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Interactive effect of temperature and resources on carbon cycling by freshwater bacterioplankton communities

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ABSTRACT: Planktonic heterotrophic prokaryotes play an essential role in all aquatic ecosystems due to their short generation times and access to dissolved organic carbon and nutrients. In order to understand how rising temperatures and nutrient loading affect the biogeochemistry of whole lake ecosystems, it is essential to understand the interactive effects that temperature and resources have on the metabolism of natural bacterioplankton communities. To address this question we sampled bacterial communities from 1 mesotrophic and 1 oligotrophic lake in Clearwater County, Minnesota, USA, in winter and summer. Each community was exposed to a combination of carbon, nitrogen and phosphorus additions at 4 different levels (ambient, 2×, 5×, and 10× ambient) and 4 temperatures (4, 14, 24 and 34°C). Community metabolic response to temperature depended on the resource treatment, and the season when the community was sampled. Bacterial respiration increased more with temperature than bacterial growth, resulting in decreased bacterial growth efficiencies at higher temperatures. This result was most pronounced in the high resource treatments, while at lower resource levels the results were more ambiguous. In addition, differences between seasons and lakes suggested that the bacterial communities had adapted or acclimated to *in situ* temperature such that communities sampled from cold temperature environments had higher respiration at high temperatures than those sampled from warmer environments. These results suggest that the effect of temperature on carbon cycling mediated by the bacterial community depends on both the *in situ* resource pool and the extent to which the bacterial community is acclimated to a specific temperature regime.

KEY WORDS: Carbon cycling · Bacterioplankton · Multiple stressors · Bacterial growth efficiency · BGE · Temperature and nutrient interactions

INTRODUCTION

In pelagic marine systems, bacterioplankton account for 60% of the organic surface area (Cho & Azam 1988) and have been shown to process up to 90% of locally produced photosynthate (Biddanda et al. 1994). Similarly in lakes, where burial and remineralization processes are significant contributors to global fluxes (Dean & Gorham 1998), bacterioplankton have been shown to be key processors of organic matter (Biddanda et al. 2001). However, our understanding of the effects of temperature on the metabolism of natural bacterioplankton communities remains poorly defined.

Traditionally, temperature dependence of metabolic processes has been described using Q_{10}s or Arrhenius-Boltzman plots, which allow for the calculation of the temperature characteristic of a specific biological process. However, it is clear that the relationship between temperature and metabolism is different over different ranges of temperature (Montagnes et al. 2003, Apple et al. 2006) and can often vary in a non-linear fashion (Felip et al. 1996), rendering Q_{10}s most appropriate for general approximations of the temperature depen-

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dence of metabolic processes, at best. Recently, general relationships between temperature and respiration have been defined using the Arrhenius equation for a broad range of organisms, including bacteria (Brown et al. 2004). Using an Arrhenius analysis to define temperature dependence can exclude a significant portion of the variability in the response because the metabolic index has been log transformed, and therefore such analyses may be best suited for use with observational data or for a general approximation of temperature dependence. A more specific analysis of temperature dependence is possible, and perhaps most intuitive, using direct plots of metabolic rates vs. temperature and comparing differences between rates from different treatments (e.g. resource level, community origin). In addition, in order to better understand how changing temperature will affect bacterially mediated carbon cycling, it is important to define how temperature affects anabolic metabolism as well as catabolic metabolism.

Organic carbon that enters the bacterial carbon pool has one of 2 immediate fates. It can be remineralized as CO_2 through bacterial respiration (BR) or incorporated into bacterial biomass as bacterial production (BP). These measurements are often combined to create a synthetic term, bacterial growth efficiency—BGE = BP/(BR + BP)—which describes the proportion of carbon entering the bacterial pool that is incorporated into biomass. Previous researchers have concluded that temperature is not a dominant regulator of BGE (del Giorgio & Cole 2000). However, 2 more recent observational studies, one in a lake ecosystem and one in the pelagic ocean, found significant negative relationships between BGE and temperature, although in each case there was a good deal of unexplained variance around the relationship (Rivkin & Legendre 2001, Biddanda & Cotner 2002).

The lack of a consistent, well-defined relationship between BGE and temperature is most likely in part due to the interactive effects of resources on the metabolic response of bacteria to changes in temperature (Pomeroy & Wiebe 2001). This interaction further complicates our ability to predict how carbon cycling mediated by the microbial loop will respond to temperature, especially in the face of concurrent eutrophication of aquatic ecosystems (Smith 2003).

In the present study, we experimentally addressed how temperature and resources interact to affect carbon cycling of bacterioplankton communities sampled in 2 seasons (winter and summer) and in 2 lakes (1 oligotrophic and 1 mesotrophic) by measuring each component of BGE at 4 experimentally generated temperatures (4, 14, 24 and 34°C) and 4 different quantities of labile resources (carbon [C], nitrogen [N] and phosphorus [P]). We asked: Does temperature affect BGE differently at different resource levels? What is the effect of temperature on each component of BGE, i.e. BP and BR? Does the origin of the bacterial community (i.e. winter vs. summer or oligotrophic vs. mesotrophic lake) affect how temperature and resources influence BGE? To answer these questions we designed our experiment to test the following hypotheses: (1) respiration and growth will increase with temperature but respiration will increase relatively more than growth (Rose 1967); (2) the relationship between bacterial metabolism and temperature will depend on the *in situ* temperature at which the communities were sampled (Fuhrman & Azam 1983, Simon et al. 1999); (3) BGE will decrease with increasing temperature (Rivkin & Legendre 2001, Biddanda & Cotner 2002).

MATERIALS AND METHODS

Overview of the experimental design and lakes. We sampled natural bacterial communities by filtration (<1 µm pore size) from 2 lakes (the mesotrophic Lake Itasca, and the oligotrophic Long Lake) in Clearwater County, Minnesota, USA, at 2 different times of year (summer August 2003 and winter January 2004). Long Lake is relatively smaller (surface area 0.59 km^2, mean depth ca. 8 m), and oligotrophic (chl *a* ca. 1.2 µg l^{-1}). Lake Itasca, the headwaters of the Mississippi, is a larger (surface area = 4.4 km^2), but on average a much shallower (mean depth ca. 2 m), meso-eutrophic (chl *a* ca. 10.6 µg l^{-1}) system. We conducted our experiments on lakes of distinct trophic status to include communities acclimated to high and low *in situ* nutrient conditions and sampled each lake in summer and winter to include communities acclimated to warm and cold temperatures. Bacterial communities were grown in batch culture at 4 different nutrient treatments (ambient, 2×, 5×, and 10× ambient, referred to as R0, R2, R5 and R10, respectively) and 4 different temperatures (4, 14, 24 and 34°C). Ambient values of DOC in Lake Itasca were measured at the time of the August experiment (385 µmol l^{-1}) and found to be similar at the time of the January experiment (423 µmol l^{-1}) (Hall 2006). We assumed 20% of measured ambient DOC to be labile (Søndergaard & Middelboe 1995) and added N and P in Redfield proportion (Redfield 1958). For the August experiments, carbon (C, equal parts acetate and glucose), nitrogen (N, NH_4Cl) and phosphorus (P, KH_2PO_4) were added at the beginning of each experiment to create the R2 treatment for a final amendment concentration of 80, 12, and 0.75 µmol l^{-1} respectively, and increased proportionally for the R5 and R10 treatments. In January, because our previous work indicated that bacteria have higher P requirements at colder temperatures (Cotner et al. 2006),

P amendments to each treatment were increased 4-fold, while C and N amendments remained the same to avoid P-limitation in the cultures. No resources were added to the ambient treatment in either season.

In order to ensure that each community had sufficient time to respond to resource additions at each temperature, a separate preliminary experiment was carried out: the week prior to each experiment, we placed bacterial communities from each lake into 20 l carboys at 2 resource levels (R0 and R5) for each experimental temperature. We measured BP every 24 h for ~8 d, while at the highest 2 temperatures (24 and 34°C) we measured BP in 12 h increments for the first 24 h, then each 24 h increment subsequently. We then looked for the first peak in BP at each experimental temperature and considered the occurrence of the first peak to be the maximum response of each community for that treatment (see Fig. 1). The time until the maximum response was then used in the subsequent experiments as the pre-determined stop time for each experimental temperature (Table 1). These preliminary experiments were conducted for each lake during each season. At the pre-determined stop time, each microcosm was sampled for BP, and bacterial abundance (BA). BR was measured in parallel experiments for each unique treatment (i.e. resource level × temperature) in a series of 9 BOD bottles (per treatment).

Lake sampling and experimental set-up. Water was sampled from ca. 1 m below the surface during the summer and directly below the ice in the winter with an 8 or 10 l Van Dorn bottle and gently poured into 20 l carboys. The carboys were then placed into a large cooler and kept in the dark until they were returned to the laboratory. All samples were returned to the laboratory within 1.5 h of collection. Before filtration, carboys were allocated to temperature control cabinets at the desired experimental temperature. Bacterioplankton communities were separated by filtration through a 147 mm diameter, 1 µm pore size polycarbonate filter using a peristaltic pump. Directly after filtration, water was placed in acid clean 1 l polycarbonate bottles. Filtration of all water at the beginning of each experiment took between 6 and 10 h. Once all water had equilibrated at the designated temperature, nutrients were added to the microcosms. The addition of resources was considered time zero for each experiment. Microcosms were then sampled at the pre-determined stop time for BP and BA.

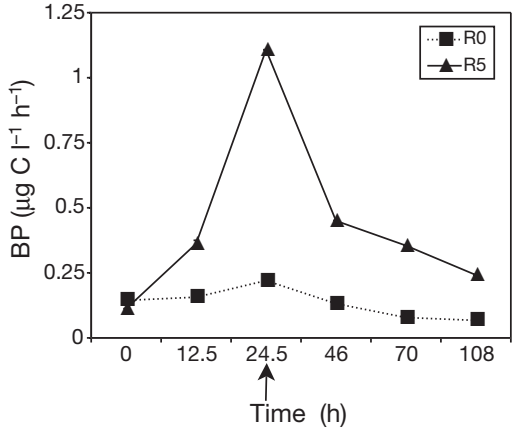

Fig. 1. Pre-experiment time series analysis conducted to determine the stop time for each experimental temperature. This example shows bacterial production (BP) vs. time for the bacterial community sampled from Long Lake in August at 24°C. Arrow indicates the stop time (t = 24 h) at which the 24°C treatment for Long Lake would be evaluated for the August community. This analysis was performed in each lake and each season at each of the 4 experimental temperatures at 2 (R0 and R5) resource levels

Table 1. Amount of time (h) required to elicit the maximum response at each experimental temperature. Experiments were conducted in each lake during each season

	4°C	14°C	24°C	34°C
August				
Long Lake	100	50	24	24
Itasca Lake	24	24	15	12
January				
Long Lake	155	64	44	68
Itasca Lake	150	72	24	12

Bacterial production and abundance. To measure BP, four 10 ml aliquots (1 control, killed with 50% TCA, and 3 samples) were taken from each bottle and incubated with a mixture of cold leucine (Aldrich no. L602500) and ^{3}H-labeled leucine (Amersham Bioscience no. RK170, 1.9 mmol MBq^{-1}) to a final concentration of 30 nmol leucine l^{-1} for August experiments and 45 nanomol leucine l^{-1} for January experiments, for 2 h. For each season, these levels of leucine were determined to be saturating by separate experiments (data not shown). Each incubation was stopped by adding 1 ml of 50% trichloroacetic acid (TCA) to the remaining 3 aliquots. Samples were filtered onto 0.22 µm nitrocellulose filters (Millipore) then rinsed twice with 1 ml of cold TCA. Filters were placed in 7 ml scintillation vials and suspended in ca. 4 ml of scintillation cocktail and counted on a scintillation counter (Coulter-Beckman). BA samples were preserved in 2% (final concentration) formalin and kept refrigerated until slides could be made using acridine orange within 48 h following the methods of Hobbie et al. (1977). All measurements of BP were converted from nmol leucine l^{-1} h^{-1} to µg C l^{-1} h^{-1} using a constant conversion factor of 3.1 (Kirchman 1993). Although it has been suggested that the relationship between leucine

incorporation and carbon incorporation might change with temperature (Tibbles 1996), at least one study notes that thymidine incorporation rates that were converted to BP using empirically determined conversion factors for each temperature fit well with leucine incorporation rates where a constant conversion factor was used (Kirschner et al. 2004); this suggests that leucine conversion factors might not be significantly affected by temperature. Here we assumed that a single conversion factor was appropriate at each experimental temperature. We recognize, however, that the relationship between leucine incorporation and carbon assimilation might indeed change with temperature and should be investigated further.

Bacterial respiration. For each treatment, nine 300 ml BOD bottles were filled from a 10 l carboy of pre-filtered (<1 µm) lake water. For the treatments that received nutrient additions, resources were mixed in the carboy before filling the BOD bottles. Sets of 3 BOD bottles were fixed for each of 3 time-points using the Winkler method (Wetzel & Likens 2000), and subsequently titrated using an autotitrator (Mettler DL21). The length of the respiration experiment (e.g. time between each time point) varied for each temperature to ensure a significant decrease in oxygen over time and encompass the stop time for the parallel microcosm experiments. Because the consumption of oxygen over time was often non-linear in the nutrient-amended treatments, polynomial regression curves were fit to the data and the first derivative of each equation was evaluated at the microcosm stop time to approximate an instantaneous respiration rate at the stop times of the parallel microcosm experiments. The value from this calculation was not significantly different from the slope of oxygen consumption for the linear portion of the curve, and therefore the linear portion of the oxygen consumption curve was used to calculate BR. Respiration rates were measured in µmol oxygen l^{-1} h^{-1} and converted to µmol C l^{-1} h^{-1} assuming a respiratory quotient of 1. BGE was calculated as:

$$BGE = \frac{BP}{BP + BR}$$

Specific growth rate and specific bacterial respiration. Differences in BP and BR are the product of differences in the biomass pool size and cellular rate processes. Because incubations were run for different lengths of time, we corrected for the possibility of accumulated differences in biomass pool size by calculating specific growth rate (SGR, h^{-1}), and specific bacterial respiration (SBR, h^{-1}). SGR was calculated by dividing the instantaneous rate of leucine uptake in units µg C l^{-1} h^{-1} (BP) by the carbon content of the biomass pool (µg C l^{-1}) measured simultaneously. The size of the biomass carbon pool was estimated from bacterial abundance counts converted to units of carbon using a per cell carbon content of 100 fg $cell^{-1}$ (Vadstein & Olsen 1989). This number is representative of nutrient-enriched freshwater communities and is similar to per cell carbon content values from other enriched natural communities as well (Fagerbakke et al. 1996 and references therein). Similarly, SBR was calculated by assuming exponential growth during the period of respiration incubations and evaluating the slope of ln(BA) at time zero and $\ln(BA)_{final}$ and then calculating the BA value for the mean time of the respiration experiment. BR was then divided by this value to give a biomass normalized respiration rate (SBR, h^{-1}). All statistical analyses were performed using JMP® (SAS Institute).

RESULTS

The bacterial community metabolic response to temperature was highly influenced by the resource treatment, the season the community was sampled in and the lake the community was sampled from. Using BP as the response, a full factorial ANOVA, including temperature, season, lake and resource level as factors, indicated that the 4-way interaction term was significant ($p < 0.0001$). Therefore, we evaluated each of our hypotheses by testing the effect of temperature on each response with the data separated by resource level, season and lake, unless otherwise noted.

Respiration

During both seasons, BR generally increased with temperature for both Long Lake and Lake Itasca communities, consistent with our first hypothesis that BR should increase with increasing temperatures (Fig. 2). In addition, the magnitude of the increase in BR with temperature was greater for the higher nutrient treatments (Fig. 2), demonstrating the interactive effect of temperature and resources on bacterial metabolism. In Lake Itasca, SBR increased with temperature to 24°C and then either increased, remained the same or decreased depending on the season and resource level, while in Long Lake, SBR generally increased from 4 to 34°C in both seasons (Fig. 3).

Our second hypothesis stated that the relationship between respiration and temperature should be different for January than for August communities. The maximum BR observed in January in each lake (Long Lake = 407 µg C l^{-1} h^{-1} and Lake Itasca = 390 µg C l^{-1} h^{-1}) was nearly twice the maximum BR observed for August communities from each lake (120 and 223 µg C

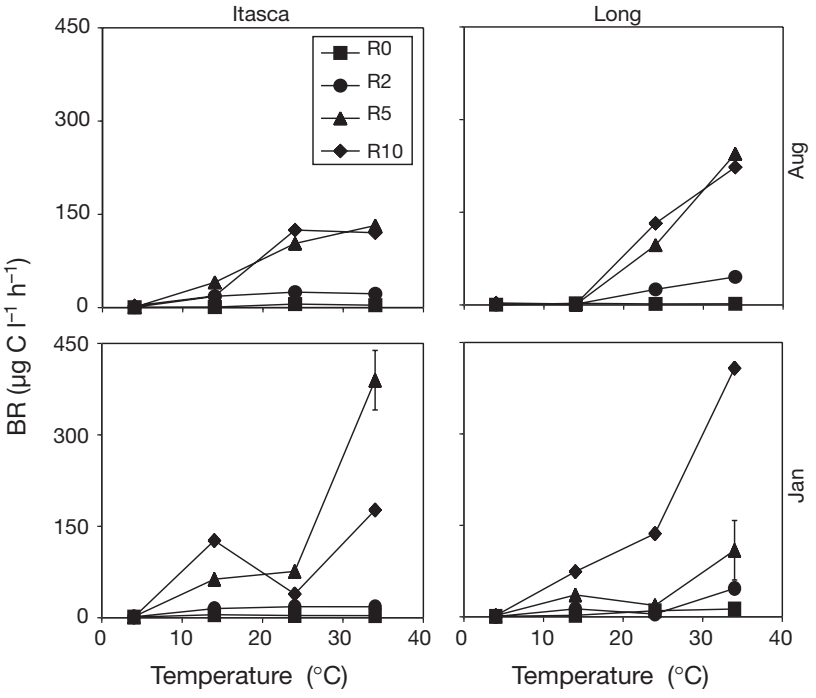

Fig. 2. Relationship between bacterial respiration (BR) and temperature separated by season and lake. Error bars indicate ±1 SD; error bars are smaller than the associated symbol when not visible

l^{-1} h^{-1}, respectively), each of which occurred at the highest temperature, suggesting that respiration may be higher in communities from cold environments exposed to warmer temperatures than for communities sampled from warm environments. However, because the length of the respiration experiments varied between seasons, we first normalized for differences in biomass between experiments and used SBR as the response. With SBR as the response, a full factorial ANOVA with lake, season, resource level and temperature as predictors indicated no significant resource level by lake interaction. Therefore, in order to test our second hypothesis, we separated our data by season only and compared the effect of temperature on SBR between August and January communities at each resource level. SBR increased significantly more with temperature in January communities relative to August communities at each resource level, with the exception of the ambient level where the difference in slopes was only borderline significant (Table 2).

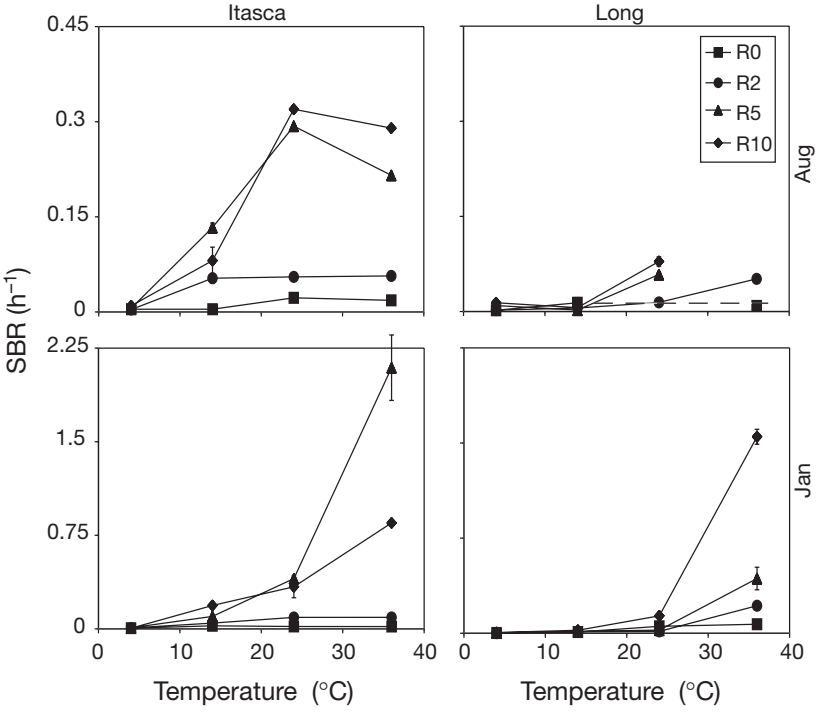

Fig. 3. Relationship between specific bacterial respiration (SBR) and temperature separated by season and lake. Error bars indicate ±1 SD. Error bars are smaller than the symbol when not visible. Dashed line for Long Lake in August indicates missing data point at 24°C for R0 treatment. Note that the scale for the January communities is greater than that for the August communities

Growth

The effect of temperature and resources on BP and SGR was influenced by the season and lakes in which the communities were sampled. With the exception of Long Lake in January, BP increased with temperature up to 24°C, consistent with our first hypothesis that BP should increase with increasing temperature (Fig. 4). Beyond 24°C, BP increased, remained the same, or decreased, depending on the resource level. Initial addition of resources (differences between R0 and R2) significantly increased BP at all temperatures in both lakes and both seasons (Fig. 4), and subsequent additions (differences between R2 and R10) only led to increased BP in certain treatments (e.g. 34°C Fig. 4).

In August, BP of the Lake Itasca community increased with temperature for all nutrient treatments with the exception of R0, which decreased at the highest temperature. Similarly, BP in Long

Table 2. Differences in the slope of specific bacterial rate (SBR) vs. temperature between communities sampled in August and January. Estimates of slope using a least squared regression fit and the probability that that the slopes are significantly different from one another as calculated with a *z*-test are listed. R0, R2, R5, R10: nutrient additions at ambient, 2×, 5× and 10× ambient, respectively

Treatment	January	August	p-value
R0	0.001	0.000	0.055
R2	0.005	0.001	0.024
R5	0.038	0.007	0.037
R10	0.036	0.009	0.005

Lake in August increased with temperature at each resource level with the exception of the highest temperature, where only R10 continued to increase with temperature and the 3 lower nutrient treatments decreased.

In January, BP of the Lake Itasca community again increased consistently with temperature at all resource levels except R0 (where it again decreased at the highest temperature) and R10 (where it decreased from 14 to 24°C and then increased again beyond 24°C). However, in Long Lake, with the exception of the unammended treatment, there was either no significant change in BP with temperature or BP decreased with increasing temperature (Fig. 4), which is the opposite of what we had predicted.

During August, SGR of the Lake Itasca community achieved maximum growth between 24 and 34°C, while the Long Lake community had a maximum SGR at 14°C (Fig. 5). In August, near the *in situ* temperature (23°C in Long Lake and 25°C in Itasca Lake) SGR increased with increasing nutrient additions; however, at the low end of the experimental temperature range, nutrient additions did not consistently increase SGR even though sufficient incubation time had occurred to elicit a response (Fig. 5). In addition, in Long Lake, the deeper and colder of the 2 lakes, nutrient additions (R2) did not increase SGR at the lowest or the highest temperature.

In January, for Lake Itasca communities, nutrient additions near the *in situ* temperature (2 to 4°C) did not result in increasing SGR (Fig. 5). SGR did increase with nutrient amendments at 24 and 34°C. At the warmest temperature (34°C), however, SGR decreased for the highest resource level. Finally, in Long Lake SGR was highest at the lowest temperature and decreased with increasing temperature at the highest 2 resource levels. At the lower resource levels, however, SGR increased until 24°C and then decreased at 34°C.

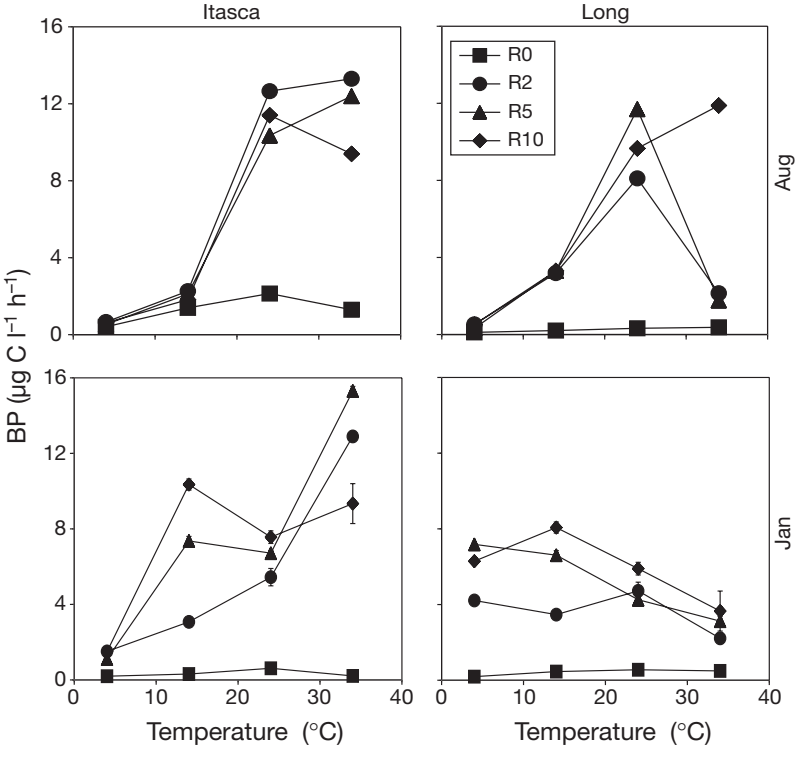

Fig. 4. Relationship between bacterial production (BP) and temperature separated by season and lake. Error bars indicate ±1 SD. Error bars are smaller than the associated symbol when not visible

Relative increase of respiration and growth with temperature

Our first hypothesis stated that SBR and SGR should increase with increasing temperature. This was true for SBR for the entire range of experimental temperatures, and for SGR (with the exception of Long Lake in January) for the experimental temperatures that fell within the range of seasonal lake temperatures (4 to 24°C). Our first hypothesis also stated that SBR should increase more with temperature than SGR. Because SGR often did not increase uniformly over the entire temperature range, i.e. it decreased beyond 24°C, we tested this hypothesis by comparing the slope of increase in SBR with temperature relative to that of SGR using only the experimental temperatures that fell within the natural range of seasonal lake temperatures (4 to 24°C).

For all treatments, lakes and seasons SBR increased more with temperature than did SGR. In January, the increase in SBR with temperature was significantly greater than that of SGR with temperature for all treatments with the exception

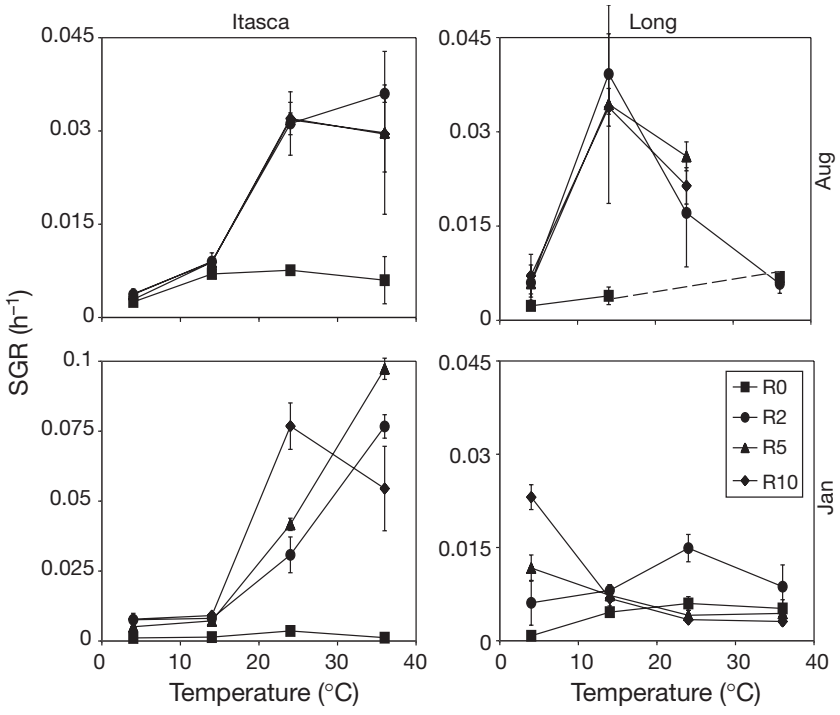

Fig. 5. Relationship between specific growth rate (SGR) and temperature separated by season and lake. Note that the scale on the *y*-axis is different for Lake Itasca in January

of the ambient levels in each lake, i.e. for 6 of 8 treatments (Table 3). In August, however, the difference between the slope of SBR and SGR with temperature was only significant for the highest 2 resource levels in Lake Itasca, i.e. only 2 of the 8 treatments (Table 3).

Table 3. Estimates of slopes of specific bacterial rate (SBR) and specific growth rate (SGR) vs. temperature using a least squared regression fit and the probability that the slopes are significantly different from one another as calculated with a *z*-test. p-values of slopes that are significantly different are in **bold**. NS: insufficient points to statistically compare slopes. Treat: treatment

	Treat	SBR	SGR	p-value
August	R0	0.0009	0.0003	0.0985
Itasca	R2	0.0026	0.0014	0.1894
Lake	R5	0.0144	0.0014	**<0.001**
	R10	0.0155	0.0015	**0.0019**
Long	R0	0.0012	0.0002	NS
Lake	R2	0.0006	0.0005	0.3783
	R5	0.0025	0.0010	0.2148
	R10	0.0033	0.0007	0.1379
January	R0	0.0007	0.0001	0.2514
Itasca	R2	0.0042	0.0012	**0.0010**
Lake	R5	0.0198	0.0018	**0.0016**
	R10	0.0166	0.0035	**<0.001**
Long	R0	0.0026	0.0014	0.1210
Lake	R2	0.0070	0.0004	**<0.001**
	R5	0.0010	−0.0004	**<0.001**
	R10	0.0066	−0.0010	**0.0032**

Bacterial growth efficiency

In general our data supported our third hypothesis that BGE would decrease with increasing temperature but the relationship between temperature and BGE depended on the season, lake and resource treatment (Fig. 6). In August, at the highest 2 resource levels (R5 and R10), Lake Itasca community BGE decreased from 4 to 14°C and then slightly increased or remained the same beyond 14°C. At the second resource level (R2), BGE decreased steeply initially from 4 to 14°C and then increased beyond 14°C. BGE in the ambient treatment, however, initially increased from 4 to 14°C and then decreased beyond 14°C. BGE of the Long Lake community increased from 4 to 14°C and then decreased or remained the same beyond 14°C, with the exception of R0. In January, for both communities, BGE was the highest at the lowest temperature and then decreased with increasing temperature, with the exception of the R2 resource level, which decreased from 4 to 14°C in each lake but increased beyond 14°C.

In summary, our results supported each of our hypotheses, although in some cases our hypotheses were not supported for specific treatments (resource level, season and lake). While BR and SBR increased with temperature in both seasons, consistent with our first hypothesis, BP and SGR only consistently increased in the communities sampled in August from 4 to 24°C. SBR of the January communities increased more with temperature than did SBR of the August communities. Respiration did increase more with temperature than growth across all treatments, leading to a general decrease in BGE, as we hypothesized. However, this trend was most pronounced for the January communities, and in Lake Itasca in August at the highest 2 resource treatments (R5 and R10; Table 3 and Fig. 6, respectively).

DISCUSSION

The results of the present study show complex responses of bacterial communities to simultaneous temperature and resource perturbations, most likely due to both physiological processes and community structure. Here, we discuss (1) possible reasons for the differences we observed in respiration and growth with respect to temperature, (2) the observed dif-

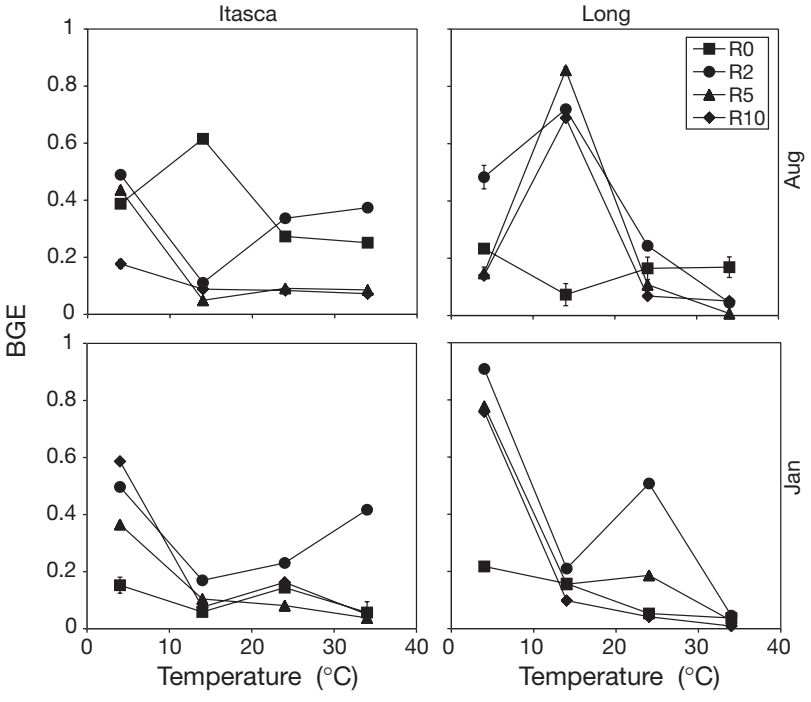

Fig. 6. Relationship between bacterial growth efficiency (BGE) and temperature separated by season and lake. Error bars indicate ±1 SD. Error bars are smaller than the associated symbol when not visible

ferences between lake communities and seasonal communities, and (3) possible biological mechanisms for these results.

These experiments were not designed to test whether the distinct temperature responses we saw were a result of changes in community composition or due to phenotypic plasticity of the extant community. Nevertheless, it is well known that bacterial community composition changes seasonally (Yannarell et al. 2003, Crump & Hobbie 2005) and it is possible that the community composition changed even during the course of the experiments. However, given the difference in response to temperature of the communities sampled between seasons and the time each community was given to acclimate to the experimental temperature (see Table 1), our data suggest that the bacterioplankton were seasonally adapted to *in situ* temperature, consistent with observations from other temperate lakes (Simon & Wunsch 1998). Therefore, because the between season differences in metabolic response were greater than any differences that occurred to the community response during the acclimation period, it suggests that the most significant differences in community composition, with respect to thermal adaptation, occurred seasonally and not on shorter time scales (i.e. days).

Although previously the effect of temperature on BGE had been reported as ambiguous (del Giorgio & Cole 2000 and references therein), more recent studies have shown it to be a decreasing function of temperature (Biddanda & Cotner 2002, Rivkin & Legendre 2001). We found that BGE consistently decreased with temperature at the highest 2 resource levels, but results for the lower 2 resource levels were less clear. More specifically, BGE decreased with increasing temperature due to a disproportionate increase in respiration relative to growth with increasing temperatures (Table 3). BP and BR are often considered to be coupled; however, it has been suggested that they might be independently regulated and can become uncoupled under specific situations (Smith & Prairie 2004). It has been well documented that bacterial growth is often phosphorus limited in a broad range of aquatic systems (Morris & Lewis 1992, Zohary & Robarts 1998, Cotner 2000, Sala et al. 2002, Granéli et al. 2004). Less work has been done to examine what limits BR in natural systems, although both C limitation (e.g. Smith & Prairie 2004) and at least one case of P limitation have been reported (Obernosterer et al. 2003). Specifically relevant to this work, recent studies in Minnesota lakes of the same region studied here also show C limitation of BR (Stets 2007)

Although it has been shown that BR can occur in the absence of anabolic metabolism (Russell & Cook 1995, Dauner et al. 2001), anabolic metabolism cannot occur in the absence of catabolic metabolism. Our results suggest that slight alleviation of resource limitation, either through increases in temperature from 4 to 14°C leading to increased affinity for substrate (Nedwell 1999) or nutrient additions at the lowest level (R2) resulted in increased growth with minimal associated increases in respiration and therefore increased BGE. At higher resource levels, however, an abundance of carbon subsidies led to an uncoupling of growth and respiration, with greater increases in respiration, relative to growth, with temperature and a pattern of decreasing BGE with increasing temperature.

Furthermore, using the grand mean of the entire dataset, it is clear that BR was affected differently by the interaction of temperature and resources than BP (Fig. 7). BR increased linearly with resource and increasing temperature increased the slope of that relationship (Fig. 7), while BP increased in a saturating manner, consistent with resource uptake experiments, and increasing temperature increased

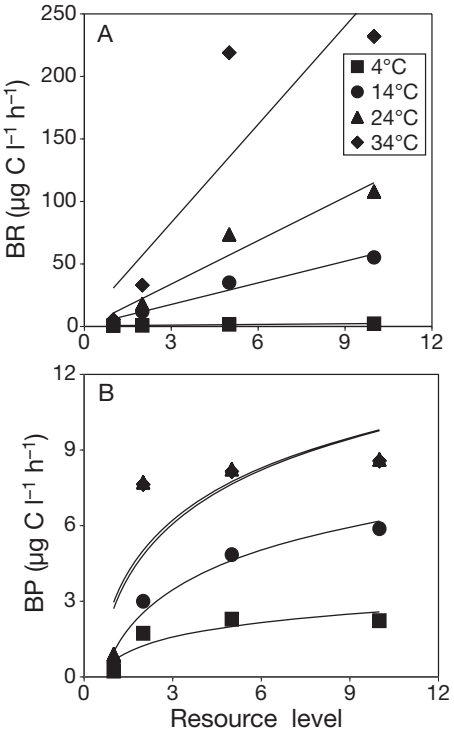

Fig. 7. Mean response from both lakes during both seasons of (A) bacterial respiration (BR) and (B) bacterial production (BP) to resources at each of the 4 incubation temperatures. Regression equations for best fit lines are: (A) 4°C, BR = 0.2R + 0.5, R^2 = 0.97; 14°C, BR = 5.8R + 0.3, R^2 = 0.97; 24°C, BR = 11.6R – 0.8, R^2 = 0.95; 34°C, BR = 26.2R + 4.5, R^2 = 0.78; (B) 4°C, BP = 0.8ln(R) + 0.7, R^2 = 0.77; 14°C, BP = 2.3ln(R) + 1.0, R^2 = 0.97; 24°C, BP = 3.0ln(R) + 3.0, R^2 = 0.67; 34°C, BP = 3.1ln(R) + 2.7, R^2 = 0.67; where R is the level of resource added

the maximum production rate (Fig. 7). We can generalize from this result that at the resource supply ratio used in this study (i.e. Redfield ratio, summer, and below Redfield ratio, winter) increasing temperature resulted in decreasing BGE due to differences in the characteristic temperature response between respiration and growth of natural bacterioplankton communities. In addition, the differences between BP and BR were most pronounced at high resource quantity. This suggests that the quantity of the labile resources in a given aquatic ecosystem is an important determinant of how BGE will change with temperature.

Mechanisms of adaptation to *in situ* temperature

There were several striking differences between the Long Lake January community and the August community. Our data show that the Long Lake community sampled in January was able to use available substrates to achieve high levels of growth at low temperatures (4 and 14°C), whereas the August community was not (Fig. 5). At the highest temperature, August communities for both lakes had lower maximum respiration than January communities (Fig. 2) and SBR increased with temperature significantly more in January communities relative to August communities (Table 2).

These differences in response to experimental temperature forcing by communities sampled from cold and warm environments are consistent with the biology of prokaryotic cellular membranes. In prokaryotic organisms, the effect of temperature on membrane structure is particularly significant because respiration and uptake are mediated by biochemical machinery integrated within the same membrane. In contrast, in eukaryotes, uptake and respiration are physically separated, with uptake occurring across the cellular membrane and respiration across the mitochondrial membrane. Therefore, prokaryotic organisms must maintain a membrane phase-state that allows active transport of substrate at low temperatures as the membrane tends toward a crystalline state, while at higher temperatures, as the membrane becomes increasingly fluid, it is necessary to maintain the membrane sufficiently rigid to prevent H^+ from returning through non-specific pathways and decreasing proton motive force. In order to maintain this specific level of viscosity across the broad temperature range experienced in temperate lake ecosystems, organisms must alter membrane composition. Although studies conducted on nutrient-replete media show that individual strains can adjust the fatty acid composition of their membranes to changes in temperature (Russell 1990, van de Vossenberg et al. 1999), alteration of membrane lipids requires de novo synthesis of RNA and proteins (Mansilla et al. 2004) and under nutrient-limited conditions lipid synthesis is often halted (White 2000). Therefore in natural systems, where nutrient limitation is common, alteration of membrane lipid composition in response to changing temperature may not be a viable option. Without the ability to change membrane composition, low temperatures would result in reduced affinity for substrates (Nedwell 1999) and at high temperatures organisms would be required to increase respiration to maintain proton motive force, which has been demonstrated in the laboratory but not yet observed in natural communities (De Vrij et al. 1988). The mechanism outlined above is consistent with our findings that the Long Lake community sampled in January was more able than communities sampled in August to use substrate to obtain growth at lower temperatures, but displayed a higher respiratory flux at higher temperatures, and is one possible mechanism for how bacterioplankton adjust to *in situ* temperature.

Implications

Because of their geographic ubiquity and highly versatile metabolism, understanding the metabolic changes that natural bacterioplankton communities undergo across a range of temperatures is key to understanding how biogeochemical cycles will be altered by increased thermal and resource inputs to aquatic ecosystems. Interpreting experimental studies such as ours in the context of climate forcing is difficult and often inappropriate. Clearly the 'global bacterioplankton community' contains sufficient metabolic diversity to grow at a wide range of temperatures (Brock 1967) and has evolved a variety a mechanisms to deal with life at higher temperatures (e.g. Na^+ pumps in thermophiles; Albers et al. 2000). As temperatures increase, ecological theory suggests that immigration by genotypically and phenotypically distinct populations will replace extant populations that cannot successfully compete for limiting resources under the new temperature regimes. This is a reasonable hypothesis if bacteria are not dispersal limited, as has been suggested (Reche et al. 2005). However, temperate lakes provide a unique environment, with respect to temperature, because of the wide range of temperatures that occur over a relatively short vertical distance, as well as seasonally. Mixing of vertical layers on short time scales coupled with consistent seasonal changes could prevent establishment of populations with highly specific temperature optima and select for communities with a wide phenotypic plasticity with respect to temperature, i.e. generalists (Simon et al. 1999). In the present study, we saw that the bacterial community from the deeper and colder of the 2 lakes (Long Lake) demonstrated physiological responses to experimental temperature forcing that were consistent with cold adaptation. This supports the idea that thermal adaptation of the bacterioplankton is more likely to be found in ecosystems with a more stable thermal regime (Simon et al. 1999).

CONCLUSIONS

Our results indicate that the metabolic response of bacterial communities to experimental temperature and nutrient forcing reflected the environment (i.e. lake and season) from which each community was sampled. BR increased more than growth with increasing temperature, causing BGE to decrease with temperature; however, this pattern was only present in the high resource treatments when excess labile carbon was present. BGE only increased in response to increasing temperature in the low nutrient treatments, consistent with the idea that increased temperature increases bacterial affinity for limiting substrates and can partially alleviate nutrient limitation of growth (Nedwell 1999). Therefore, our results suggest that the quantity and quality of the dissolved resource pool will strongly affect how BGE changes with changing thermal inputs.

In addition, we found that January community respiration increased more in response to temperature than did respiration of August communities, suggesting that one cost of acclimation to colder environments is higher respiratory costs at higher temperatures. Presumably, community responses similar to those observed here are more likely to be indicative of lakes where seasonal and spatial variability in temperature are common (i.e. temperate dimictic lakes). In these systems, organisms are exposed to a wide range of temperatures annually and on shorter time scales due to mixing events. In order to develop a more robust understanding of how temperature affects carbon cycling by aquatic bacterial communities, more studies should be conducted in lake ecosystems of high and low latitudes and should focus on how a stoichiometrically variable resource base differentially affects anabolic and catabolic bacterial processes.

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LITERATURE CITED

Albers SV, van de Vossenberg JLCM, Driessen AJM, Konings WN (2000) Adaptations of the archaeal cell membrane to heat stress. Frontiers Biosci 5:813–820

Apple JK, del Giorgio PA, Kemp WM (2006) Temperature regulation of bacterial production, respiration, and growth efficiency in a temperate salt-marsh estuary. Aquat Microb Ecol 43:243–254

Biddanda BA, Cotner JB (2002) Love handles in aquatic ecosystems: role of dissolved organic carbon drawdown, resuspended sediments and terrigeonous inputs in the carbon balance of Lake Michigan. Ecosystems 5:431–455

Biddanda BA, Opsahl S, Benner R (1994) Plankton respiration and carbon flux through bacterioplantkon on the Lousiana Shelf. Limnol Oceanogr 39:1259–1275

Biddanda BA, Ogdahl ML, Cotner JB (2001) Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. Limnol Oceanogr 46:58–67

Brock TD (1967) Life at high temperatures. Science 158: 1012–1019

Brown JH, Gillooly JF, Savage VM, B WG (2004) Toward a metabolic theory of ecology. Ecology 85:1771–1789

Cho BC, Azam F (1988) Major role of bacteria in biogeochemical fluxes in the ocean's interior. Nature 332:441–443
Cotner JB (2000) Heterotrophic bacterial growth and nutrient limitation in large oligotrophic lakes and oceans. Verh Int Ver Limnol 27:1–5
Cotner JB, Makino W, Biddanda BA (2006) Temperature affects stoichiometry and biochemical composition of Escherichia coli. Microb Ecol 52:26–33
Crump BC, Hobbie JE (2005) Synchrony and seasonality in bacterioplankton communities of two temperate rivers. Limnol Oceanogr 50:1718–1729
Dauner M, Storni T, Sauer U (2001) *Bacillus subtilis* metabolism and energetics in carbon-limited and excess-carbon chemostat culture. J Bacteriol 183:7308–7317
Dean WE, Gorham E (1998) Magnitude and significance of carbon burial in lakes, reservoirs, and peatlands. Geology 26:535–538
del Giorgio PA, Cole JJ (2000) Bacterial energetics and growth efficiency. In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley-Liss, New York, p 289–325
De Vrij W, Bulthuis RA, Konings WN (1988) Comparative study of energy-transducing properties of cytoplasmic membranes from mesophilic and thermophilic *Bacillus* species. J Bacteriol 170:2359–2366
Fagerbakke KM, Heldal M, Norland S (1996) Content of carbon, nitrogen, oxygen, sulfur and phophorus in native aquatic anc cultured bacteria. Aquat Microb Ecol 10:15–27
Felip M, Pace ML, Cole JJ (1996) Regulation of planktonic bacterial growth rates: the effects of temperature and resources. Microb Ecology 31:15–28
Fuhrman JA, Azam F (1983) Adaptions of bacteria to marine subsurface waters studied by temperature response. Mar Ecol Prog Ser 13:95–98
Granéli W, Bertilsson S, Philbert A (2004) Phosphorus limitation of bacterial growth in high Arctic lakes and ponds. Aquat Sci 66:430–439
Hall EK (2006) Interacting effects of temperature and nutrients on the metabolism and biomass composition of natural cacterioplankton communities. PhD thesis, University of Minnesota, Saint Paul, MN
Hobbie JE, Daley RJ, Jasper S (1977) Use of nucleopore filters for counting bacteria by fluorescence microscopy. Appl Environ Microbiol 33:1225–1228
Kirchman DL (1993) Leucine incorporation as a measure of biomass production by heteroptrphic bacteria. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, FL, p 509–512
Kirschner AKT, Wihlidal P, Velimirov B (2004) Variability and predictability of the empirical conversion factor for converting ^{3}H-thymidine uptake into bacterial carbon production for a eutrophic lake. J Plankton Res 26:1559–1566
Mansilla MC, Cybulski LE, Albanesi D, Mendoza D (2004) Control of membrane lipid fluidity by molecular thermosensensors. J Bacteriol 186:6681–6688
Montagnes DJS, Kimmance SA, Atkinson D (2003) Using Q_{10}: Can growth rates increase linearly with temperature? Aquat Microb Ecol 32:307–313
Morris DP, Lewis WJ Jr (1992) Nutrient limitation of bacterioplankton growth in Lake Dillon, Colorado. Limnol Oceanogr 37:1179–1192
Nedwell DB (1999) Effect of low temperature on microbial gowth: lowered affinity for sustrates limits growth at low temperature. FEMS Microbiol Ecol 30:101–111
Obernosterer I, Kawasaki N, Benner R (2003) P-limitation of respiration in the Sargasso Sea and uncoupling of bacteria from P-regeneration in size-fractionation experiments. Aquat Microb Ecol 32:229–237
Pomeroy LR, Wiebe WJ (2001) Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. Aquat Microb Ecol 23:187–204
Reche I, Pulido-Villena E, Morales-Baquero R, Casamayor EO (2005) Does ecosystem size determine aquatic bacterial richness? Ecology 86:1715–1722
Redfield AC (1958) The biological control of chemical factors in the environment. Am Sci 46:205–221
Rivkin RB, Legendre L (2001) Biogenic carbon cycling in the upper ocean: effects of microbial respiration. Science 291: 2398–2400
Rose AH (1967) Thermobiology. Academic Press, London
Russell JB, Cook GM (1995) Energetics of bacterial growth: balance of anabolic and catabolic reactions. Microbiol Rev 59:48–62
Russell NJ (1990) Cold adaptation of microorganisms. Phil Trans R Soc Lond B 326:595–611
Sala MM, Peters F, Gasol JM, Pedrós-Alió C, Marrasé C, Vaqué D (2002) Seasonal and spatial variations in the nutrient limitation of bacterioplankton growth in the northwestern Mediterranean. Aquat Microb Ecol 27: 47–56
Simon M, Wunsch C (1998) Temperature control of bacterioplankton growth in a temperate large lake. Aquat Microb Ecol 16:119–130
Simon M, Glockner FO, Amann R (1999) Different community structure and temperature optima of heterotrophic picoplantkon in various regions of the Southern Ocean. Aquat Microb Ecol 18:275–284
Smith EM, Prairie YT (2004) Bacterial metabolism and growth efficiency in lakes: the importance of phosphorus availability. Limnol Oceanogr 49:137–147
Smith VH (2003) Eutrophication of freshwater and coastal marine ecosystems—a global problem. Environ Sci Pollut Res 10:126–139
Søndergaard M, Middelboe M (1995) A cross-system analysis of labile dissolved organic carbon. Mar Ecol Prog Ser 118: 283–294
Stets EG (2007) Autotrophic–heterotrophic coupling in lake ecosystems: the importance of organic carbon and food-web interactions. PhD thesis, University of Minnesota, Saint Paul, MN
Tibbles BJ (1996) Effects of temperature on the incorporation of leucine and thymidine by bacterioplankton and bacterial isolates. Aquat Microb Ecol 11:239–250
Vadstein O, Olsen Y (1989) Chemical composition and phosphate uptake kinetics of limnetic bacterial communities cultured in chemostats under phosphorus limitation. Limnol Oceanogr 34:939–946
van de Vossenberg JLCM, Driessen AJM, da Costa MS, Konings WN (1999) Homeostasis of the membrane proton permeability in *Bacillus subtilis* grown at different temperatures. Biochim Biophys Acta 1419:97–104
Wetzel RG, Likens GE (2000) Limnological analyses. Springer Verlag, New York
White D (2000) The physiology and biochemistry of prokaryotes. Oxford University Press, New York
Yannarell AC, Kent AD, Lauster GH, Kratz TK, Triplett EW (2003) Temporal patterns in bacterial communities in three temperate lakes of different trophic status. Microb Ecol 46:391–405
Zohary T, Robarts RD (1998) Experimental study of microbial P limitation in the eastern Mediterranean. Limnol Oceanogr 43:387–395

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