Temperature control of bacterioplankton growth in a temperate large lake

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ABSTRACT: In order to examine the significance of temperature in controlling growth of bacterioplankton in a large lake with a pronounced pelagic zone we performed 12 temperature shift-up experiments year-round in mesotrophic Lake Constance, Germany, with samples from 1, 6, 10 or 20 and 50 m depth. We measured incorporation of ^{14}C -leucine (Leu) and ^3H -thymidine (TdR) at 5, 10, 15, 20 and 25°C including the *in situ* temperature $\pm 2^\circ\text{C}$. The results show that during most of the year bacteria at 1 and 6 m were well adapted to the ambient temperature, ranging between 4 and 23°C, because *in situ* and optimum growth temperatures agreed within 1 to 5°C. At 20 and 50 m, *in situ* and optimum growth temperatures usually diverged by more than 5°C, and often by more than 10°C, indicating that bacterial growth at these depths was clearly limited by temperature. Hence, in the upper water column, temperature controlled bacterial growth by selecting for a well-temperature-adapted community whereas in the lower part of the water column temperature directly controlled bacterial growth. In several experiments we observed 2 temperature optima. We interpret this observation as an indication of the existence of 2 different subpopulations with different temperature preferences. Q_{10} values of Leu incorporation and TdR incorporation, ranging from 1.4 to 42.5, exhibited systematic differences. At 1, 6 and 10 m, Q_{10} of Leu was higher than that of TdR except in 3 cases, whereas at 20 and 50 m Q_{10} of TdR was higher than that of Leu except in 3 cases. This observation suggests that protein synthesis (Leu) and DNA replication (TdR) are controlled differently by temperature in differently adapted bacterial communities. In order to test the hypothesis that utilization of the most important substrates is optimized at the optimum growth temperature we carried out 5 additional experiments in which we measured uptake and respiration of dissolved free amino acids and monosaccharides. In 3 experiments we found that the fraction of amino acids respired had a distinct minimum at the optimum growth temperature whereas respiration of monosaccharides had such a minimum only in 1 experiment. The experiments gave no indication of enhanced respiration percentages at minimum growth temperatures.

KEY WORDS: Temperature · Bacterial production · Amino acid uptake · Monosaccharide uptake · Respiration · Large lake

INTRODUCTION

The growth of heterotrophic bacteria in natural environments usually is limited by some factor like the resource which exhibits the greatest discrepancy between requirements and availability. Depending on the trophic state and seasonal situation of the given environment, organic carbon or nitrogen, ammonium or inorganic phosphate most often have been identi-

fied as limiting bacterioplankton growth (Kirchman 1990, Coveney & Wetzel 1992, Morris & Lewis 1992, Zweifel et al. 1993, Schweitzer & Simon 1995). Besides resources, temperature has been found to limit growth of planktonic bacteria, in particular at relatively low temperature. Positive correlations between temperature and bacterioplankton growth parameters below a certain temperature have been found in estuarine environments, the subtropical convergence of the Atlantic and in lakes (Morris & Lewis 1992, Hoch & Kirchman 1993, Shiah & Ducklow 1994a, Coveney & Wetzel 1995, Tibbles 1997). Above these temperatures, abundance and growth were independent of temperature, implying that other factors like resources con-

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trolled bacterioplankton growth. In a cross-system overview of freshwater and marine environments White et al. (1991) also found a positive correlation between temperature and the bacterioplankton specific growth rate. On the other hand, Rivkin et al. (1996) in a more recent cross-system overview of marine environments did not find a significant positive correlation between temperature and bacterioplankton specific growth rate.

The studies mentioned indicate that there is a general control of bacterioplankton growth by temperature, directly or indirectly. There is, however, evidence based on temperature shift-up and shift-down experiments that the bacterioplankton communities often are not well adapted to the ambient temperatures, in particular in colder environments (Lovell & Konopka 1985, Li & Dickie 1987, Autio 1992, Kirchman et al. 1993, Shiah & Ducklow 1994b, 1997, Felip et al. 1996, Pakulski et al. 1996).

It is important to examine the temperature adaptation of planktonic bacteria because of the implications for the cycling of dissolved organic matter (DOM) and the global carbon cycle. Wiebe et al. (1992, 1993) examined the growth rates of bacterial isolates as a function of temperature and substrate concentration and found that the concentrations needed to sustain a certain growth rate increased with decreasing temperature. Hence, we hypothesize that at temperatures far below the optimum growth temperature, bacteria use relatively more of the substrates for energy requirements, which means that the growth efficiency at such low temperatures would be reduced compared to growth close to the optimum temperature. A consequence of this would also be that the amount of DOM recycled into the food web via the microbial loop or respired to CO₂ depends on how well bacteria are adapted to the ambient temperature. It would be interesting to examine whether similar relationships also hold true for temperatures exceeding the optimum. This hypothesis also would imply that bacterioplankton communities strive to adapt closely to the ambient temperature, if the conditions allow, in order to optimize utilization of substrates for biosynthetic processes. Hence, it is interesting to examine how the dominant substrates, amino acids and carbohydrates (Jørgensen et al. 1993, Rosenstock & Simon 1993, Kroer et al. 1994, Middelboe et al. 1995, Hanisch et al. 1996, Rich et al. 1996, Simon et al. 1998) are metabolized with respect to biosynthesis and energy requirements. Uptake and respiration of these substrates should reflect most comprehensively how bacteria obtain their biosynthetic and energetic requirements.

In order to examine the role of temperature in the bacterial cycling of DOM we studied the adaptation of planktonic bacteria to the ambient temperature

throughout the year in Lake Constance, Germany, a large mesotrophic lake. By using the dual-label approach we simultaneously measured rates of thymidine-based bacterial cell multiplication (BCM) and of leucine-based biomass production (BPP-C) in the epi-, meta- and hypolimnion at 5, 10, 15, 20 and 25°C. In addition we measured respiration of dissolved free amino acids and monosaccharides to test the hypothesis that respiration is minimized at the optimal growth temperature.

MATERIAL AND METHODS

The study was carried out between May 1995 and March 1997 at the center and deepest point of Lake Überlingen, the northwestern arm of mesotrophic and warm-monomictic Lake Constance, Germany. The maximum depth of Lake Constance is 254 m whereas that of Lake Überlingen reaches 147 m. The structure and microbial dynamics of the plankton community in Lake Constance have been studied extensively during the last decade (e.g. Gaedke & Straile 1994, Simon et al. 1998, Weisse & Müller 1998). Samples were collected with a clean van Dohrn bottle at 1, 6, 10 or 20 and 50 m. From May until 1 August 1995 the depth of 10 m was sampled as the layer of the thermocline and thereafter a sample from 20 m was taken due to the deepening of the thermocline. Subsamples were withdrawn into clean 1 l polyethylene bottles and kept in a cooling box until further processing in the lab. Experiments started within 2 h of sampling. *In situ* temperatures were continuously recorded at the sampling station with thermistor chains. Calculated 3 h means are given in Fig. 1.

Twelve experiments were carried out in which the growth of planktonic bacteria as a function of temperature was examined at the 4 depths indicated. In the first experiment incubation temperatures were 5, 10, 18 and 23°C and in the others 5, 10, 15, 20 and 25°C. These temperatures included the ambient temperature $\pm 2^\circ\text{C}$. The bacterioplankton growth in samples from 50 m was examined only in a range from 5 to 20°C. Additional experiments with surface samples (12 and 19 September 1996) and samples from 1 m (3 December 1996, 14 January 1997) and 10 m (25 March 1997) were performed in which uptake and respiration of dissolved free amino acids and monosaccharides in this temperature range was measured as well.

Rates of biomass production and cell multiplication of heterotrophic bacterioplankton were determined by the incorporation of ¹⁴C-leucine (Leu; Kirchman et al. 1985, Simon & Azam 1989) and ³H-thymidine (TdR; Fuhrman & Azam 1980) using the dual-label approach (Chin-Leo & Kirchman 1988). Subsamples of 5 ml were

Fig. 1. *In situ* temperatures at 1, 6, 10, 20 and 50 m in Lake Constance between April 1995 and April 1996. Arrows indicate when temperature shift-up experiments were carried out

withdrawn into clean polystyrene test tubes. Triplicates and a Formalin-killed control were labelled with ^{14}C -Leu ($11.5 \text{ GBq mmol}^{-1}$, Amersham) and TdR (3 TBq mmol^{-3} , Amersham, diluted 1:5 with non-labelled TdR) at a final concentration of 30 nM each. Experiments in various seasons confirmed that this concentration maximized incorporation of Leu and TdR. Incubation at the temperatures indicated above in the dark was stopped after 90 min by adding Formalin (2% final concentration). Samples were not preadjusted to these temperatures because of the small sample volume. Depending on the ambient and incubation temperature the samples adjusted to the incubation temperature within 5 to 10 min. After fixation, samples were filtered onto $0.45 \mu\text{m}$ nitrocellulose filters (Sartorius), rinsed with ice-cold particle-free lake water and extracted with ice-cold 5% trichloroacetic acid (TCA) for 5 min. After rinsing the extracted filters twice with ice-cold 5% TCA, the filters were dissolved with ethylacetate and radioassayed by liquid scintillation counting. Bacterial production (BPP-C, $\text{g C l}^{-1} \text{ h}^{-1}$) was calculated from Leu incorporation rates by using a conversion factor of $2.63 \text{ kg C (mol Leu)}^{-1}$ (Simon & Rosenstock 1992). This conversion factor includes a 2-fold intracellular isotope dilution of Leu and a protein:C ratio of 0.86. To convert TdR incorporation into rates of bacterial cell multiplication (BCM) a conversion factor of $2 \times 10^{18} \text{ cells (mol TdR)}^{-1}$ (Simon & Rosenstock 1992) was used.

Uptake rates of a mixture of 16 ^{14}C -amino acids (Amersham, mean specific activity $1.97 \text{ GBq milli-atom}^{-1} \text{ C}$) and an equal molar mixture of ^{14}C -monosac-

charides (glucose, galactose, fructose, mannose, N-acetyl-glucosamin, mean specific activity $10.2 \text{ GBq mmol}^{-1}$, Amersham) were measured basically in the same way as described for measurements of Leu and TdR incorporation (see above) but separate incubations were used for each label. Final concentrations of total amino acids and monosaccharides added were 5 nM , respectively. After stopping the incubation, samples were not extracted by ice-cold TCA but filtered directly onto $0.45 \mu\text{m}$ nitrocellulose filters and rinsed with particle-free lake water. Respiration of ^{14}C -amino acids and ^{14}C -monosaccharides was measured according to Hobbie & Crawford (1969) in 50 ml samples in 100 ml Erlenmeyer flasks with screw caps. These samples were preadjusted to the incubation temperature for 30 min. Triplicates and a Formalin-killed control were labelled with 5 nM (final concentration) of the respective radiotracer and incubated for 90 min. After stopping the incubation the evolved $^{14}\text{CO}_2$ was trapped in phenethylamine-soaked filterpapers (Whatman no. 1) fixed in a wire hook inside the neck of the Erlenmeyer flask. Samples were acidified by adding $250 \mu\text{l}$ 0.1 N HCl with a syringe through the silicon septum of the screw cap and shaken overnight such that $>98\%$ of the $^{14}\text{CO}_2$ produced was trapped in the filter paper. The filter papers were radioassayed by liquid scintillation counting.

Gross turnover rates of dissolved free amino acids and monosaccharides were calculated as the sum of the respective uptake and respiration rates. The relative proportions of respired substrates were calculated as the ratio of respiration rates/gross turnover rates.

Fig. 2. Temperatures *in situ* and at optimum growth of bacterioplankton determined as rates of protein-based bacterial carbon production (BPP-C) in Lake Constance. (*) Experiments in which samples were taken at 20 m

The temperature optimum was defined as the lowest temperature tested which yielded rates of bacterial production not statistically different from the highest rates. Responses of BPP-C and BCM to increasing temperatures were calculated on the basis of their respective Q_{10} values calculated as $Q_{10} = (K_1/K_2)^{10/(t_1-t_2)}$ (Sherr & Sherr 1996). K_1 and K_2 are bacterial production rates at temperatures t_1 and t_2 (in °C), where $t_1 > t_2$, and t_1 covered the entire temperature range from 5°C to the first optimum or that of increasing temperature below the second, higher optimum.

RESULTS

At 1, 6 and 10 m the temperature increased fairly continuously between April and early August such that highest temperatures of 23, 20 and 15°C, respectively, were reached (Fig. 1). At the end of August there was a sharp drop in temperature to 12 to 15°C at these depths due to a strong storm. At 20 m the temperature

remained below 9°C until early September and slightly increased further until October whereas at 50 m 7°C was not exceeded throughout the year.

The response of bacterioplankton growth to the different temperature regimes exhibited 1 or 2 optima at varying temperatures and thus indicated pronounced differences in the adaptation patterns depending on the seasonal situation and depth. At 1, 6 and 10/20 m the second optimum was always at 25°C and that at 50 m in 2 cases at 20°C (Fig. 2). Because temperatures higher than 25°C at 1, 6 and 10/20 m and than 20°C at 50 m were not tested we do not know whether these temperatures, in fact, were the optimum or whether it was even higher. For reasons of conformity, however, we keep the expression 'optimum temperature'. Because in most cases optimum temperatures for BPP-C and BCM were similar, only the temperature patterns of BPP-C are shown (but see below for differences of BCM). In the vast majority of the experiments at 1, 6 and 10 m covering temperatures between 5 and 25°C, *in situ* temperatures and optimum temperatures, or when 2 optima occurred the lower one, ranged within 5°C. At 20 and 50 m rates of BPP-C and BCM at *in situ* and optimum temperatures more often diverged by >5°C, indicating that at these depths the bacterioplankton was less well adapted to the ambient temperatures. However, at 50 m at certain times the *in situ* temperature and

Table 1. Linear regression analysis of *in situ* and optimum temperature (T) versus bacterioplankton growth at *in situ* and optimum temperatures ($p < 0.01$). Given is r^2 of temperature versus both rates of protein-based bacterial carbon production (BPP-C) and bacterial cell multiplication (BCM)

Depth	x-value	y-value	r^2 (BPP-C)	r^2 (BCM)
1 m	$T_{in situ}$	Growth _{<i>in situ</i>}	0.59	0.64
	T_{opt}	Growth _{<i>in situ</i>}	0.17	0.18
	$T_{in situ}$	Growth _{opt}	0.60	0.58
	T_{opt}	Growth _{opt}	0.25	0.18
6 m	$T_{in situ}$	Growth _{<i>in situ</i>}	0.26	0.35
	T_{opt}	Growth _{<i>in situ</i>}	0.04	0.05
	$T_{in situ}$	Growth _{opt}	0.30	0.35
	T_{opt}	Growth _{opt}	0.14	0.15
10/20 m	$T_{in situ}$	Growth _{<i>in situ</i>}	0.32	0.65
	T_{opt}	Growth _{<i>in situ</i>}	0	0.23
	$T_{in situ}$	Growth _{opt}	0.60	0.47
	T_{opt}	Growth _{opt}	0.11	0.15

lower optimum of rates of BPP-C or BCM or of both parameters were similar, such as on 10 October and 6 February (Fig. 2), indicating that at least a subpopulation of the ambient bacterioplankton was well temperature-adapted. At several dates and depths the temperature optima of BPP-C and BCM were different such as on 3 and 16 May and on 6 and 26 February at a few depths. At 50 m the optimum temperature of BCM rates did not exceed 15°C except on 4 July and 10 April. In contrast, rates of BPP-C exhibited optima at 20°C in 5 of 12 experiments (Fig. 2).

Examples of the temperature shift-up patterns are given in Fig. 3. The different patterns on 3 and 16 May indicate the pronounced change in the temperature adaptation of bacterioplankton growth throughout the water column within 2 wk. On 3 May there was only 1 optimum at 10°C from 1 to 10 m and even an inhibition of bacterial growth above 20°C at 1 and 6 m. Two weeks later, however, a second optimum at 25°C occurred even though the *in situ* temperatures remained fairly similar (Figs. 1 & 3). On 29 August after a strong storm the *in situ* and optimum temperatures of bacterial growth at 1 and 6 m were 20°C even though before this storm in early August the optimum temperature had been 25°C (Fig. 2). On 26 February the temperature adaptation exhibited bi-phasic patterns at 1, 6 and 20 m with lower optima at 5 and 10°C.

The effect of temperature adaptation on bacterioplankton growth for cycling of organic matter and its transfer into the food web becomes evident from the comparison of rates of BPP-C and BCM at *in situ* and optimum temperatures (Fig. 4). During the growing season from May until October BPP-C rates at 1 and 6 m varied not more than 1.5-fold except on 10 October at 6 m. In most cases rates at both temperatures agreed within 25%. Interestingly, the highest rates of BPP-C and BCM at *in situ* temperature occurred on 3 May at 1 and 6 m at the peak of the phytoplankton spring bloom and at *in situ* temperatures of 10 to 12°C. On this day the bacterioplankton was well adapted to the ambient temperature (Figs. 2 & 3). During the growing season at 10 m and below and throughout the water column from November until April rates at *in situ* and optimum temperatures often differed by more than 2-fold.

A linear regression analysis demonstrated that rates of BPP-C and BCM at 1, 6 and 10/20 m at the *in situ* as well as at the optimum temperature were significantly correlated to the *in situ* temperature but such a corre-

Fig. 3. Rates of protein-based bacterial carbon production (BPP-C) and bacterial cell multiplication (BCM) $\pm$ standard deviation in the temperature shift-up experiments on 3 May, 16 May, 29 August and 26 February in Lake Constance

lation did not exist between these bacterial growth parameters and the optimum temperature (Table 1). At 1 m the *in situ* temperature explained 60% of the variability in BPP-C and BCM at *in situ* and optimum temperature. At 10/20 m the *in situ* temperature also explained around 60% of the variability of BCM at *in situ* temperature and of the variability of BPP-C at optimum temperature. At 6 m this correlation was much weaker than at 1 and 10/20 m as shown by an r^2 of only 50% of that at the other depths.

The direct effect of temperature on BPP-C and BCM was determined from the Q_{10} values. For BPP-C, Q_{10} values ranged between 1.4 and 30.4 and for BCM between 1.4 and 42.5. Eighty-six percent of the Q_{10} values for BPP-C and 81% of those for BCM ranged between 1.5 and 6.0. The temperature ranges for both parameters sometimes varied but in 42 cases they were

similar (Fig. 5). At 1, 6 and 10 m Q_{10} values of BPP-C rates were higher than those of BCM rates except in 3 cases (Fig. 6). In contrast, at 20 and 50 m Q_{10} values of BCM rates were at least as high but often higher than those of BPP-C except in 3 cases (Fig. 6).

Uptake and respiration of amino acids and monosaccharides also exhibited quite different responses to the varying thermal regime. *In situ* temperatures at the respective depth were 17°C on 12 and 19 September, 7°C on 3 December and 4°C on 14 January and 25 March. In September, rates of bacterial growth, gross uptake of amino acids and monosaccharides were much higher than on the other dates during winter. Generally, the temperature response of amino acid and monosaccharide uptake followed that of BPP-C and BCM, with 1 or 2 optima ranging from 15 to 25°C even though some differences occurred with respect to the optimum temperature, such as on 19 September and 14 January.

Respiration percentages of amino acids and monosaccharides also exhibited pronounced differences to increasing temperatures. The data, however, did not exhibit a consistent trend with amino acid and monosaccharide uptake and bacterial growth. On 12 September the percentage of amino acids respired was inversely related to increasing temperature and thus to bacterial growth and amino acid gross uptake (Fig. 7). The relative respiration of monosaccharides was also inversely related to temperature between 10 and 20°C but increased at 25°C at the maximum of bacterial growth. On 19 September the percentage of amino acids respired was positively correlated to rates of BPP-C ($r^2 = 0.96$, $p < 0.01$) and BCM ($r^2 = 0.86$, $p < 0.01$) whereas that of monosaccharides showed only little differences with temperature. On 3 December the percentage of amino acids respired was fairly constant over the entire temperature range whereas that of monosaccharides was around 20% between 5 and 10°C and around 50% at 15°C and above (Fig. 8). On 14 January the relative respiration of both amino acids and mono-

Fig. 4. Rates of (A) protein-based bacterial carbon production (BPP-C) and (B) bacterial cell multiplication (BCM) $\pm$ standard deviation at *in situ* and optimum temperatures in Lake Constance. (*) Experiments in which samples were taken at 20 m

Fig. 5. Q_{10} of rates of protein-based bacterial carbon production (BPP-C) and bacterial cell multiplication (BCM) in Lake Constance. Only data from experiments in which the ranges for calculating Q_{10} values of BPP-C and BCM matched are shown. (*) Experiments in which samples were taken at 20 m

saccharides showed a distinct peak at 10°C at low gross uptake rates (Fig. 8). On 25 March the relative respiration of both amino acids and monosaccharides was highest at 25°C, the temperature of the higher growth optimum, whereas it was lowest at 15°C, that of the lower optimum.

The results gave no indication of a general trend of reduced respiration percentages at the temperature optimum of bacterial growth. Only on 12 September for amino acids, on 14 January for both amino acids and monosaccharides and on 25 March for amino acids did the respiration percentage show a distinct minimum at the optimum temperature of bacterial growth.

DISCUSSION

Our results indicate that bacterioplankton growth at 1, 6 and 10 m usually was well adapted to ambient temperatures during most of the growing season. This was shown by the fact that the temperature optimum, or when 2 optima occurred the lower one, agreed with

the *in situ* temperature within 5°C. We interpret the occurrence of 2 optima as an indication of 2 bacterial subpopulations with different temperature preferences from which one was well adapted to the ambient temperature and thus most relevant for bacterioplankton growth and substrate turnover under *in situ* conditions (see below). At 20 and 50 m bacterioplankton growth usually was not well adapted to the fairly low ambient temperatures. Hence, temperature proved to be important in controlling bacterioplankton growth in the upper and lower water column but in 2 different ways. In the upper water column during the growing season the varying temperature led to the establishment of an adapted bacterial community or subpopulation but had a minor effect in directly controlling bacterial growth. In the lower and permanently cold, i.e. <9°C, part of the water column, temperature usually directly controlled bacterial growth. This dual temperature effect is also shown by the significant correlation between the *in situ* temperature and both the rates of BPP-C and BCM at *in situ* and at optimum temperature, which, however, explained at most 65% of the variability of these rates (Table 1). Hence, there is room for other controlling factors such as resources (see below).

Other studies have also shown that temperature can be important in controlling bacterioplankton growth. In one approach positive correlations were established between ambient growth or production rates of bacterioplankton at *in situ* temperatures (White et al. 1991, Morris & Lewis 1992, Hoch & Kirchman 1993, Shiah & Ducklow 1994a, Coveney & Wetzel 1995, Tibbles 1997). In a

Fig. 6. Scatter plot of Q_{10} values of protein-based bacterial carbon production (BPP-C) and bacterial cell multiplication (BCM) in Lake Constance

Fig. 7. Protein-based bacterial carbon production (BPP-C) and bacterial cell multiplication (BCM), gross uptake and respiration (% of gross uptake) of dissolved free amino acids (AA) and monosaccharides (CHO) $\pm$ standard deviation on 12 and 19 September 1996 at 0 m in Lake Constance

cross-system overview White et al. (1991) found that the effect of temperature on bacterioplankton growth is most pronounced in estuarine environments, followed by marine systems and freshwater lakes. This finding is consistent with observations in the Delaware estuary on the Atlantic coast of the USA that the effect of temperature on bacterial growth was most pronounced in the upper estuary whereas downstream and towards the mouth into the Atlantic temperature had a smaller effect on bacterial growth (Hoch & Kirchman 1993).

Various studies on temperature shift-up experiments have provided direct evidence of temperature control of bacterioplankton growth. The pioneering work of Sieburth (1967), on the basis of growth characteristics of bacterial isolates, demonstrated that the temperature adaptation of bacterial communities in Narragansett Bay, Rhode Island, USA, varied seasonally but lagged behind the amplitude of the *in situ* temperature. *In situ* and optimum temperatures matched only from June to September. In a small eutrophic lake, Lovell & Konopka (1985) found that between May and

October bacteria were only well adapted to the ambient temperature in the epilimnion in July whereas at other times and in the meta- and hypolimnion temperature strongly limited bacterial growth. Felip et al. (1996) in one experiment in a small lake in winter showed that the optimum temperature for bacterial growth was $16^{\circ}C$ but a second subpopulation had an optimum at the ambient temperature of $4^{\circ}C$. Autio (1992) found that in the Baltic Sea the *in situ* and optimum temperature of bacterioplankton growth varied considerably from May to August, but only at the end of July was bacterial growth well adapted to the *in situ* temperature. Li & Dickie (1987) found that in the Bedford Basin, Nova Scotia, the optimum temperature of bacterioplankton growth varied seasonally but remained always substantially above the *in situ* temperature. Studies in the Chesapeake Bay (Shiah & Ducklow 1994b, 1997) and the subarctic Pacific (Kirchman et al. 1993) showed that the ambient bacterial communities were not well adapted to the *in situ* temperatures because the optimum temperatures were much higher. On the other hand, studies in the Gerlach Strait of the Southern Ocean (Pakulski et al. 1996) and in the Benguela upwelling (Tibbles 1997) found that bacteria were well adapted to the ambient temperatures of 12 and $0^{\circ}C$, respectively, even though the highest temperature tested in the Gerlach Strait experiments was only $1^{\circ}C$. Another recent

study reports that bacterioplankton growth was well adapted to ambient temperatures in the Antarctic circumpolar current and the marginal ice zone but not at the Polar Front (Simon et al. 1999).

The available information indicates that in most environments bacterioplankton growth is not well adapted to the ambient temperature or only for short periods of time, mainly in summer. Therefore, it appears remarkable that the bacterioplankton in the upper water column of Lake Constance usually is well adapted to the ambient temperature throughout the growing season. In estuarine and many marine environments and also in small lakes a more or less continuous mixing and exchange of various water masses and the seeding of allochthonous bacterial communities seem to prevent the establishment of bacterial communities that are well temperature-adapted. Only lakes with a pronounced pelagic zone and a long water retention time and marine systems with constant environmental conditions not affected by the advection of foreign water masses appear to provide the constant conditions for the establishment of bacterial popula-

Fig. 8. Protein-based bacterial carbon production (BPP-C) and bacterial cell multiplication (BCM), gross uptake and respiration [% of gross uptake] of dissolved free amino acids (AA) and monosaccharides (CHO) $\pm$ standard deviation on 3 December 1996 and 14 January and 25 March 1997 in Lake Constance. Sampling depths were 1 m on 3 December and 14 January, and 10 m on 25 March

tions well adapted to the ambient temperature. We hypothesize that only in these types of aquatic ecosystems can the recycling of dissolved organic matter through the microbial loop into the food web be optimized as a function of temperature with respect to biomass production and respiration (growth yield, but see below). The quality of the substrates utilized, however, is another important factor affecting the growth yield (Kristiansen et al. 1992, Middelboe & Søndergaard 1993).

We assume that the occurrence of 2 temperature optima in a temperature shift-up experiment was an indication of the existence of 2 bacterial subpopulations with different temperature preferences. One subpopulation was well adapted to and active at the ambient temperature and the other one with a higher temperature optimum can be considered as a seed for higher temperature conditions but does not dominate the activity at the ambient conditions. We do not have direct evidence for the existence of 2 subpopulations. However, to our knowledge, there is no report of biphasic temperature adaptation curves of bacterial pure cultures. Hence it is improbable that homogenous bacter-

ial populations with 2 temperature optima exist. In support of this assumption Simon et al. (1999) showed that at the Polar Front in the Southern Ocean, in the Antarctic circumpolar current and in the marginal ice zone bacterial communities with different temperature preferences exhibited pronounced differences in their structural composition as demonstrated by *in situ* hybridization with fluorescent rRNA-specific oligonucleotide probes. Whereas in the Antarctic circumpolar current and the marginal ice zone bacteria of the *Cytophaga/Flavobacterium* cluster dominated with 40 and 72% of the DAPI-stainable bacteria, this group constituted only 19 to 24% at the Polar Front.

The temperature response of thymidine-based rates of BCM and leucine-based rates of BPP-C generally agreed well. An interesting finding, however, was that the Q_{10} values of rates of BCM and BPP-C exhibited systematic differences. At 1, 6 and 10 m, Q_{10} values of rates of BPP-C were higher than those of BCM, except in a few cases in winter, whereas at 20 and 50 m, Q_{10} values of rates of BCM in most cases were higher than those of BPP-C. This observation implies that in the upper water column rates of BPP-C increased rela-

tively more with temperature than rates of BCM. In contrast, in the lower part of the water column rates of BCM increased relatively more with temperature than rates of BPP-C. Tibbles (1997) and Shiah & Ducklow (1994b) also found that leucine incorporation (equivalent to BPP-C) increased more with temperature than thymidine incorporation (equivalent to BCM). In accordance to our data, Shiah & Ducklow (1997) report that in an estuary at temperatures below 20°C thymidine incorporation increased with temperature more than that of leucine whereas at an ambient temperature of more than 20°C the opposite was true. These comparisons suggest that protein synthesis and DNA replication are controlled differently by temperature. Protein synthesis appears to be favored over cell multiplication at higher temperatures, which leads to larger cells, whereas the opposite is true in colder conditions, leading to relatively smaller cells.

Temperature control of bacterioplankton growth cannot be considered without taking resource limitation into account. If temperature directly controls bacterial growth, bacteria will consume more substrates at higher temperature, thus enhancing the turnover of dissolved organic matter and inorganic nutrients such as P and N. If the bacterioplankton community is well temperature-adapted, resources directly control bacterial growth within a certain temperature range above which substrate utilization is constant or even reduced. However, in any case bacterial biomass production will be limited as a function of available nutrients relative to the requirements for growth, i.e. biosynthesis and respiration. Recently it has been shown in various studies that growth of heterotrophic bacterioplankton in lakes and the Baltic Sea is mostly limited by P or co-limited by P+C (Morris & Lewis 1992, Zweifel et al. 1993, Coveney & Wetzel 1995, Schweitzer & Simon 1995) whereas in the marine environment growth of heterotrophic bacterioplankton appears more limited by N and C (Kirchman 1990). In studies with facultatively psychrophilic and mesophilic bacterial isolates Wiebe et al. (1992, 1993) found that growth at temperatures $\leq 10^\circ\text{C}$ was substantially enhanced when substrate concentrations were increased by a factor of 1000 or more whereas at the optimum temperature this was not the case. This observation suggests that substrate utilization at low temperatures and low substrate concentrations is less effective than at high concentrations far exceeding the *in situ* concentrations, implying that the growth efficiency is reduced under these conditions. Possibly, more energy is required when enzymes function far below their optimum temperature. In fact, Harder & Veldkamp (1971) reported that obligately psychrophilic bacteria grew faster than facultatively psychrophilic bacteria at 4°C both at low and high concentrations of the limiting substrate, indicat-

ing that the former were able to optimize substrate utilization at the low temperature. In studies with mixed bacterial communities Felip et al. (1996) and Shiah & Ducklow (1994b) did not find enhanced growth at high substrate concentrations and temperatures below 10°C. Because adaptation of bacterioplankton growth to temperature means an optimization with respect to growth rates, one should expect a most efficient utilization of substrates for biosynthetic requirements at the optimum growth temperature.

In the experiments on uptake and respiration of amino acids and monosaccharides, which are the most important substrates for growth of heterotrophic bacterioplankton in Lake Constance (Schweitzer & Simon 1995, Hanisch et al. 1996, Simon et al. 1998), we found some, but no consistent, evidence that substrate utilization is optimized at the temperature optimum of bacterial growth. Respiration percentages of amino acids exhibited distinct minima at optimum temperatures on 12 September, 14 January and 25 March, whereas those of monosaccharides had a distinct minimum only on 14 January (Figs. 7 & 8). There was, however, no general trend towards higher respiration percentages at low temperature nor for preferential utilization of monosaccharides as the energy source. In 3 out of 5 experiments, respiration percentages of amino acids and monosaccharides were fairly similar, whereas in 2 experiments amino acids were respired to even higher degrees than monosaccharides. One difficulty in our experiments was that in 3 out of 5 experiments 2 bacterial communities with different temperature optima were present, overshadowing a clear-cut response of one population to substrate utilization with increasing temperature. However, even a homogenous bacterial population with respect to temperature adaptation consists of an unknown number of bacterial species with presumably different substrate preferences and affinities, making it difficult to get consistent results of substrate utilization and respiration at various temperatures.

In summary, our results showed that the growth of bacterioplankton in Lake Constance is usually well adapted to the ambient temperature in the upper water column, whereas in the lower water column temperature directly limits growth. The biphasic temperature response curves are strong indications of 2 existing bacterial subpopulations with different temperature preferences. In some cases utilization of amino acids and monosaccharides was optimized, i.e. respiration minimized, at the optimum temperature of bacterial growth, whereas in other cases no such optimization was detected. Hence, our results provided some, but no general, support for the hypothesis that utilization of substrates is optimized at the optimum growth temperature.

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