  56. Silver, M. W., J. G. Mitchell, and D. L. Ringo. 1980. Siliceous nanoplankton. II. Newly discovered cysts and abundant choanoflagellates from the Weddell Sea, Antarctica. *Mar. Biol. (Berlin)* **58**:211–217.
  57. Smith, R. E. H., P. Clement, and G. F. Cota. 1989. Population dynamics of bacteria in the Arctic sea ice. *Microb. Ecol.* **17**:63–76.
  58. Tseytlin, B. V. 1976. Gigantism in deep-water plankton-consuming organisms. *Oceanology* **15**:499–503.
  59. Verity, P. G. 1985. Grazing, respiration, excretion, and growth rates of tintinnids. *Limnol. Oceanogr.* **30**:1268–1282.