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Temperature controls on aquatic bacterial production and community dynamics in arctic lakes and streams

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Summary

The impact of temperature on bacterial activity and community composition was investigated in arctic lakes and streams in northern Alaska. Aquatic bacterial communities incubated at different temperatures had different rates of production, as measured by ^{14}C-leucine uptake, indicating that populations within the communities had different temperature optima. Samples from Toolik Lake inlet and outlet were collected at water temperatures of 14.2°C and 15.9°C, respectively, and subsamples incubated at temperatures ranging from 6°C to 20°C. After 5 days, productivity rates varied from 0.5 to ~13.7 μg C l^{-1} day^{-1} and two distinct activity optima appeared at 12°C and 20°C. At these optima, activity was 2- to 11-fold higher than at other incubation temperatures. The presence of two temperature optima indicates psychrophilic and psychrotolerant bacteria dominate under different conditions. Community fingerprinting via denaturant gradient gel electrophoresis (DGGE) of 16S rRNA genes showed strong shifts in the composition of communities driven more by temperature than by differences in dissolved organic matter source; e.g. four and seven unique operational taxonomic units (OTUs) were found only at 2°C and 25°C, respectively, and not found at other incubation temperatures after 5 days. The impact of temperature on bacteria is complex, influencing both bacterial productivity and community composition. Path analysis of measurements of 24 streams and lakes sampled across a catchment 12 times in 4 years indicates variable timing and strength of correlation between temperature and bacterial production, possibly due to bacterial community differences between sites. As indicated by both field and laboratory experiments, shifts in dominant community members can occur on ecologically relevant time scales (days), and have important implications for understanding the relationship of bacterial diversity and function.

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Introduction

Bacteria are important organisms in ecosystems, performing the critical tasks of decomposing organic matter, regenerating nutrients and forming the base of microbial food webs. The rates of these critical tasks can be modified by temperature, and understanding the bacterial response to temperature is necessary for understanding ecosystem function. For example, at higher temperatures, metabolic rates increase considerably and bacteria are able to break down organic substrates more rapidly (Pomeroy and Wiebe, 2001) and increase secondary production (Kirchman and Rich, 1997). Here we examine the links between temperature and ecosystem function as measured by aquatic bacterial production both directly and indirectly through alteration of bacterial community composition.

The metabolic activity of aquatic bacteria is directly limited by temperature in many systems. A strong relationship between bacterial-specific growth rate and temperature was found in a metadata analysis of 57 studies in freshwater, coastal and marine habitats (White *et al.*, 1991), and temperature limitation has been found in estuaries (Shiah and Ducklow, 1994; Almeida *et al.*, 2001; 2007), sea grass beds (Danovaro and Fabiano, 1995), rivers (Freese *et al.*, 2006), coastal systems (Sherr *et al.*, 2001), marine systems (Longnecker *et al.*, 2006), and lakes of various nutrient regimes (Sommaruga and Conde, 1997; Simon and Wunsch, 1998; Friedrich *et al.*, 1999; Gurung and Urabe, 1999; Ram *et al.*, 2005; Vrede, 2005) including high arctic lakes (Panzenbock *et al.*, 2000). A few studies found that temperature is not limiting to bacteria; in these studies only small increases in temperature were tested (Ducklow *et al.*, 1999), or it was found that carbon quality was more important (Mcknight *et al.*, 1993).

Temperature can also interact with carbon availability or substrate affinity to control bacterial activity. At colder temperatures, bacteria begin to lose substrate affinity, leading to carbon limitation despite available sources in

the environment, possibly due to stiffening lipid membranes (Nedwell, 1999). Wiebe and colleagues (1992) found that psychrophilic bacteria have an increased need for substrate at lower temperatures when tested from −1.5°C to 35°C. However, Yager and Deming (1999) found no enhanced substrate requirement by bacteria in arctic polynyas during incubations from −1°C to 5°C. Likewise, Kirchman and colleagues (2005) found that cold-adapted bacteria have shown responses to temperature and carbon similar to those in temperate habitats, although leucine incorporation rates per cell did tend to be lower at lower temperatures within their experiments. Substrate affinity of bacterial enzymes is fundamental to activity, and elevated temperature increases activity provided there is ample substrate (carbon). Arctic aquatic systems receive relatively large amounts of terrestrial carbon subsidies which can increase the amount of bioavailable dissolved organic matter (DOM), and thus with ample DOM it is likely that temperature constrains bacterial activity.

Bacterial response to temperature can go beyond a general metabolic effect and can be population or community specific. Psychrophiles grow optimally at temperatures < 15°C, with an upper cardinal temperature of 20°C (Morita, 1975), and are found in a wide range of environments such as the coastal Arctic Ocean (Connelly *et al.*, 2006), sea ice (Kottmeier and Sullivan, 1988; Huston *et al.*, 2000) and Antarctic saline lakes (McMeekin and Franzmann, 1988). Psychrotolerant bacteria are also found in cold habitats, but have higher optimal growth temperatures (~20°C) and can withstand temperatures as low as −10°C (Bowman *et al.*, 1997; Bakermans *et al.*, 2006). Psychrophilic and psychrotolerant bacteria respond differently to a given temperature based on functional constraints such as membrane fluidity and enzyme structure (Feller *et al.*, 1997; Russell, 2000; D'Amico *et al.*, 2006). Both of these groups of bacteria are likely present in many regions, and their competition due to population-specific growth rates may also be controlled by temperature, resulting in measured community-specific temperature responses.

While individual cells shift their physiology to respond to temperature changes, the dominance of a population within a community will change based on competition between groups that have different growth rates at different temperatures or environmental conditions. Bacteria living at suboptimal temperatures may be present, albeit at low densities or relatively inactive, but may increase in dominance when conditions become favourable. In natural communities, the observed optimal temperature for a community can shift seasonally (Tison *et al.*, 1980), indicating shifts in abundance between bacterial populations with different optimal temperatures and substrate affinities (Ogilvie *et al.*, 1997). A community should adapt to the temperature ranges for its habitat, given enough time for shifts in populations to occur.

The rate of changing environmental conditions is likely to impact both bacterial activity and community composition. Water temperatures change rapidly with storm events, leaving bacterial communities little time to adjust composition to populations with different temperature optima. Pettersson and Baath (2003) found in humic soil that the rate of community compositional change was not related to temperature, although bacterial activity still indicated temperature limitation in the soils. However, Upton and colleagues (1990) found that temperatures could determine outcomes of competition between different bacterial populations. The impact of changes in temperature is likely to be most immediate on productivity by acting directly on enzymatic reactions. Shifts in bacterial community composition in response to temperature should be slower because the dominance of bacteria results from differential growth rates between populations in response to environmental conditions. However, bacterial populations also vary in enzymatic capabilities and preferred carbon substrates in addition to preferred temperature ranges. Therefore, changes in community structure resulting from temperature changes will also change potential levels of activity.

Evidence of temperature limitation and coexistence of bacterial populations with different preferred temperatures leads to the question of how temperature impacts bacterial production (BP), both directly by affecting enzyme activity and other cellular functions and indirectly through shifts in community composition. In this article we show that the direct mechanism impacts BP at very short timescales (hours). The indirect mechanism operates through shifts in community composition, and we support our hypothesis that communities consist of bacterial populations that have different temperature optima (e.g. phylogenetically distinct psycrophilic and psychrotolerant populations), resulting in multiple temperature optima for the community as a whole. We also demonstrate that communities shift dominant members based on water temperature, and these shifts can occur rapidly over periods of days.

Results

Temperature quotient

Laboratory incubations indicated direct temperature control on bacterial activity at several sites. Bacteria from Toolik Inlet and Lake I-8 inlet and outlet increased in activity when incubated at higher temperatures; the calculated Q_{10} values for these sites ranged from 1.3 to 2.9 (Fig. 1). Q_{10} averages 1.5 for aquatic bacterial specific growth rates (Rivkin *et al.*, 1996), and has been calcu-

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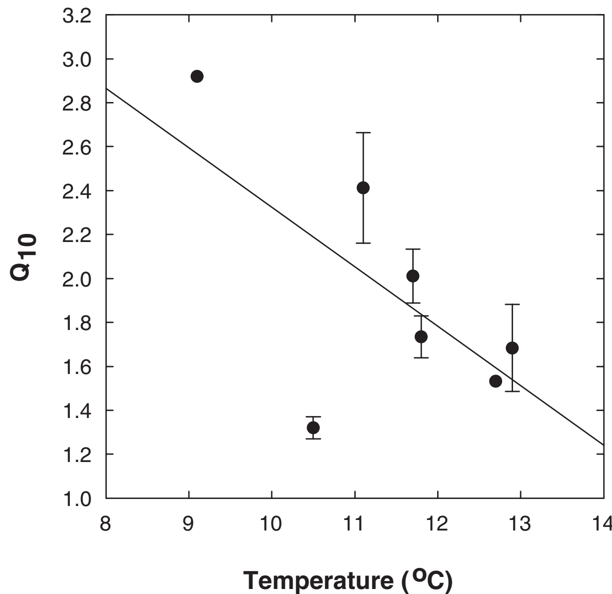

Fig. 1. Bacterial Q_{10} versus *in situ* collection temperature, $r^2 = 0.41$, $P = 0.12$. Values of Q_{10} greater than 1 indicate temperature control of leucine incorporation. Error bars are ± 1 standard error (SE, $n = 6$, $n = 7$ for 15 July 2005 sample), calculated from replicates of different temperature pairs within a Q_{10} measurement. SE is not calculated for I-8 inlet and I-8 outlet Q_{10} which were based on two incubation temperatures.

lated as high as 4.8 for leucine incorporation rate of arctic bacteria (Bussmann, 1999). At Lake I-8, Q_{10} was much higher for the inlet site (2.92) compared with the outlet (1.53) on the same collection date in July 2003. There was greater spatial than temporal variation; the Q_{10} for Toolik Inlet during summer 2005 had a range from 1.32 to 2.41 with an average SE (standard error of the mean) of 0.14, smaller than the range among the sites. The Q_{10} values had a negative relationship with *in situ* collection temperature ($r^2 = 0.41$, $P = 0.12$), and a positive relationship with other environmental variables such as pH ($r^2 = 0.64$, $P = 0.03$), conductivity ($r^2 = 0.36$, $P = 0.15$) and NO_3 ($r^2 = 0.36$, $P = 0.29$) but not with stream flow, DOC, TDN, DON, NH_4 or PO_4 ($r^2 < 0.3$ and $P > 0.2$).

Temperature experiments

The importance of community composition was seen in re-growth experiments at different temperatures. In experiment A, performed on water from Toolik Inlet, there was an initial, short-term (day 1) increase in activity at all temperatures (Fig. 2, top). However, by day 3 distinct peaks in activity developed only at 12°C and 20°C (Fig. 2, bottom). The same water and bacteria incubated at 8°C and 16°C had significantly lower BP than those incubated at 12°C and 20°C, and at 16°C the activity actually declined from that measured on day 1. Experiment B produced similar results, but used a wider range of incubation temperatures (every 2°C) at both the inlet and outlet of Toolik Lake. In this experiment all combinations of bacterial inoculum and DOM source water showed the development of multiple temperature optima over time (Fig. 3), suggesting that temperature was more important to growth than were the different DOM sources found at the lake inlet and outlet. Similar to experiment A, peaks in activity were most distinct after 5 days and at 12°C or 20°C. There were, however, slight differences in activity among the treatments (inlet versus outlet DOM source water) after 5 days. These differences may be due to DOM chemistry or to community composition; the inlet and outlet communities were 66.7% similar at the time of field collection based on denaturant gradient gel electrophoresis (DGGE) banding patterns, and while the two sites had similar protein and phenolic concentrations the DOC concentration and DOM absorbance were slightly higher at Toolik Inlet.

Temperature also influenced bacterial community composition. Community fingerprinting performed on experiment A, where Toolik Inlet samples were incubated at 8°C, 12°C, 16°C and 20°C and re-grown from a 50% inoculum for 5 days, indicated the development of more similar communities within a temperature treatment (Figs 4 and S1). Replicates grown at the same temperature had 85–93% average similarity (1.1–6.8% standard deviation) while those grown at 8°C and 20°C had the lowest average similarity compared with each other (79% similarity after 5 days, 5.1% standard deviation). In experiment C, when Toolik Lake inlet and outlet samples were combined in a factorial of inoculum and DOM source at temperatures of 2°C, 12°C and 25°C for 5 days, community fingerprinting again clustered samples by temperature (Figs 5 and S2) within either the inlet or outlet community inoculum. At the end of the experiment, these bacterial communities had statistically more operational taxonomic units (OTUs) in common with other inocula from their same collection sites (starting community) or with samples incubated at the same temperature, despite incubation DOM source as indicated by ANOVA on pairwise similarities and dummy variables representing incubation conditions (Table 1). As was first seen in experiment B, in this experiment the influence of temperature was clearly a stronger driver of community structure than was DOM source during the 5-day incubation period.

Psychrophilic and psychrotolerant bacterial populations were distinguished by BP peaks and by community similarity (DGGE) in laboratory incubations at different temperatures. While genomic sequences and structural differences may also distinguish these two populations, we assign the operational description of communities based on the observed optimal temperature of productivity. Within collection habitat, bacteria incubated at 2°C had an average 69% similarity to those incubated at 12°C and

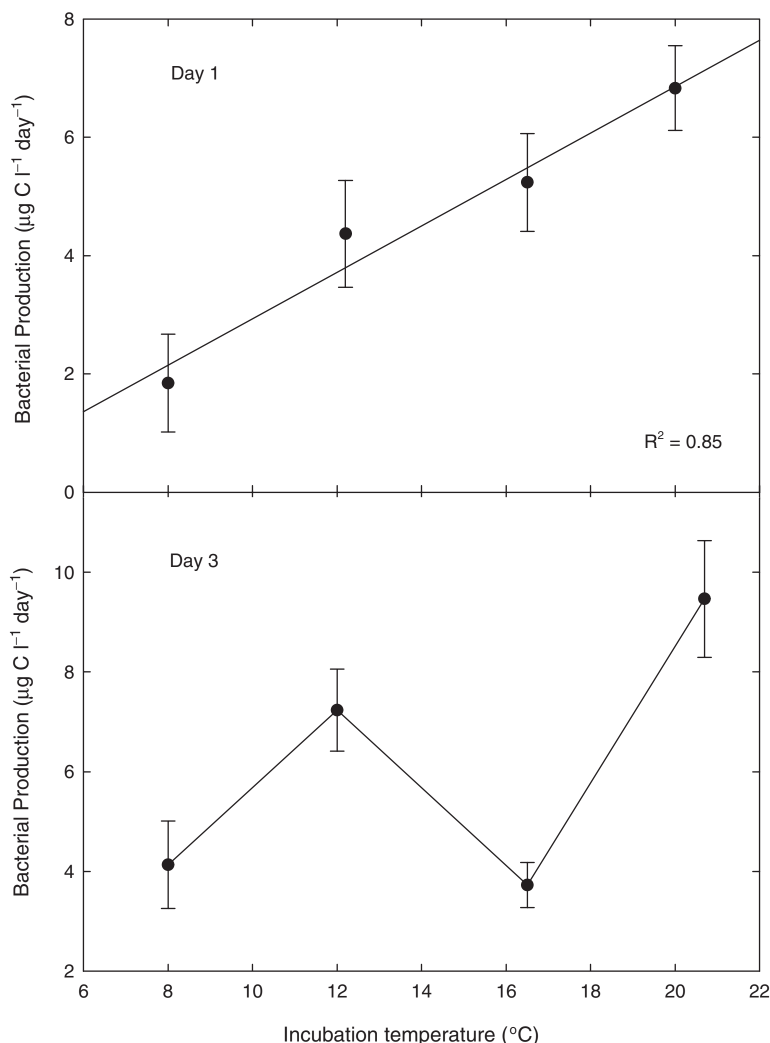

Fig. 2. Bacterial production versus temperature from Toolik Inlet samples incubated at different temperatures in experiment A. Bars indicate standard deviation of three replicates of each treatment.

a slightly lower average similarity (58%) to those incubated at 25°C, while the 12°C and 25°C incubations had an average 61% similarity at the end of experiment C. Based on Morita's (1975) definition of cardinal temperature ranges, psychrophilic bacteria would be able to persist in the 2°C and 12°C incubations which had the highest similarity between temperatures, while psychrotolerant bacteria can survive at all tested temperatures and should dominate at 25°C. Given these definitions and incubation temperatures, out of 79 total OTUs identified (Table 2) there were 4–12 detectable psychrophilic OTUs and 6–24 psychrotolerant OTUs within the environment at the time of sample collection.

In situ *responses of BP to temperature*

From 2003 to 2007, summer (mid-June through August) stream water temperatures at Toolik Inlet ranged from 4°C to 19°C, averaging 11°C, with a mean daily temperature fluctuation of 3.5°C that ranged from 0.4°C to 10.4°C diel change in water temperature. Stream discharge was also quite variable and ranged from 0.02 to 10.2 $m^3 s^{-1}$ (Table 3). Path analysis of discharge, temperature and BP at Toolik Inlet ($P < 0.001$ for the entire model) for 2003–2006 showed a standardized correlation coefficient of 0.45 ($P < 0.001$) for stream discharge (natural log transformed) on BP and of 0.25 ($P = 0.053$) for water temperature on BP. Upstream of Toolik Inlet at Lake I-8, path analysis of discharge, water temperature and BP for 2003–2006 at both the inlet and outlet ($P < 0.001$) had a similar correlation between discharge and BP ($\beta = 0.43$, $P < 0.001$) and a higher correlation between temperature and BP ($\beta = 0.60$, $P < 0.001$). In this variable habitat, BP showed strong spatial and seasonal patterns across several sites and over longer time periods.

I-8 outlet BP was consistently higher than the inlet of I-8, but both the inlet and the outlet responded synchronously during most summer seasons (Fig. 6). In 2003, ultraviolet (UV) absorbance by DOM, an indicator of the extent of

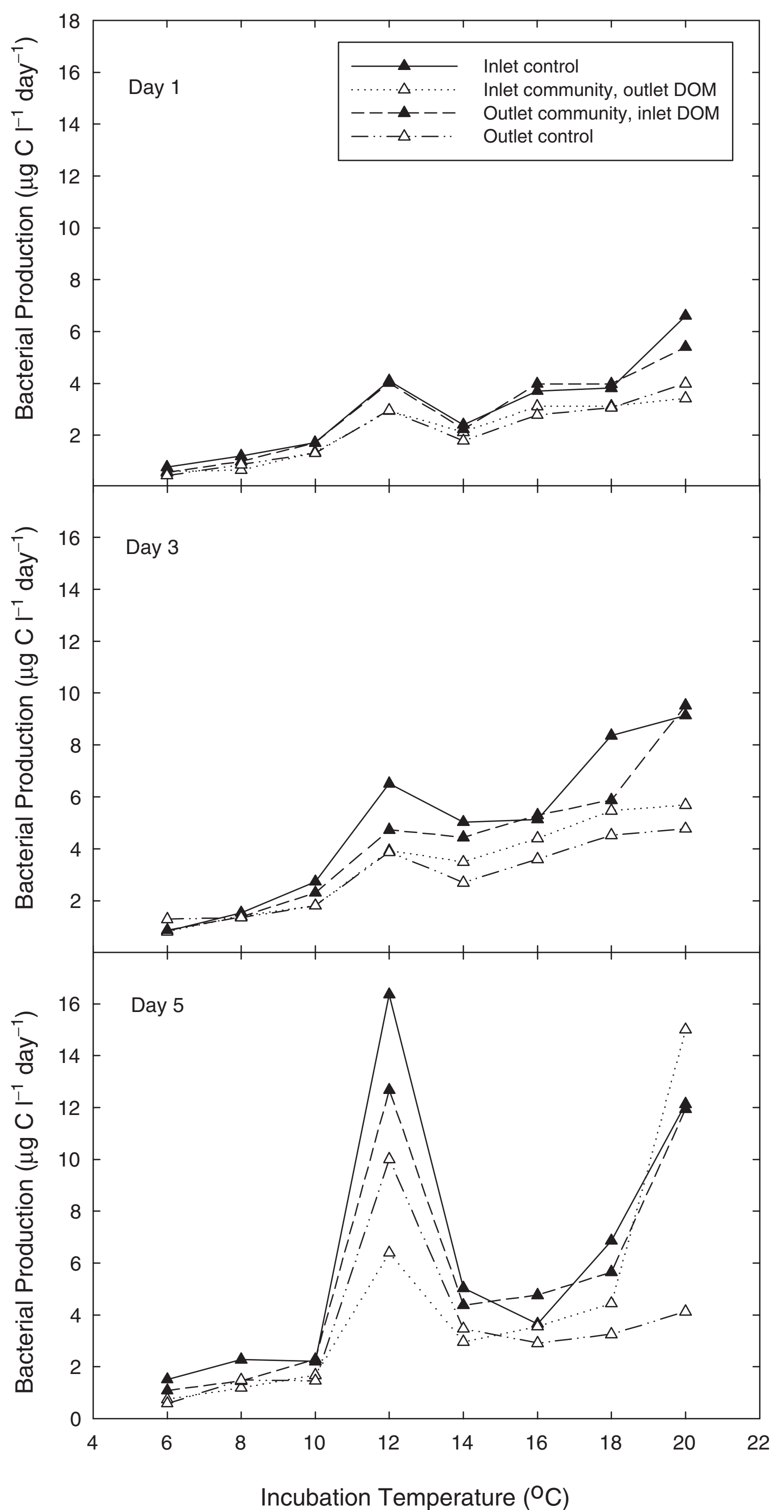

Fig. 3. Bacterial production versus temperature from Toolik inlet and outlet samples incubated at different temperatures in experiment B. Initial collection temperatures at Toolik inlet and outlet were 14.2°C and 15.9°C, respectively, with a daily range 13.3–16.7°C at the inlet.

photo-degradation (Moran and Covert, 2003), increased with the first summer rain event indicating freshly exposed DOM from terrestrial habitats. The BP initially increased along with UV absorbance; however, later storm events were accompanied by smaller increases in BP despite continued high UV absorbance of DOM (Fig. 6). Temperature was colder during these later rain events, and consequently temperature may have constrained the bacterial response at Lake I-8 in the later part of the season.

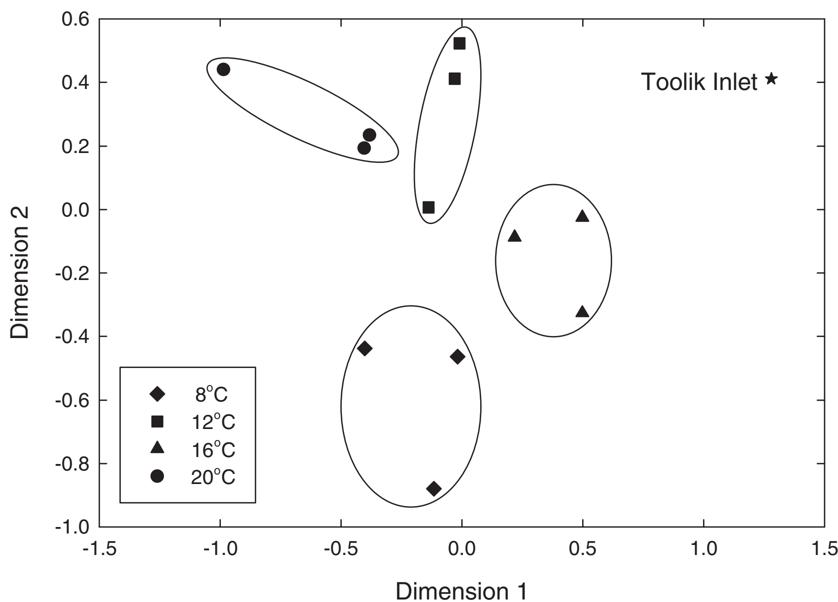

Fig. 4. Non-metric multi-dimensional scaling (MDS) of Toolik Inlet DGGE community fingerprints from post-incubation samples held at different temperatures from experiment A. Proximity of samples indicates a higher degree of community similarity as indicated by overlap in OTUs determined by the DGGE banding patterns of PCR-amplified products from 16S rRNA genes. Normalized Raw Stress = 0.0265.

Data from three sampling surveys performed on lakes across the larger catchment also indicate that bacteria probably switched from carbon limitation in the early season to temperature limitation later in the season in 2003, but this pattern varied annually. Across the I-series catchment, path analysis (Fig. 7) of BP, temperature, DOC and chlorophyll *a* (chl) in all the lakes and inlet sites showed both a direct effect of temperature on BP and an indirect effect of temperature on BP through chl. The DOC concentrations were not statistically significant in our model for any of the sample sets examined (data not shown), and it is likely that chl reflects the labile portion of the DOC pool in our system. In 2003, 2004 and 2006, BP in the entire catchment correlated with chl in the early season (late June) before the first rain event ($\beta = 0.69$, Table 4). Later in the summer of 2003, temperature was the strongest correlate for BP ($\beta = 0.41$, Table 4), verifying that the patterns observed at Lake I-8 (Fig. 6) were synchronous across the larger catchment. For the data set 'all', the indirect pathway of temperature to chl to BP is statistically significant, as is the model as a whole (Table 4). However, separating lake and stream inlet sites into early, mid and late summer seasons as well as by year indicates that the direct effect of temperature on BP is usually greater than the indirect effect through chl. Additionally, the beta coefficient for the direct effect of

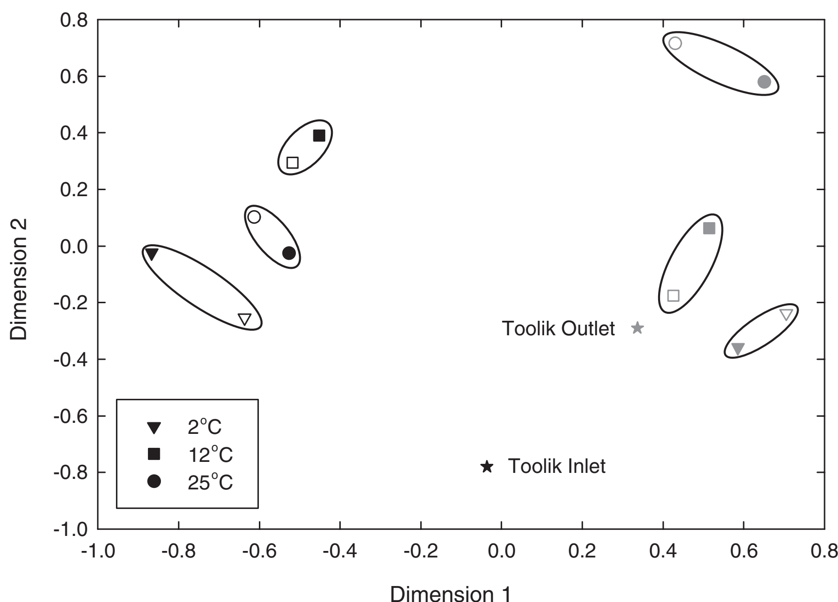

Fig. 5. Non-metric MDS of DGGE community similarity from experiment C. Proximity of samples indicates a higher degree of community similarity as indicated by the banding patterns of OTUs. Closed symbols designate samples where bacterial communities were given DOM from their source habitat and open symbols designate samples with a DOM source from the opposite site. Black symbols indicate bacterial communities from Toolik Inlet and grey from Toolik Outlet. Normalized Raw Stress = 0.02054.

Table 1. ANOVA results from community similarity of samples from Fig. 5.

Between-subjects factors

		Value label	n
Starting community	0	Different	40
	1	Same	33
Incubation temperature	0	Different	49
	1	Same	24
DOM source	0	Different	40
	1	Same	33

Tests of between-subjects effects

Dependent variable: ending community similarity

Source	Type III sum of squares	df	Mean square	F	Significance
Corrected model	1.36	6	0.23	23.49	**0.00**
Intercept	19.9	1	19.9	2060	**0.00**
Starting community	1.02	1	1.02	105.8	**0.00**
Incubation temperature	0.22	1	0.22	23.14	**0.00**
DOM source	< 0.01	1	< 0.01	0.02	0.89
Community * Temperature	0.02	1	0.02	1.67	0.20
Community * DOM source	< 0.01	1	< 0.01	0.01	0.94
Temperature * DOM source	< 0.01	1	< 0.01	0.02	0.89
Error	0.64	66	0.01		
Total	23.34	73			
Corrected total	1.99	72			
	R squared = 0.681 (adjusted R squared = 0.652)				

Community samples were scored as having either the same or different experimental conditions as their comparison samples (dummy variables). Similarity values used to create Fig. 5 were then compared with the commonalities in conditions between samples. Starting community indicates whether or not both samples were collected at the inlet or at the outlet of Toolik Lake. Incubation temperature indicates if compared samples were both incubated at 2°C, 12°C or 20°C. The DOM source category indicates if both samples were incubated in 0.2 μm filtered DOM collected from the same site.

temperature on BP has a weak inverse correlation with temperature range among all sites in each of the I-series samplings, similar to the trend of higher Q_{10} at lower *in situ* temperatures. While the pattern of early season carbon limitation holds across several years, the strength of correlation of temperature with BP varies seasonally and annually in the I-series catchment.

Discussion

Both field and laboratory experiments indicate variability in the temperature control on bacterial activity during the ice-free summer. Temperature dependence of BP (leucine incorporation) was directly measured with Q_{10} values greater than 1. However, despite the general indication of temperature limitation, the range in Q_{10} was quite large (Fig. 1) and varied both spatially and temporally. Such variation is commonly found; for example, Rivkin and colleagues (1996) calculated an average Q_{10} of 1.5 in a literature review of aquatic bacterial-specific growth rate, while Bussmann (1999) measured a Q_{10} of 4.8 for leucine incorporation rate of arctic bacteria. Atkin and Tjoelker (2003) found that the Q_{10} of plant respiration decreases with increases in measurement temperature across biomes. They explained this difference across biomes as enzymatic capacity impacting temperature response at low temperature, and substrate supply constraining Q_{10} at higher temperatures. Shiah and Ducklow (1994) found temperature limitation to be more important than substrate limitation for specific growth rate of estuarine bacteria in colder (non-summer) seasons, with a high Q_{10} of 2.72. Similarly, Bridgeman and colleagues (2000) found that substrate supply of DOM constrained BP at high temperatures, and that the quality of DOM, which influences enzymatic capacity, constrained BP at low temperatures. However, it is also possible that there is an enzymatic capacity of the entire community, where different populations adapted to processing different biochemical compounds (i.e. quality of DOM) comprise a range of capacity to enzymatically degrade DOM. In this case, shifts in community composition would be consistent with shifts in the enzymatic capacity of individuals or populations, and could explain the spatial and temporal variation in Q_{10} that we observed.

The spatial variation in Q_{10} was characterized by a negative relationship of Q_{10} with *in situ* temperature (Fig. 1, $r^2 = 0.41$; $P = 0.12$). This higher Q_{10} at lower temperature has been found in growth rates of estuarine bacteria (Hoch and Kirchman, 1993) and is consistent with studies showing that cold-grown plants acclimatize with higher Q_{10} values (Atkin *et al.*, 2005), and that soil respiration acclimatizes to warmer temperatures with lower Q_{10} values (Luo *et al.*, 2001). This temperature acclimatization is analogous to changes in Q_{10} caused by shifts in bacterial communities with different enzymatic capacities, such as populations of psychrophilic or psy-

Table 2. Number of unique OTUs found only at certain temperatures.

Temperature	No. bands
Only at 2°C	4
2°C and 12°C	8
Only at 12°C	7
12°C and 25°C	12
Only at 25°C	6
All temperatures	6

The DGGE bands are from samples incubated for 5 days at 2°C, 12°C and 25°C, out of 79 total identified bands (OTUs).

Table 3. Stream discharge and water temperature of Toolik Inlet from the ice-free summer season, generally measured from 15 June to 20 August except 20 June to 16 August in 2003 and 21 June to 16 August in 2004.

	Water temperature (°C)			Stream discharge (m^3 s^{-1})		
Year	Minimum	Mean	Maximum	Minimum	Mean	Maximum
2003	4.6	9.6	19.3	0.04	1.65	9.01
2004	6.8	12.2	18.7	0.12	1.24	7.03
2005	5.0	11.2	18.9	0.02	0.31	3.22
2006	4.6	10.7	14.7	0.18	0.86	3.33
2007	8.3	12.9	18.8	0.01	0.31	2.83

chrotolerant bacteria. Populations also likely differ in optimal pH, which may explain the strong positive relationship between pH and Q_{10}. Therefore, the differences we observed in Q_{10} could be attributed to the differences we measured in bacterial community composition at different sites. For example, the Q_{10} at I-8 inlet was the highest (2.9) and had the lowest *in situ* temperature, while at I-8 outlet the Q_{10} was 1.5 and had one of the highest *in situ* temperatures (Fig. 1). Furthermore, these two sites had very low community similarity at the time of measurement (18%) and, in fact, the bacterial communities at these two sites are consistently different and had a mean similarity of only 42% during the 2003 summer season.

The variation in bacterial response to temperature was seen not only among sites in environmental samples, but also within communities incubated at different temperatures. Multiple temperature optima developing within the same community indicate the presence of both psychro-

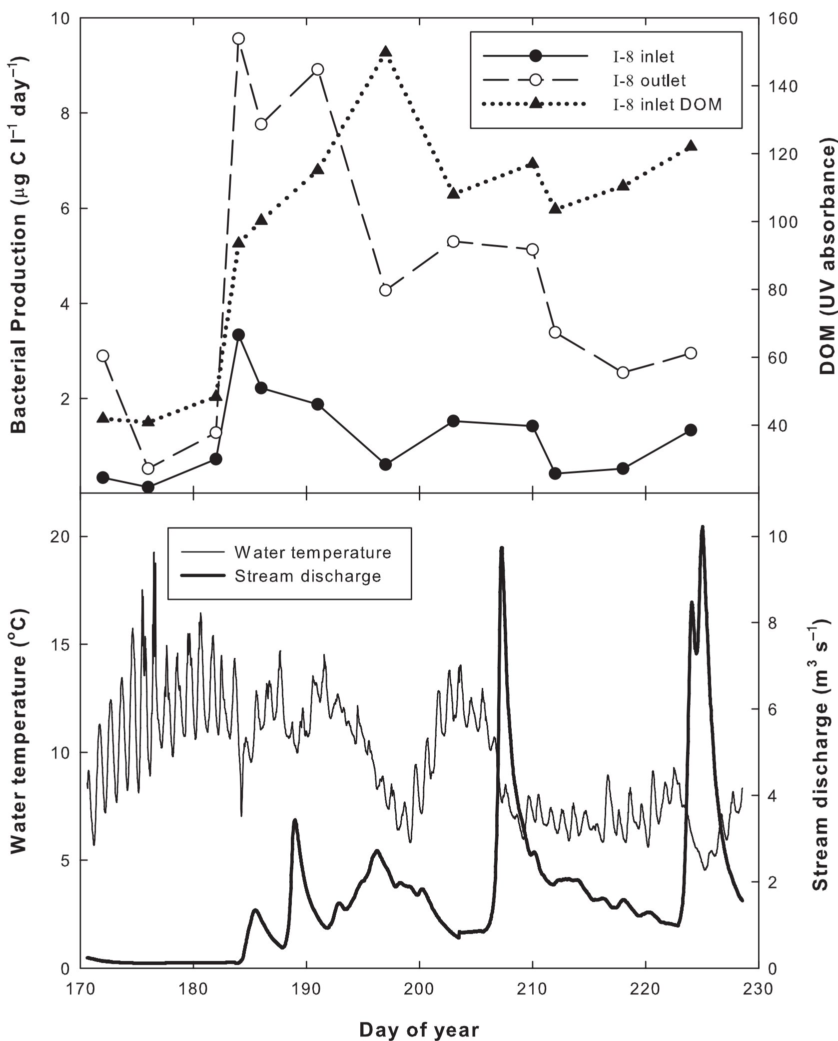

Fig. 6. Top panel: DOM UV absorbance at I-8 inlet and BP at I-8 inlet and outlet in 2003. Bottom panel: Stream discharge and water temperature at Toolik Inlet in 2003.

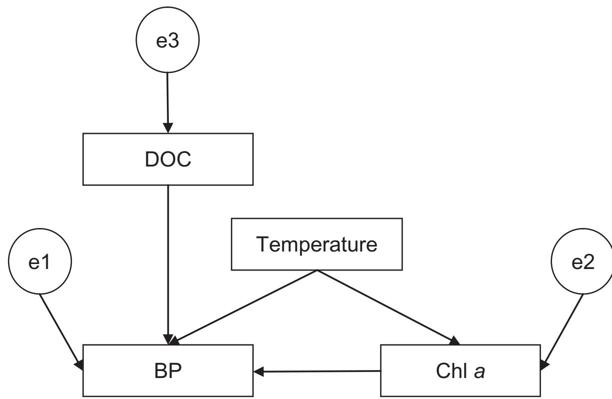

Fig. 7. Path model for I-series lakes in summers 2003–2006. Boxes indicate variables and circles are error terms associated with endogenous variables. Straight arrows indicate presumed causal relationships.

philic and psychrotolerant bacteria within the initial community. This suggests that the inoculated bacterial populations best adapted to incubation temperatures outcompete other populations via increased growth. Predation could also influence the outcome of bacterial competition, but viral activity should increase most in those incubations with highest bacterial productivity (Thingstad, 2000; Hewson and Fuhrman, 2007) which would tend to dampen any observed temperature effect. Any introduction of grazers during the experiment would have a similar effect (Corno and Jurgens, 2008), although no grazers were detected via microscopy. In both re-growth temperature experiments, the 12°C and 20°C treatments developed the highest BP relative to other incubation temperatures (Figs 2 and 3). Since psychrophilic bacteria have optimal temperatures < 15°C and maximal temperatures of < 20°C (Morita, 1975), the populations with optimal growth in our experiment of 12°C can be characterized as psychrophilic and the populations with optimal growth at 20°C can be characterized as psychrotolerant. Under field conditions, the relative dominance of psychrophilic and psychrotolerant bacteria is likely to shift continuously towards the group whose optimal temperatures are closest to current water temperature, with BP shifting accordingly.

The DNA evidence also indicates shifts in dominance by different bacterial populations at different temperatures. Bacterial community fingerprints changed after incubation at different temperatures, within the relatively short timescale of 5 days. In both the temperature re-growth experiment at 8°C, 12°C, 16°C and 20°C (Fig. 4) and the incubations at 2°C, 12°C and 25°C (Fig. 5), populations shifted in dominance and the community as a whole shifted in levels of productivity. There

Table 4. Path analysis results.

Sample set	*n*	χ^2	*P*-value	Standardized regression weights, *P*-value						Indirect effects
				Temp => Chl *a*		Chl *a* => BP		Temp => BP		
All	283	46.848	***	0.280	***	0.247	***	0.009	0.884	0.069
All, lakes	120	18.785	***	–0.095	0.302	–0.002	0.98	–0.187	*	0.000
All, streams	163	27.583	***	0.396	***	0.181	*	0.12	0.142	0.072
All, early	93	12.993	**	–0.111	0.284	0.342	***	0.204	*	–0.038
All, mid	96	17.197	***	0.487	***	0.404	***	–0.189	0.085	0.196
All, late	94	8.842	*	0.493	***	0.119	0.312	–0.031	0.795	0.059
2003	70	3.638	0.162	0.308	**	0.468	***	0.169	0.111	0.052
2003, early	23	2.901	0.235	–0.173	0.411	0.694	***	0.276	0.062	–0.032
2003, mid	24	8.058	*	0.614	***	0.461	*	0.320	0.074	0.283
2003, late	23	0.471	0.790	0.370	0.062	0.317	0.066	0.406	*	0.117
2004	72	35.164	***	0.429	***	0.475	***	0.051	0.650	0.204
2004, early	24	1.906	0.386	0.308	0.121	0.543	**	0.010	0.956	0.167
2004, mid	24	6.514	*	0.620	***	0.264	0.233	0.335	0.130	0.093
2004, late	24	5.739	0.057	0.566	**	0.474	*	–0.066	0.770	0.142
2005	69	10.679	**	0.065	0.593	0.051	0.643	0.418	***	0.003
2005, early	22	5.843	0.054	0.105	0.630	0.037	0.855	0.281	0.163	0.004
2005, mid	24	4.557	0.102	0.512	**	0.123	0.573	0.280	0.199	0.063
2005, late	23	8.384	**	0.059	0.781	–0.123	0.541	0.145	0.472	–0.007
2006	72	1.024	0.599	0.134	0.257	0.190	0.091	0.277	*	0.026
2006, early	24	1.699	0.428	–0.021	0.921	0.483	**	0.266	0.119	–0.004
2006, mid	24	0.942	0.624	0.100	0.638	0.023	0.898	0.465	**	0.001
2006, late	24	2.406	0.300	0.564	**	–0.081	0.741	0.220	0.371	–0.046

***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$.

Sample set 'All' includes data from all I-series sampling from 2003 to 2006. Early, mid and late season samples were split by month of collection date (June, July or August). Standardized regression weights are equivalent to the standardized total and direct effects for temperature to chl, and chl to BP, while temperature to BP is only the direct effect. The indirect effects are for temperature to BP via chl. The DOC concentration was included in the model, but was not statistically significant for the sample sets analysed.

was no clear relationship between the total number of bands ('taxonomic diversity') and temperature. However, some OTUs in the community fingerprinting analysis (Table 2) were only present at certain temperatures, which indicate that certain OTUs in the community can only reach PCR-detectable levels when held at a constant low or high temperature.

The competitive interactions of psychrophilic and psychrotolerant groups of bacteria in response to temperature affect productivity because the groups have different levels of activity dependent on temperature. Bennett and Lenski (1997) found that *Escherichia coli* acclimated at different temperatures were competitively superior at the temperature at which the bacterial populations had evolved. Rutter and Nedwell (1994) found that two psychrotolerant bacterial species differed in the rate of their response to changing temperature, with *Brevibacterium* sp. responding rapidly and *Hydrogenophaga pseudoflava* showing a time lag in growth after a temperature change. Under steady temperature conditions, or slow shifts in temperature, *H. pseudoflava* was able to out-compete the *Brevibacterium*, while *Brevibacterium* performed best when temperatures changed dramatically. This suggests that competition between psychrophilic and psychrotolerant bacteria may also be affected by the rates of temperature changes, not just the mean daily water temperature. Upton and colleagues (1990) also found that stable versus fluctuating temperatures could control the outcome of bacterial competition in Antarctic lake sediments, with greater diversity occurring under fluctuating temperatures. Using laboratory-bred *E. coli*, Cooper and colleagues (2001) found that cold-adapted bacteria did poorly when moved to higher temperatures, but that warmer-adapted bacteria did not suffer the same relative decrease when moved to colder temperatures. This implies that psychrophilic bacteria are worse off at higher temperatures than psychrotolerant bacteria are at lower temperatures, potentially giving psychrotolerant bacteria the competitive edge during times when temperatures are warmer. Fluctuating temperatures, then, may allow the persistence of both types of bacteria.

In the field, bacterial communities are subject to daily changes in temperature as well as frequent changes in stream flow, which carries DOM and nutrients from upstream. At Toolik Inlet, both temperature and discharge were significantly related to BP for the path analysis of 2003–2006, even though all four years had very different temperature and storm event patterns (Table 3). Path analysis is useful for distinguishing between direct (causal) and indirect (correlational) effects (Wright, 1934; Sokal and Rohlf, 1995) through an analysis of partial (standardized) regression correlation coefficients (β) of potentially important independent variables; the strength of direct versus indirect effects on a dependent variable can be compared in different pathways of effect-response within a specified model (e.g. Fig. 7). The path analysis (Table 4) showed that discharge had a larger effect (β coefficient = 0.45) than water temperature ($\beta = 0.25$) on BP, although the effect of discharge may be due to drivers of BP that co-vary with discharge such as DOM and nutrients. These results were similar to those found at Lake I-8 inlet and outlet, where path analysis of discharge, water temperature and BP showed a strong correlation ($\beta = 0.60$) of water temperature and BP, probably related to consistent differences in temperature between the inlet and outlet of the lake.

Even though temperature was related to BP over all sites over all years, the specific response of sites varied from year to year. This variation is probably due to interactions of temperature with carbon availability, although nutrient availability, viral lysis or grazing could also be important differences between sites. For example, at Lake I-8 in the summer of 2003, BP increased as DOM entered the system early in the summer when water temperatures were warm (Fig. 6). However, DOM absorbance (i.e. freshness or quality) remained high later in the summer but BP dropped; it appears that lower water temperatures during late summer storm events constrained the bacterial response to the available DOM by reducing the ability of bacteria to take up substrates. The impact of DOM quality on BP can also depend on the source and prior processing of DOM upslope (Judd *et al.*, 2007), and the quality of DOM may vary among different storm events. In experiment B, the magnitude of response in BP at 12°C and 20°C in different DOM treatments may be due to differences in DOM absorbance or other unmeasured differences in DOM (Fig. 3), although replication would be needed to confirm this effect. Despite this potential variation, similar interactions between temperature and carbon limitation have been observed elsewhere. Rivkin and colleagues (1996) found empirical evidence of an inverse relationship between temperature and carbon limitation in a meta-analysis, and we found alternation of temperature and chl (a proxy for algal carbon source) as a significant effect on seasonal differences in BP within the path analysis. Kirschner and Velimirov (1997) also found that temperature and chl explained 69% of the seasonal variation in BP in the Danube River. Temperature and DOC accounted for 50% of the bacterial growth rate in the Greenland Sea (Middelboe and Lundsgaard, 2003), and were correlated with bacterial abundance in the Northern Adriatic Sea (Paoli *et al.*, 2006). It is clear that both temperature and carbon are important controls of BP at individual sites in natural ecosystems.

The interaction between temperature and carbon limitation we observed at the individual site Lake I-8 was found also in all I-series streams and lakes in summer 2003 (Table 4). Summer rain events flush terrestrial

carbon subsidies into the aquatic system, and because from 2003 to 2006 there was low flow (and warm water temperature) in the early summer season, BP was likely constrained by carbon availability; this was indicated by positive correlations of BP with chl (which reflects the labile portion of the DOC pool in our system; Table 4). Later in the summer, air and water temperatures often decline with rain events, and thus temperature constrains the metabolic activity of the bacteria and limits the response of BP to terrestrial carbon subsidies supplied by rain events. However, the pattern of a strong shift to temperature limitation late in the summer season was seen only in 2003, perhaps because this was the wettest and coolest year of the study and thus provided the strongest contrasts between potential temperature versus carbon limitation.

The variable effect of temperature on BP seen in the field results over all years may be due to (i) variation in chl, (ii) the range of water temperature or (iii) differences in community composition among sites. The control by temperature across the catchment was indicated directly by correlations of temperature with BP and indirectly through chl. The indirect effect of temperature on BP through chl appears to be driven by results from stream sampling sites, where direct effects of temperature on chl were higher and statistically significant (Table 4). This correlation could be due to the covariance of temperature and light levels (through storms and cloudiness), and light is well known to impact algal productivity and autochthonous DOM in streams (Dodds, 2007). However, the indirect effect for the pathway from temperature to BP was usually weak (beta values < 0.2), and stronger direct pathways from temperature to BP and chl to BP were more common.

The second possible explanation for the variation in control by temperature on BP may be related to the range of water temperature. Higher beta values for the direct effect of temperature on BP were calculated during time periods when catchment surface waters had lower ranges in temperature. More stable water temperatures would allow bacterial populations and communities time to respond physiologically to the current water temperature. This increased impact of temperature on BP during periods of more stable temperature is consistent in effect to the inverse relationship between Q_{10} and *in situ* temperature observed at Lake I-8 and Toolik Inlet. Storm events tend to have both less variable and lower temperatures, suggesting that the variation in trade-off between carbon versus temperature limitation may be due to the timing and magnitude of storm events.

Finally, we have shown that bacterial communities vary at sites across the catchment, and that different communities respond differentially to temperature. For example, community similarities among all sites in the catchment during July 2003 ranged from 23% to 95% and averaged 61% (see also Crump *et al.*, 2007). The variation in communities reflects distances between sites as well as the degree of hydrologic connectivity and habitat similarity (Crump *et al.*, 2007). As indicated by the laboratory experiments discussed above, bacterial communities shift when temperature changes, and the new communities may have either higher or lower BP depending on the specific community optimum (Figs 2 and 3). This interaction between temperature, community composition and activity optima may partially explain the fact that the strong temperature dependence of BP measured in our lab experiments was weaker and more variable in the field observations.

The impact of temperature on bacteria community composition and activity has implications for the response to climate warming in the Arctic. If air temperatures increase 5°C, lake temperatures are predicted to increase ~3°C and the ice-free season to lengthen by 7 weeks (Hobbie *et al.*, 1999). Warmer, more stable temperature conditions would decrease the predominance of psychrophiles during the summer season and psychrotolerant bacteria may become dominant. However, psychrophilic marine bacteria have been shown to have enhanced heat tolerance at 17°C under starvation conditions (Preyer and Oliver, 1993), a possible mechanism of psychrophilic persistence in oligotrophic waters during conditions which should favour psychrotolerant bacteria. A shift to more psychrotolerant bacteria does not necessarily mean higher overall activity, because moderate warming of surface waters may depress psychrophilic activity but temperatures could still be too low for optimal activity of psychrotolerant bacteria as seen by the bi-modal responses in our temperature re-growth experiments. In other words, a shift of the average water temperature in aquatic habitats from 12°C to 16°C may actually result in lower bacterial activity (see Figs 2 and 3). This emphasizes the central role of shifts in bacterial community composition and their interactions with temperature in determining rates of activity in natural bacterial communities.

Conclusions

Temperature controls on aquatic bacteria are more complex than previously thought due to the interactions of changing temperature and rapid shifts in community composition. We found seasonal temperature limitation of bacterial activity across several spatial scales as well as multiple temperature optima of populations within bacterial communities. In addition, temperature plays a key role controlling bacterial community dynamics because composition shifts rapidly with changing temperature. These shifts occur at ecologically relevant timescales (days),

and bacterial community characteristics will ultimately constrain the potential BP resulting from increasing temperatures or from changes in temperature variability.

Experimental procedures

Site description

Sites are located on the North Slope of Alaska, at the Toolik Field Station Arctic LTER (long-term ecological research) site. Toolik Lake is located at 68°38′00″N, 149°36′15″W at an elevation of 720 m, and has an ice-free season lasting from mid-June through September (O'Brien *et al.*, 1997). The terrestrial system is largely tussock tundra with dwarf birch-willow riparian zones and wet sedge wetlands with large amounts of organic matter accumulation in histel soils (Chapin *et al.*, 1995). The area has continuous permafrost and thaw depth typically varies from ~15 cm in June to a maximum of ~40 cm depth in August (data available at http://ecosystems.mbl.edu/ARC/). Due to the presence of continuous permafrost, there is very little water exchange between surface waters and deeper, frozen soils or aquifers. Snow melt in the spring and pulsed storm events throughout the summer bring terrestrial carbon subsidies into aquatic systems; this results in much higher BP relative to primary production in lakes than is found in temperate systems (O'Brien *et al.*, 1997).

The I-series catchment, the largest sub-basin of the Toolik Lake catchment, is 46.6 km^2 and contains 10 lakes as well as the largest inlet to Toolik Lake, Toolik Inlet (see Kling *et al.*, 2000 for further descriptions). Toolik Lake is a multi-basin lake draining a catchment of 66.9 km^2, and has a single outlet (Macintyre *et al.*, 2006). Upstream, in a sub-basin of the I-series catchment, Lake I-8 (0.182 km^2 in area) was sampled frequently, and has a large catchment area (29.1 km^2) without upstream lakes. All of the lakes in the Toolik Lake catchment are oligotrophic, with mean primary productivity of 3.23 μmol C l^{-1} day^{-1} and mean chl of 1.02 μg l^{-1}. There are typically 2–3 storm events during the summer season, post snow-melt, which carry terrestrial and upstream DOM into downstream habitats. Concentrations of DOC are typically much higher (~500 μM, Kling *et al.*, 2000) than those found in coastal ocean areas (Ducklow *et al.*, 1999), and storm events can have a large impact on DOM available downstream.

Field measurements

All I-series lakes and major inlet streams were sampled three times each summer from 2003 to 2006 over 2-day periods (late June, mid-July and early August) for BP, temperature, DOC and chl; precise sampling locations followed Kling and colleagues (2000). Stream samples were collected in 2 l bottles from under the water surface but without disturbing the stream bottom, and lake samples were taken either from ~0.5 m depth by boat or at the lake outlet which represents an integrated sample of epilimnetic water (details in Kling *et al.*, 2000). Lake I-8 inlet and outlet as well as Toolik Inlet were sampled weekly for DNA, BP and temperature. Temperature was measured with a digital thermometer during sample collection. Bacterial production was measured immediately or within ~1 h of field collection using ^{14}C-labeled leucine uptake following Kirchman (1992) with an isotopic dilution of 1, resulting in a conversion factor of 1.55 kg C mol^{-1}. Each measure was calculated from the incubation of three unfiltered 10 ml subsamples (taken from 2 l collection bottles) and one 10 ml trichloroacetic acid (TCA)-killed control with ^{14}C leucine for approximately 3 h before ending with addition of 5% TCA. Samples were filtered onto 0.22 μm nitro-cellulose filters and extracted using ice-cold TCA. Filters were then dissolved with 1 ml of ethylene glycol monoethyl ether in scintillation vials, and analysed with a liquid scintillation counter (Packard Tri-Carb 2100TR) after the addition of 5 ml of Scintisafe scintillation cocktail. The BP measurements had an average coefficient of variation (CV) of 18%. Ultraviolet absorbance of DOM was measured on unfiltered samples using a quartz cell with a 5 cm path length on a Shimadzu 1601-UV scanning spectrophotometer in the wavelength range of 220–400 nm. DOC samples were filtered through Whatman GF/F filters in the field, acidified to pH 3, and stored in the dark at 4°C until analysis on a Shimadzu TOC 5000 using platinum-catalysed high-temperature combustion to CO_2 followed by infrared detection. The chl samples were filtered onto Whatman GF/F filters in the field and kept dark until frozen upon returning from the field. After overnight freezing at ~20°C, filters were extracted for 24 h with acetone, read on a Turner Designs 10 AU fluorometer configured with a chlorophyll optical kit (10-037R), and corrected for phaeopigment using acidification with HCl. Stream discharge and temperature were monitored every 30 min at Toolik Lake inlet using a Stevens PGIII Pulse Generator (and manual discharge measurements to generate a rating curve) and a Campbell Scientific Model 247 conductivity and temperature probe connected to a Campbell Scientific CR510 datalogger.

Path analysis of I-series data was conducted using a structural equation modelling program (AMOS 6.0) to calculate standardized regression coefficients; natural log transformations of BP, chl and stream discharge measurements were performed to normalize input data when required and any discrepancies were addressed via maximum likelihood. Temperature quotient (Q_{10}) was calculated for samples collected from Lake I-8 inlet and outlet on 3 July 2003 and at Toolik Inlet on 24 June, 1, 15, 22 July, and 5 August 2003. Bacterial production was measured in samples incubated at a range of temperatures within 1 h of sample collection: Lake I-8 inlet and outlet samples were incubated at 6°C and 16°C and Toolik Inlet samples were incubated at 6°C, 9°C, 12°C, 14°C, 17°C and 20°C. The Q_{10} value was calculated as $Q_{10} = e^{10k}$ where k = slope of log-transformed R versus T, where R = leucine incorporation rate (pmol l^{-1} h^{-1}), and T = incubation temperature (°C) as described by Atkin and colleagues (2005).

Temperature experiments

Three experiments were conducted to test the influence of water temperature on BP and bacterial community composition. The experiments were performed in different years, were designed to answer different questions, and included different measurements; they are presented here in logical progression of answering our hypotheses. All experiments

included temperature manipulations: experiment A measured BP and community composition, experiment B measured BP, and experiment C measured community composition. Experimental incubations used whole water collected from the field and partitioned to include 50% 1.0 μm filtered water (used as the bacterial inoculum) with 50% 0.2 μm filtered water (particle-free water, used as the DOM source). Treatment mesocosms were started within 8 h of water collection and kept in the dark in HDPE amber Nalgene bottles at set temperatures (± 1°C). The starting time point of the incubations was considered to be the time of inoculation, and collection times were within a few hours of 24 h (1 day), 68 h (nominal '3 days') or 116 h (nominal '5 days') due to the length of time required to sample all treatments (2–4 h). Filtration intentionally excluded both grazers and larger primary producers, while dark incubation minimized photosynthesis; this was done to focus on the impacts of temperature and bacterial community composition. Experiment A was conducted from 26 to 31 July 2006. Three replicate bottles were collected in the field from Toolik Lake inlet and filtered as described above to result in each replicate having 1 l total volume. From each replicate, 40 ml of subsamples were taken at the end of 1, 3 and 5 days for BP measurement, and on the fifth day 500 ml was filtered for DNA analysis. Experiment B was performed on Toolik Lake inlet and outlet samples from 26 to 31 July 2004, with factorial combinations of bacterial inoculum and DOM source from each habitat for a total volume of 250 ml, with 40 ml of subsamples taken for BP after 1, 3 and 5 days (no DNA collected). Experiment C was set-up the same as experiment B and run from 28 June to 3 July 2004, but with a total 1 l volume and DNA collected on day 5. In summary, experiment A was fully replicated; experiments B and C were performed first, without field replicates, but the time-course of changes and the end-of-experiment BP and DNA results were similar in all three experiments and consistently support our conclusions.

Community fingerprinting

DNA samples were collected from the field and from laboratory mesocosms by filtering ~500 ml of sample through a 0.2-μm-pore-size Sterivex filter. Filters were preserved using a DNA extraction buffer described by Crump and colleagues (2003) and stored at −80°C until extraction. Bacterial DNA was extracted using a phenol-chloroform extraction and PCR amplified using 357f with a G-C clamp and 519r universal 16S rRNA gene bacterial primers on a Bio-Rad thermocycler (Crump *et al.*, 2003; 2007). The amplified DNA was then separated using DGGE with 8% acrylamide gels cast with a 30–40% to 50–70% gradient of urea and formamide. Standard lanes were created using a mixture of previously identified Toolik Lake clone isolates (Crump *et al.*, 2003) and used to standardize within and between gels. Gels were run on a CBS scientific system for 18–24 h at 75 volts and 65°C. Imaging was performed with Quantity One software on a Chemi-Doc gel documentation system (Bio-Rad), and gel bands were identified using GelCompar software to create a presence–absence matrix as described by Crump and Hobbie (2005). Each band represents an OTU of bacteria. Dice transformation (SPSS 14.0, 15.0) was used to condense presence–absence data into percent community similarities between samples. The software PROXCAL (SPSS Categories, v. 14.0, 15.0) was used to create multi-dimensional scaling (MDS) proximity graphs.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Image of DGGE gel from experiment A. Image has had colours inverted and contrast adjusted with text overlay.
Fig. S2. Image of DGGE gel from experiment C. Image has had colours inverted and contrast adjusted with text overlay.

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