

Pelagic microbial activity in an arctic polynya: Testing for temperature and substrate interactions using a kinetic approach

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Abstract

To test the hypothesized inhibition of high-latitude marine bacteria living at lower temperatures when organic matter is scarce, the effects of temperature and organic substrate concentration on pelagic microbial heterotrophy were investigated in the perennially cold surface waters of a summertime arctic polynya. Utilization (incorporation plus respiration) of radiolabeled amino acids was measured as a function of increasing added substrate concentration at in situ (subzero) temperature and under short-term warming. Results analyzed using analysis of variance (ANOVA) showed that increased substrate concentration had a significant effect on utilization rates at all stations, suggesting that communities were always living well below saturating levels of substrate. About half of the communities sampled revealed a significant temperature response using ANOVA; interactions between temperature and substrate concentration were detected only rarely. Kinetic parameters, used to link microbial activity and substrate utilization with temperature sensitivity, exhibited mixed responses to short-term warming. Maximum specific utilization rates showed the greater temperature sensitivity, with Q_{10} values ranging from 0.25 to 13. Specific affinities responded to temperature significantly at only about half of the stations in the polynya. Psychrophilic behaviors (e.g., highest specific affinities and oligotrophic capacities at lower incubation temperatures) were observed at stations most likely to be influenced by direct Arctic Ocean outflow. Complete agreement with the hypothesis of enhanced substrate requirement by bacteria living at subzero temperature was not found. An improved understanding of the diversity of cold-tolerant and cold-loving microorganisms is needed before generalizing or predicting the role of temperature in the cycling of organic matter at high latitudes.

Temperature–substrate interactions may underlie seasonal cycles of marine bacterial activity at all latitudes, greatly influencing the cycling of organic matter through marine food webs (Pomeroy and Wiebe 1993). A bacterial growth limitation at low temperatures is thought to occur at the level of substrate uptake via reduction of reaction rates and membrane fluidity (*see* Gounot 1991 for review) and by slower diffusion rates (Jumars et al. 1993). In some perennially cold regions, a correspondence has been observed between in situ temperature and substrate concentrations needed for measurable bacterial production and substrate utilization (Pom-

eroy et al. 1990). Some seasonally cold subarctic waters require greater organic amendments at low temperatures before respiration is detected (Pomeroy et al. 1991). Bacterial production and substrate utilization in warm equatorial waters also exhibit a requirement for enhanced substrate concentrations when cooled (Kirchman and Rich 1997). In laboratory cultures, mesophilic (moderate temperature-loving) and psychrotolerant (cold-tolerant) bacterial isolates from cold environments show enhanced substrate requirements for growth at low temperatures but grow equally well on any substrate concentration if warmed (Wiebe et al. 1992, 1993; Nedwell and Rutter 1994). The temperature–substrate-dependent growth responses of psychrophilic (cold-loving) isolates appear to be more varied (L. Pomeroy and W. Wiebe pers. comm.) and to show some dependence upon the carbon source (Herbert and Bell 1977). The dominance of psychrophilic bacteria in perennially cold marine environments remains uncertain (Morita 1997), however, and the degree to which temperature–substrate interactions limit microbial activity in high-latitude oceans continues to be an active area of research.

Different measures of microbial activity often give different indications of a pelagic community. The percentage of cells capable of substrate uptake, as measured by microautoradiography, does not often correspond well with the percentage of cells stained with the metabolic indicator 5-cyano-2,3-ditoyl tetrazolium chloride (CTC; Karner and Fuhrman 1997), despite the good correspondence between CTC results and O_2 -based respiration rates (Smith 1998).

Acknowledgments

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Measures of bacterial production do not always correlate with measures of substrate uptake or respiration (Jahnke and Craven 1995). Evaluations of parameter effects on activity may thus be methods-dependent as well. Selection of uptake kinetics with labeled substrates for assessing temperature effects on microbial activity was based on the direct link that the approach provides between substrate uptake and availability. Recent advances in microbial uptake kinetic theory (Button 1998; Koch 1998) have also allowed for fine-tuned interpretations of such experimental results, particularly as they relate to microbial activity at low substrate concentration.

The ability to take up substrate (S) depends on membrane transport systems and thus on transporter density and capacity and can be described by the following cell-specific kinetic parameters (Button 1998): maximum specific utilization rate, V_{max}^S (g-S g-cells⁻¹ h⁻¹); half-saturation or Michaelis constant, K_m (g-S L⁻¹), and specific affinity, a_s^o (L g-cells⁻¹ h⁻¹). V_{max}^S reflects the density of membrane transporters as well as the most rate-limiting step in cytoplasmic enzymatic processing. K_m (or its inverse) has been used to describe substrate affinity (see review by Button 1985), even though by definition (the concentration at half-maximal utilization) it is not independent of V_{max}^S . a_s^o , the initial slope of a specific uptake rate versus substrate concentration curve, measures the ability of cells to respond to small increases in substrate availability and may relate to membrane permease activity.

While limitations to their strict mechanistic interpretation exist (Koch 1998), these kinetic parameters in various forms have been used for decades to investigate substrate utilization in pure and mixed cultures, in bulk seawater and lake water, and in sediments, using both single- and mixed-substrate additions (see reviews by Karl 1986 and Button 1998). In this study, we used a mixture of ¹⁴C-amino acids (AAs) to test for temperature effects on the uptake kinetics of pelagic microbial communities in a perennially cold, arctic coastal environment characterized by episodic food inputs. We expected that specific affinity, as the first step in microbial activity, would be the critical parameter for testing substrate limitation due to low temperature. We also expected that V_{max}^S would show less sensitivity to temperature than a_s^o , because reported rates of respiration or growth at high substrate concentrations were similar for all temperatures below about 5°C (Pomeroy et al. 1991; Wiebe et al. 1992, 1993). Use of kinetic theory to interpret the results of previously reported field and pure culture experiments led us to hypothesize a strong temperature sensitivity in specific affinity (a_s^o increasing with warming) in keeping with the current low-temperature inhibition concept, as long as the cells were not diffusion-limited (Jumars et al. 1993; Koch 1998). Our alternative hypothesis was that the observation of cold-adaptation (a_s^o favored at low temperatures) would dominate. Our observed deviations from the low-temperature-inhibition paradigm suggest that additional or alternative mechanisms may control the microbial fate of organic matter in this perennially cold region.

Methods

Sample collection—The Northeast Water (NEW) Polynya on the continental shelf of Northeast Greenland (Fig. 1) was

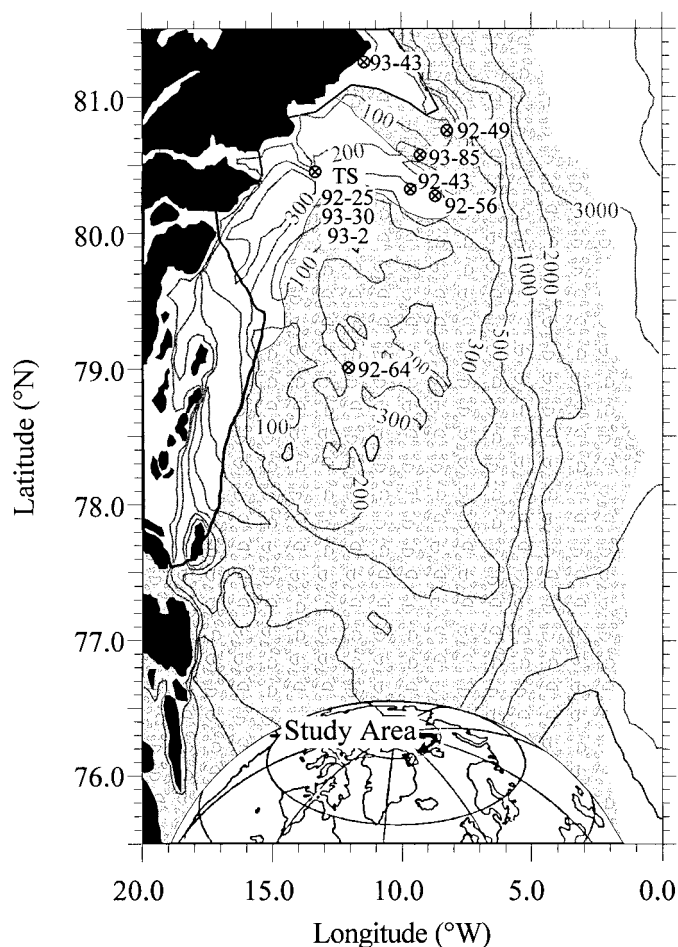


Fig. 1. Map of the Northeast Water Polynya with locations for samples collected. Station numbers are shown preceded by the last two digits of the year they were sampled. Several locations, including the time series (TS) location, were sampled multiple times during the two field seasons (see Table 1 for individual station information). Depth contours in meters; black area, land; white area, open water; gray area, land-fast ice; stippled area, ice cover (temporal variability in ice cover not shown).

the site of a major international and multidisciplinary research project during the boreal summers of 1992 and 1993 (NEWater Investigators 1993). Samples for this study were collected during expeditions of the United States Coast Guard Cutter *Polar Sea* (15 July–15 August 1992; 18 July–18 August 1993) and one leg of the RV *Polarstern* cruise in 1993 (ARK IX/2, 20 May–29 June). Unless otherwise stated, our water samples were recovered in Niskin bottles from near-surface fluorescence maxima, as measured by a SeaTech fluorometer attached to the SeaBird CTD/Rosette, at depths ranging from 0 to 60 m (Fig. 1; Table 1). The in situ temperature for the euphotic depths sampled was fairly uniform throughout the summer, averaging $-1.30^{\circ}\text{C} \pm 0.242$ ($\pm 95\%$ confidence interval).

Samples were removed to sterile polyethylene containers immediately upon arrival on deck. All manipulations with live samples, prior to experimental incubations in temperature-controlled waterbaths, were carried out using ice/sea-

Table 1. NEW Polynya station information and data for samples collected.

Sta.	Date	Latitude (N)	Longitude (W)	Sample depth (m)	BNL ID No.*	In situ temp. (°C)	Salinity (psu)	DCAA (nM)	Chl <i>a</i> (µg kg ⁻¹)	Bacteria × 10 ⁷ L ⁻¹ (±1 SE)†
92-25A‡	07/26/92	80°26.56'	13°19.89'	16	394	+0.266	32.316	67	0.38	5.0 (0.9)
92-25B‡	07/28/92	80°27.15'	13°23.04'	25	476	-1.087	32.322	86	8.22	6.9 (0.2)
92-43A	07/31/92	80°18.71'	09°41.87'	38	733	-1.477	32.334	31	5.46	9.8 (0.9)
92-43B	08/01/92	80°17.80'	09°25.55'	17	804	-1.308	31.770	47	2.07	6.3 (1.7)
92-49	08/05/92	80°46.20'	08°10.86'	50	880	-1.344	31.901	63	1.94	5.2 (0.4)
92-56	08/07/92	80°15.82'	08°40.02'	16	985	-1.390	31.578	143	2.32	3.9 (0.6)
92-64	08/09/92	79°01.34'	12°02.98'	27	1,115	-1.552	31.997	—	1.14	5.2 (0.5)
93-30‡	05/30/93	80°26.34'	13°38.66'	0§	NA	-1.700	32.310	—	0.18	1.9
93-43	06/05/93	81°15.00'	11°30.00'	26	NA	-1.570	31.580	—	0.13	1.6
93-85	06/14/93	80°35.00'	09°22.00'	58	NA	-1.620	32.060	—	0.40	2.8
93-02‡	07/23/93	80°26.40'	13°20.58'	12	3,047	-1.194	32.237	—	0.97	6.4

* Brookhaven National Laboratory identification number, used throughout *Polar Sea* cruises; not applicable (NA) for samples collected aboard *Polarstern*.

† Standard errors for 1992 measurements calculated from counts on three replicate samples; 1993 samples damaged during return transit to laboratory; bacterial abundance estimated from chlorophyll concentrations using the model 2 linear regression from 1992 (Smith et al. 1995). Data for Sta. 93-02 from sample taken by different Niskin bottle at approximately the same depth, time, and location.

‡ Time-series location; A and B refer to repeated visits to same location.

§ Sample collected away from ship during ice-camp using tripod-mounted Niskin bottle from just under ice after drilling.

|| The 1993 DCAA samples were damaged during return transit to laboratory, data not available (—).

water baths to prevent sample warming. This goal was furthered by performing nearly all operations inside refrigerated (0–2°C) laboratory vans. Because artificial increases in dissolved organic matter (DOM) concentrations are known to occur during filtration of large volumes of seawater (Fuhrman and Bell 1985), water samples were not prefiltered prior to incubation; potential grazers were not removed.

Substrate availability and biomass inventories—Subsamples were taken for dissolved combined AAs (DCAA) and total organic carbon (TOC). DCAA concentrations were measured using high-performance liquid chromatography (HPLC; Cowie and Hedges 1992a) on small volumes (<10 ml) of seawater that had been gently filtered (0.2 µm cellulose acetate; Corning), acidified using 100 µl double-distilled HCl, and then stored refrigerated until processed in Seattle. TOC samples were collected and analyzed using standard techniques (Knap et al. 1996). Separate water samples taken directly from Niskin bottles were analyzed by standard methods (Smith and Nelson 1990; Deming et al. 1997) for chlorophyll (Chl) *a* concentration, inorganic nitrogen, particulate organic carbon (POC) and nitrogen (PON), and bacterial abundance from the 1992 and 1993 *Polar Sea* cruises. These results are available in data reports accessible via the National Snow and Ice Data Center database (<http://www-nsidc.colorado.edu/>), and summarized for 1992 in the literature (Smith et al. 1995). Additional bacterial abundance estimates were made from our subsamples processed similarly; reported values are averages of all estimates made for a given sample time, location, and depth.

Bacterial cell size and morphology were determined for 20 samples from the euphotic zone of the times-series station (80°26'N, 13°20'W, Sta. 92-25 and 93-37) by measuring the length and width of up to 750 cells from each sample (3,500 total cells measured) that was fixed, filtered, and stained with acridine orange (Deming et al. 1997), photographed, projected onto a flat white surface, and measured with calipers

(Lee and Fuhrman 1987). A calibration was done as above using fluorescent 5-µm microspheres, resulting in a 6.4% downsizing of all linear measurements due to fluorescent halo effects. Biovolumes were calculated assuming cells were either coccoid ($\frac{4}{3}\pi r^3$, where *r* is radius) or rod-shaped (a cylinder plus two hemispheres: $\pi[w/2]^2[l-w] + \frac{2}{3}\pi[w/2]^3$, where *w* is the width and *l* is the length of the cell). Cell sizes for both rods and cocci measured from a single sample exhibited strongly right-skewed frequency distributions, so the average cell volume for each sample was determined using logarithmic transformation (Sokal and Rohlf 1995). Biomass (wet) was calculated from cell volumes using a power function for cell density derived from data for marine bacteria (Simon and Azam 1989; $r^2 = 0.950$): cell density (g cm⁻³) = cell volume (µm³)^{-0.0489}. Bacterial biomass for each station was calculated using average abundances for each station times the average cell size and density.

Kinetics—We measured the community utilization of a mixture of ¹⁴C-labeled AAs (New England Nuclear; NEC-445E, L[¹⁴C(U)]AA mixture, 3.80 µmol mCi⁻¹) using a method modified from Deming (1993). The prepared mixture of AAs was uniformly labeled (all carbon molecules were ¹⁴C), correlates favorably on a mole-percent basis ($P < 0.01$) with AA compositions of coastal marine phytoplankton (Cowie and Hedges 1992b), *Escherichia coli* protoplasm (Neidhardt and Umbarger 1996), and pelagic bacteria (Simon and Azam 1989), and has a combined molecular weight of 125 g AA mol AA⁻¹ (or 57.3 g C mol AA⁻¹).

For kinetic experiments, endpoint incubations were set up for 18 treatments (6 substrate concentrations and 3 temperatures) with either 2 (in 1992) or 3 (in 1993) replicate incubations for each treatment. Replicate rate measurements for each substrate concentration were determined by subtracting the average value of time-zero bottles from each of the endpoint bottles. This approach was repeated for each temperature treatment (time-zero replicates for each sub-

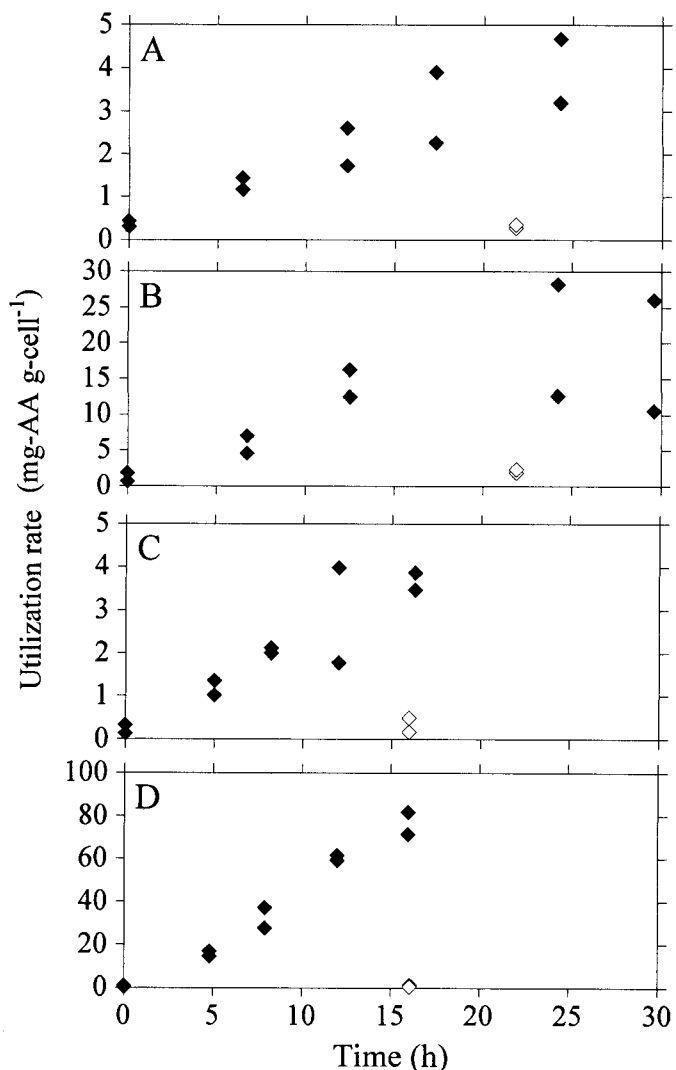


Fig. 2. Utilization of added ^{14}C -AA mixture ($\text{mg-AA g-cells}^{-1}$; solid diamonds) as a function of time for seawater samples from Stas. 92-25A (A, B) and 92-43A (C, D), with addition of $0.125 \mu\text{g AA L}^{-1}$ (A, C) or $12.5 \mu\text{g AA L}^{-1}$ (B, D). Open diamonds are 2%-formaldehyde killed controls.

strate concentration were shared by all temperature treatments) for a total of 48 or 72 incubations per experiment.

Early in the first expedition and then occasionally throughout all of the cruises, time-course experiments, using endmember temperatures and substrates, were carried out to determine the best incubation time to use for endpoint kinetic experiments. Rate measurements were determined by up to seven time points, two or three replicate bottles each, including time-zeros for each substrate concentration; samples were incubated for 3–24 h (Fig. 2). From these experiments, 12 h was chosen for endpoint experiments to get the strongest signal within the linear range.

Twenty-milliliter subsamples were dispensed for individual incubations in 50-ml, acid-washed, autoclaved, borosilicate glass serum bottles. Each subsample then immediately received a small volume (0.54 ml) of sterile stock solution to obtain the desired final AA concentration that ranged from

tracer to saturation levels ($0.5\text{--}100 \text{ nM AA}$, or $0.03\text{--}6 \mu\text{g }^{14}\text{C L}^{-1}$). Samples were quickly capped with sterile gray-butyl stoppers and aluminum crimps that allowed for submerged incubation in dark waterbaths at three temperatures between -1.5 and 5.0°C ; a range reflecting the minimum in situ temperature and the maximum to which organisms might be exposed in the surface waters of the polynya (3.5°C in 1992 and 6.4°C in 1993 at 1 m depth).

At the end of each incubation (including time-zero and 2% formaldehyde-killed controls), inorganic carbon was extracted from each sample. Serum bottle caps were quickly replaced with sterile rubber septa equipped with center wells containing a fluted $2 \times 5\text{-cm}$ piece of Whatman No. 1 filter paper wick saturated with 0.2 ml of the CO_2 absorber, phenethylamine (PEA). Control experiments indicated that no significant $^{14}\text{CO}_2$ was lost during cap replacement (data not shown). Once wick and well were in place and sealed with rubber cement, incubations were stopped by injecting 0.4 ml of 4 N H_2SO_4 into the liquid sample to reduce pH to <2 . To collect the respired $^{14}\text{CO}_2$ onto the wick, samples were kept cold in the dark and gently shaken on a shaker table ($\sim 100 \text{ rpm}$) for at least 1 h (or for 12 h without shaking when the shaker table malfunctioned; controls indicated no difference between the two treatments, data not shown). Following the extraction of $^{14}\text{CO}_2$ from the seawater, the Whatman filter paper was transferred to a scintillation vial. CO_2 capture efficiency was determined by adding $\text{NaH}^{14}\text{CO}_3$ to a series of $0.2\text{-}\mu\text{m}$ -filtered, sterile seawater samples, incubating over the same range of temperatures for 12 h in the dark, and extracting $^{14}\text{CO}_2$ as above. The 86.5% average efficiency we measured ($\pm 1.25\%$; $n = 22$) agrees with the 86.5% efficiency previously reported (Thompson and Hamilton 1974) using a similar technique. Extraction efficiency was independent of CO_2 concentration and incubation temperature (data not shown).

The ^{14}C -labeled cells from the remaining (20-ml) seawater sample were then collected onto a $0.2\text{-}\mu\text{m}$ Millipore nitro-cellulose filter by gentle vacuum filtration. The sample was followed by a 40-ml rinse (via the sample bottle itself) of cold, $0.2\text{-}\mu\text{m}$ filtered seawater of the same salinity. The filters were transferred to a scintillation vial; all filters were allowed to air dry before scintillation cocktail (Ecolume) was added. Counts were performed 12 h after addition of cocktail on Beckman scintillation counters, using standard curves and H# corrections to account for quench. Total utilization was determined by adding together the radiolabeled carbon respired (on the Whatman filter; corrected for efficiency) and incorporated (on the Millipore filter), and changed to appropriate units using the specific activity and molecular weight of the substrate. Cell-specific utilization was calculated by dividing the total utilization rate ($\text{mg-AA L}^{-1} \text{ h}^{-1}$) by the bacterial biomass (g-cells L^{-1}) measured for each station and depth.

Data analysis and assumptions—All statistical analyses were performed using SYSTAT 5.2.1 for the Macintosh (SYSTAT 1992). Unless otherwise noted, all statistics are reported with ± 1 standard error ($\pm 1 \text{ SE}$) and the number of samples, n . Multivariate analyses were done for each experiment using analysis of variance (ANOVA) with cell-specific

Table 2. Results of ANOVA test for treatment effects on utilization rate.*

Sta.	<i>n</i>	<i>r</i> ²	<i>P</i> _[A] (substrate)	<i>P</i> _T (tempera- ture)	<i>P</i> _{[A]×T} (combined)
92-25B	35	0.893	<0.001**	0.003**	0.157
92-43B	36	0.736	<0.001**	0.390	0.620
92-49	36	0.883	<0.001**	0.002**	0.195
92-56	35	0.571	0.022*	0.798	0.920
92-64	36	0.912	<0.001**	0.002**	0.039*
93-30	50	0.964	<0.001**	<0.001**	<0.001**
93-43	52	0.810	<0.001**	<0.001**	0.545
93-85	100	0.818	<0.001**	<0.001**	0.289
93-02	90	0.714	<0.001**	0.651	0.930

* Significant effects marked for 99% (**) or 95% (*) confidence levels.

utilization rate, v_s (mg-AA g-cells⁻¹ h⁻¹), as the dependent variable and incubation temperature, T , and added substrate concentration, A (μg AA L⁻¹), as factors. Nonlinear three-dimensional surface fits to the data were done using the distance-weighted least-squares technique and a tension of 1.

To solve for V_{max}^S and K_m at each temperature, nonlinear two-dimensional curve fits to a hyperbolic model equation were made using the SIMPLEX estimation method. The choice of a nonlinear curve-fitting method was made over the standard double-reciprocal Lineweaver–Burke or the Hanes–Wolf plot because of its superior accuracy and precision (Berges et al. 1994). The model equation that we used initially was based on a mass balance for substrate at the cell surface (Pasciak and Gavis 1974; Jumars et al. 1993); solving the full equation allowed us to determine that diffusion limitation was unlikely (Yager 1996). Because the cell-specific uptake rate was slower than the diffusive flux of substrate to the cell surface, the full equation simplified to the more common Michaelis–Menton-type equation that is independent of cell number:

$$v_s = \frac{V_{max}^S C_\infty}{K_m + C_\infty} \quad (1)$$

where C_∞ is the substrate concentration (mg-S L⁻¹) far from the cell surface.

Because we did not have measures of in situ substrate concentration for every station and current measures of DOM do not well reflect bioavailable DOM (Karl 1986), we substituted added substrate concentration, A , for C_∞ . As such, our estimation of the half-saturation concentration, K'_m , equals the true half-saturation coefficient plus the concentration of similar (competing) substrates ($K_m + S$) rather than a true K_m . Although this assumption had little effect on the calculation of V_{max}^S (see discussion of in situ kinetic constants by Button 1994), it meant that we could only compare K'_m values within a single station and not across all stations nor with other published literature. Such relative values were sufficient for our goal to examine treatment (temperature) effects at individual stations.

Standard errors were computed by estimating the Hessian (second derivative) matrix at the end of the SIMPLEX iteration. Differences due to temperature in computed values for V_{max}^S were assessed using planned, two-tailed pairwise t -

tests for equality of samples when variances are assumed unequal (Sokal and Rohlf 1995). We used the Bonferroni method (Sokal and Rohlf 1995) for limiting the experimentwise error rate. For a 95% confidence level per experiment, the critical α equals $0.05 \div 3$ pairwise comparisons = 0.017. For a 99% confidence level per experiment, the critical α equals $0.01 \div 3$ pairwise comparisons = 0.003. Q_{10} values (Segel 1975) were computed from the slope of linear regressions of $\log V_{max}^S$ versus $1/T$ (°K) over the entire incubation temperature range.

We calculated a_s^o by performing a sequence of linear regressions (Sokal and Rohlf 1995) on the A versus v_s curve from each experiment, starting near the origin of each hyperbolic uptake curve and adding data points for higher added substrate concentrations until the slope started to decrease (saturate). Though this was typically a very straightforward process, it could be subjective at stations with noisy data. Our strategy in these cases was to choose the maximum slope attained while achieving a significant fit (r^2). Temperature effects on a_s^o were determined using the test for equality of slopes of several regression lines (Sokal and Rohlf 1995). Pairwise comparisons like those done for V_{max}^S were also performed (although with the conservative Bonferroni correction, they revealed fewer significant differences). Assuming a perfect hyperbolic model, we also calculated $a_s^o = V_{max}^S/K'_m$, but such a calculation compounds errors associated with both V_{max}^S and K'_m estimations (Bevington 1969).

To calculate cell-specific rates and affinities, we had to assume that all of the cells counted were capable of taking up AAs. While this assumption is similar to estimates derived from microautoradiography (90%; Ouverney and Fuhrman 1999), our cell-specific rates and affinities would be underestimates if a significant percentage of the directly counted bacteria were not active. In terms of treatment effects at a given station, where initial sample biomass was the same and differential growth was likely negligible over the short-term incubations (data not shown; Yager 1996), this assumption did not alter interpretation of temperature effects on kinetic parameters.

Results

Substrate availability—Total DCAA concentrations at the near-surface fluorescence maximum ranged from 31 to 143 nM in 1992 (Table 1; 1993 samples were damaged in transit). The individual AAs that dominated the composition were aspartic acid, serine, glycine, and methionine, with glutamic acid, tyrosine, alanine, histidine, and valine sometimes detected (data not shown). No significant correlation ($P > 0.1$) was observed between the concentration of Chl *a*, ranging from 0.13 to 8.22 μg kg⁻¹, and DCAA concentrations for the samples measured. Although the samples we collected for total organic carbon analyses were contaminated, data collected by others aboard *Polarstern* overlapped occasionally with our sampling depths and suggested that DOC and DON concentrations in our 1993 samples ranged from 94 to 108 μM (1.1–1.3 mg C L⁻¹) and 5.1 to 6.4 μM (71–90 μg N L⁻¹), respectively (A. Skoog pers. comm.).

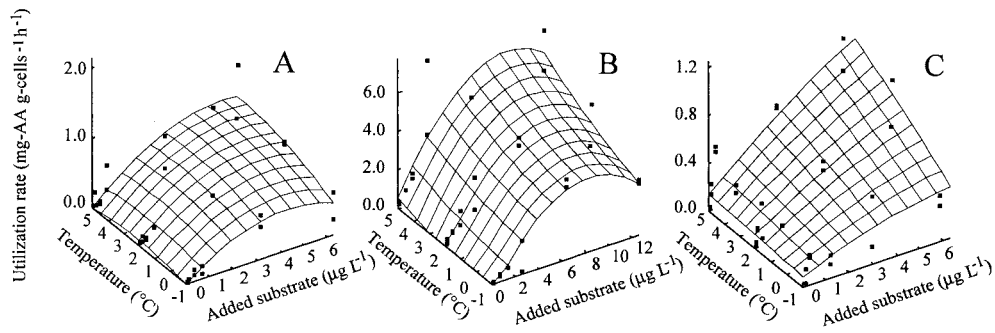


Fig. 3. Distance-weighted, least-squares three-dimensional surface fits (tension = 1) of specific utilization rate (mg-AA g-cell⁻¹ h⁻¹) as a function of added substrate concentration (μg-AA L⁻¹) and temperature (°C) for selected stations chosen to exemplify the three behaviors described in the text according to statistical information in Table 2: (A) substrate alone had a significant effect (Sta. 92-43B); (B) substrate and temperature both had significant effects (Sta. 92-25B); and (C) substrate, temperature, and an interaction between substrate and temperature had significant effects (Sta. 92-64).

Bacterial abundance and cell size—Bacterial abundances measured in our samples ranged from 3.9 to 6.9 × 10⁷ cells L⁻¹ (Table 1). The average bacterial volume in the euphotic zone of the time-series station was 0.126 (±0.003; n = 3,500) μm³. The average cell density was then calculated (Simon and Azam 1989) to be 1.11 g cm⁻³, giving a wet weight of 140 fg cell⁻¹. Communities were dominated by rod-, crescent-, and spiral-shaped cells; noncocoid cells

comprised an average of 70.8% (±3.4%; n = 20 samples) of the cells measured.

Substrate utilization—The ¹⁴C radioactivity signal from Millipore-filter blanks at time zero averaged 110 (±75) dpm, 10% or less of typical values for incorporation of radiolabeled carbon at the lowest added substrate concentration. The signal from Whatman-filter blanks at time zero averaged

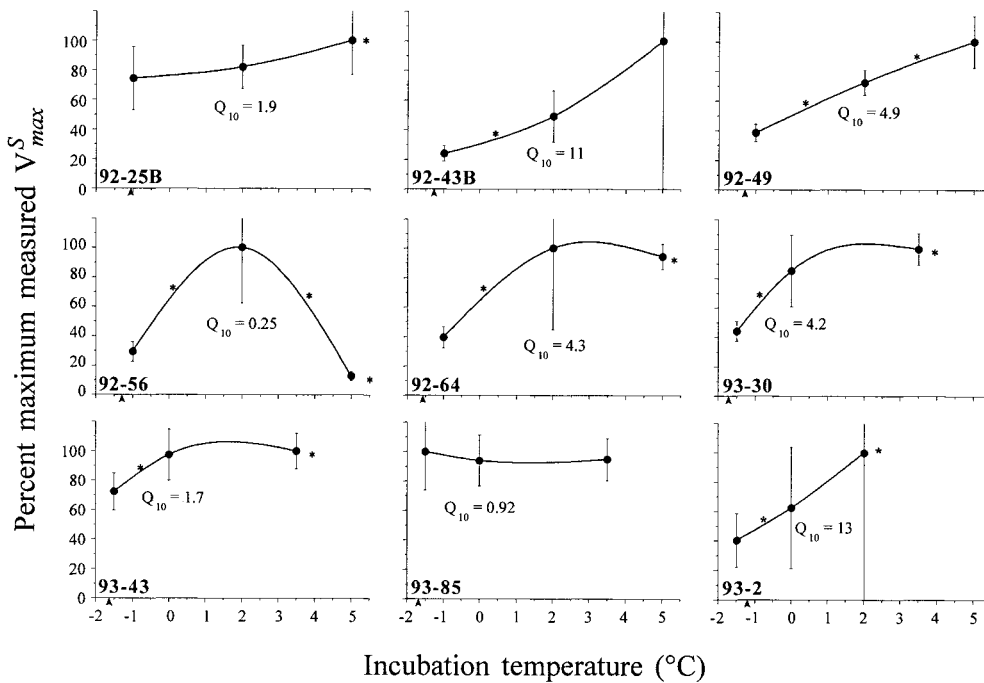


Fig. 4. Temperature dependence of V_{max}^S (mg-AA g-cell⁻¹ h⁻¹), expressed as a percentage of the maximum V_{max}^S measured at each of the nine stations. Error bars indicate ±1 SE (also scaled; see Table 3 for absolute values for V_{max}^S , error, and n). Asterisks along the curve indicate significant differences (**P < 0.01; *P < 0.05) between pairs of points. Asterisks to the right of the warmest temperature indicate significant differences between warmest and coldest incubations. Q₁₀ values were calculated over the entire temperature range, providing an average. Arrowheads on the x-axis indicate in situ temperature at time of sampling.

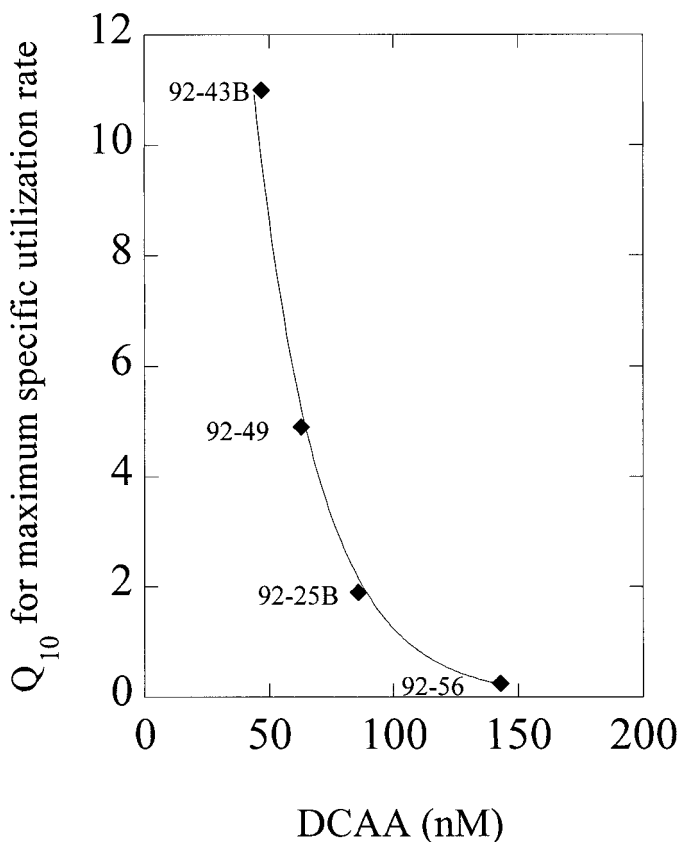


Fig. 5. Q_{10} values for V_{max}^S compared with in situ concentrations of DCAA: Q_{10} values were best explained by an exponential curve fit ($r^2 = 0.990$), with temperature sensitivity becoming more psychrophilic as DCAA concentrations increased.

65 (± 36) dpm, 30% or less of measured values for respiration at the lowest added substrate concentration. One disintegration per minute translated to about 0.1 pmol AA L^{-1} (5 pg C L^{-1}). Time-zero samples and formaldehyde-killed controls did not differ significantly from each other (Fig. 2), indicating no significant abiotic adsorption of isotope. Linearity in the time-course experiments was typically observed up to about 16 h (Fig. 2). An incubation time of about 12 h, therefore, was used for subsequent endpoint experiments.

In all nine endpoint kinetic experiments analyzed using ANOVA (Table 2), added substrate concentration had a significant effect on utilization rate ($P_{\text{substrate}} < 0.001$, except for one station where it was slightly higher but still significant at $P_{\text{substrate}} = 0.022$). Temperature had a significant ($P_{\text{temp}} < 0.05$) effect at six of the nine stations but had a significant combined effect with substrate concentration ($P_{\text{combined}} < 0.05$) at only two of the nine stations. On average, added substrate concentration and temperature explained 81.1% ($\pm 4.05\%$; $n = 9$) of the variance seen in these experiments.

Kinetics—All of the pelagic communities sampled showed rapid increases in utilization rate at low added AA concentrations and approached saturation at higher added AA concentrations (Fig. 3), allowing good curve fits (r^2 averaged 0.73 ± 0.11 ; $n = 27$; Table 3) to the Michaelis-

Menton uptake model and calculation of the kinetic parameters V_{max}^S , K_m' , and a_s^o .

V_{max}^S values ranged from 0.367 to 4.51 mg-AA $g\text{-cell}^{-1} h^{-1}$ at subzero temperatures and were as high as 6.61 mg-AA $g\text{-cell}^{-1} h^{-1}$ when warmed to 5°C (Table 3). Unscaled community V_{max} (heterotrophic potential) values ranged from 21.4 to 349 pmol-AA $L^{-1} h^{-1}$ at subzero temperatures, in keeping with similarly wide ranges from other temperate and polar seas (Morita 1975; Griffiths et al. 1978; Suttle et al. 1991). Temperature effects on V_{max}^S were mixed (Fig. 4), with Q_{10} values ranging from 0.25 to 13. Most stations showed either a monotonic increase or a step up in V_{max}^S with increased incubation temperature. One station (92-56) to the far northeast, however, showed extremely psychrophilic behavior with a strong maximum at 2°C and minimum at 5°C. Another station (93-85) showed no difference in V_{max}^S due to temperature. Note that these temperature effects are independent of biomass scaling. Temperature response, as measured by Q_{10} , corresponded well with DCAA concentrations (Fig. 5) for the four stations where both kinetics and AA data were available. There were no significant correlations ($P > 0.05$), however, between Q_{10} and any of the other estimates of organic matter quantity or quality (Chl *a*, phaeopigments, POC, PON, nitrate concentrations, or their ratios).

K_m' (or $K_m + S$), ranged from 0.299 to 11.0 μg AA L^{-1} (or 2.32–82.5 nM AA) at subzero temperatures and tended to have large associated standard errors (Table 3), preventing detection of temperature sensitivity in most cases.

a_s^o ranged from 287 to 1,830 L $g\text{-cells}^{-1} h^{-1}$ at subzero temperatures or up to 4,080 L $g\text{-cells}^{-1} h^{-1}$ when warmed to 2°C. The response of a_s^o to temperature was not uniform across the stations sampled (Table 4; Fig. 6). Five of the nine stations showed significant temperature effects on specific affinity. Only three stations from the central polynya region showed the expected increase in a_s^o with warming, with Q_{10} values ranging from 3 to 5. Two stations from the northeastern region showed extremely psychrophilic behavior (as well as the highest affinities observed in this study) with maximum affinities at either -1 or 2°C that decreased with warming to 5°C. Four stations exhibited no significant affinity response to warming. Note that these temperature effects are independent of biomass scaling. Q_{10} values, ranging from 0.23 to 6.1, correlated significantly ($P < 0.05$) with the C/N ratio of the particulate matter in the same sample (Fig. 7), but showed no significant relationship ($P > 0.05$) to any of the other estimates of organic matter quantity or quality (Chl *a*, phaeopigments, POC, PON, DCAA, nitrate concentrations, or other ratios). The mean computed value for an oligotrophic capacity index ($\log_{10}[a_s^o/K_m']$; Button 1994) was $8.5 (\pm 0.73; n = 9)$ for subzero temperatures. Comparisons between values for a_s^o determined by our linear regression technique and a straight division of V_{max}^S/K_m' suggested that our method may underestimate the absolute value of a_s^o by about 25%. The error estimates for the calculation, however, propagating standard errors for V_{max}^S and (particularly) K_m' tended to be about 10 times higher than those from the linear regression technique.

Table 3. Temperature effects on maximum specific utilization rate and Michaelis constant.

Sta.	Incubation temperature (°C)	<i>n</i>	V_{max}^S (mg-AA g-cell ⁻¹ h ⁻¹) (±1 SE)	K'_m (μg-AA L ⁻¹) (±1 SE)	r^{2*}
92-25B	-1.0	11	4.51 (0.903)	3.70 (1.85)	0.817**
	+2.0	12	5.42 (0.977)	3.43 (1.56)	0.823**
	+5.0	12	6.61 (1.51)	2.34 (1.51)	0.673**
92-43B	-1.0	12	0.597 (0.130)	1.28 (0.819)	0.724**
	+2.0	12	1.22 (0.429)	1.94 (1.76)	0.618**
	+5.0	12	2.48 (4.77)	9.98 (28.9)	0.656**
92-49	-1.0	12	2.20 (0.336)	0.978 (0.475)	0.821**
	+2.0	12	4.14 (0.481)	0.509 (0.203)	0.816**
	+5.0	12	5.72 (1.00)	1.41 (0.714)	0.825**
92-56	-1.0	12	1.79 (0.419)	0.821 (0.595)	0.617**
	+2.0	10	6.11 (2.29)	3.28 (2.79)	0.743**
	+5.0	11	0.774 (0.187)	0.393 (0.295)	0.514*
92-64	-1.0	10	0.367 (0.067)	0.290 (0.199)	0.620**
	+2.0	10	0.934 (0.515)	1.56 (2.96)	0.807**
	+5.0	10	0.881 (0.081)	0.371 (0.134)	0.887**
93-30	-1.5	14	2.46 (0.378)	3.14 (1.28)	0.820**
	+0.0	15	4.75 (1.35)	9.36 (5.25)	0.856**
	+3.5	15	5.59 (0.597)	4.03 (1.05)	0.922**
93-43	-1.5	18	1.62 (0.285)	2.95 (1.43)	0.762**
	+0.0	16	2.19 (0.392)	3.90 (1.71)	0.787**
	+3.5	18	2.25 (0.272)	1.22 (0.501)	0.710**
93-85	-1.5	32	5.07 (1.32)	11.0 (5.06)	0.794**
	+0.0	34	4.75 (0.880)	7.10 (2.70)	0.801**
	+3.5	34	4.81 (0.724)	4.64 (1.65)	0.757**
93-02	-1.5	30	1.79 (0.820)	6.68 (5.05)	0.619**
	+0.0	30	2.78 (1.87)	8.00 (8.45)	0.513**
	+2.0	29	4.44 (5.33)	15.5 (28.6)	0.527**

* Testing for significance of regression; marked at 99% (**) or 95% (*) confidence level.

Discussion

Although they are community averages, values for the critical kinetic parameter of specific affinity measured in our study compared well with (and frequently exceeded) those reported by Button (1994, 1998), suggesting that the microorganisms in the polynya region were ready and able to respond to small increases in substrate availability, even at subzero temperatures. Responsiveness was further confirmed by high oligotrophic capacities that overlapped with and exceeded those reported for pure cultures of oligotrophs and did not differ significantly from the value (9.0; Button 1994) described for organisms well-adapted for survival in the pelagic ocean. Since our study, high turnover rates of AAs at subzero temperatures have been detected in other regions of the Arctic (Rich et al. 1997). Note that the acidification process that we used to extract ¹⁴CO₂ also disassociates loosely bound substrate (Griffiths et al. 1974*a,b*; Connelly and Yager in prep.). Thus, our measured specific affinities reflect the ability of an in situ microbial community to respond to and utilize low levels of additional substrate rather than the true affinity of an enzyme-substrate complex (Segel 1975). Given that all of the assumptions required to calculate a_s^o (all cells active, A equivalent to $A + S$, filtration after acidifi-

Table 4. Temperature effects on specific affinity.

Sta.	Incubation temperature (°C)	<i>n</i>	a_s^o (L g-cells ⁻¹ h ⁻¹) (±1 SE)	r^{2*}	Affinities equal (<i>P</i>)†
92-25B	-1.0	9	672 (24.5)	0.992**	<0.001**
	+2.0	8	867 (26.6)	0.728**	
	+5.0	7	1,325 (112)	0.978**	
92-43B	-1.0	6	405 (33.9)	0.779*	0.127
	+2.0	10	324 (13.9)	0.632**	
	+5.0	7	630 (188)	0.614*	
92-49	-1.0	8	1,813 (188)	0.902**	0.002**
	+2.0	8	4,079 (282)	0.904**	
	+5.0	6	2,843 (242)	0.909**	
92-56	-1.0	8	1,831 (372)	0.916**	0.029*
	+2.0	8	787 (48.3)	0.951**	
	+5.0	8	868 (95.9)	0.875**	
92-64	-1.0	6	858 (43.4)	0.879**	0.026*
	+2.0	5	1,257 (218)	0.964**	
	+5.0	6	1,868 (133)	0.973**	
93-30	-1.5	9	436 (112)	0.554*	<0.038*
	+0.0	6	1,361 (71.4)	0.994**	
	+3.5	9	1,263 (189)	0.954**	
93-43	-1.5	9	585 (198)	0.804**	0.170
	+0.0	9	373 (106)	0.786**	
	+3.5	9	973 (177)	0.796**	
93-85	-1.5	12	355 (27.0)	0.428*	0.298
	+0.0	12	520 (107)	0.539**	
	+3.5	12	645 (176)	0.465*	
93-02	-1.5	9	287 (22.2)	0.958**	0.080
	+0.0	9	350 (53.0)	0.696**	
	+2.0	9	558 (77.0)	0.956**	

* Testing for significance of linear regression; marked at 99% (**) or 95% (*) confidence level.

† Testing for equality of slopes; significant differences marked at 99% (**) or 95% (*) confidence level.

cation capturing all bound substrate) might tend to underestimate its absolute value, NEW Polynya microbial communities, on average, clearly showed a strong response to small increases in the concentration of mixed AAs.

Perhaps because of these high specific affinities, the measured in situ AA concentrations were fairly low compared to some other arctic regions (Rich et al. 1997) and temperate oceans (Andrews and Williams 1971), though their composition was typical for marine waters (e.g., Hubberten et al. 1994). Although a small buildup of DOC (23 μM) was observed in the NEW polynya over the early summer of 1993 (Daly et al. 1999), no coincident buildup of DON occurred, despite potentially significant DON excretion by local zooplankton (Daly et al. 1999). These low DON concentrations may provide further evidence to support high microbial affinities for any nitrogen-rich substrate that becomes available, although quantitative conclusions await better understanding of DON production rates in this region.

The effects of temperature on both a_s^o and V_{max}^S provided mixed support for our working hypotheses. The generally significant effects of short-term warming on V_{max}^S were contrary to our initial prediction (based on the results of pub-

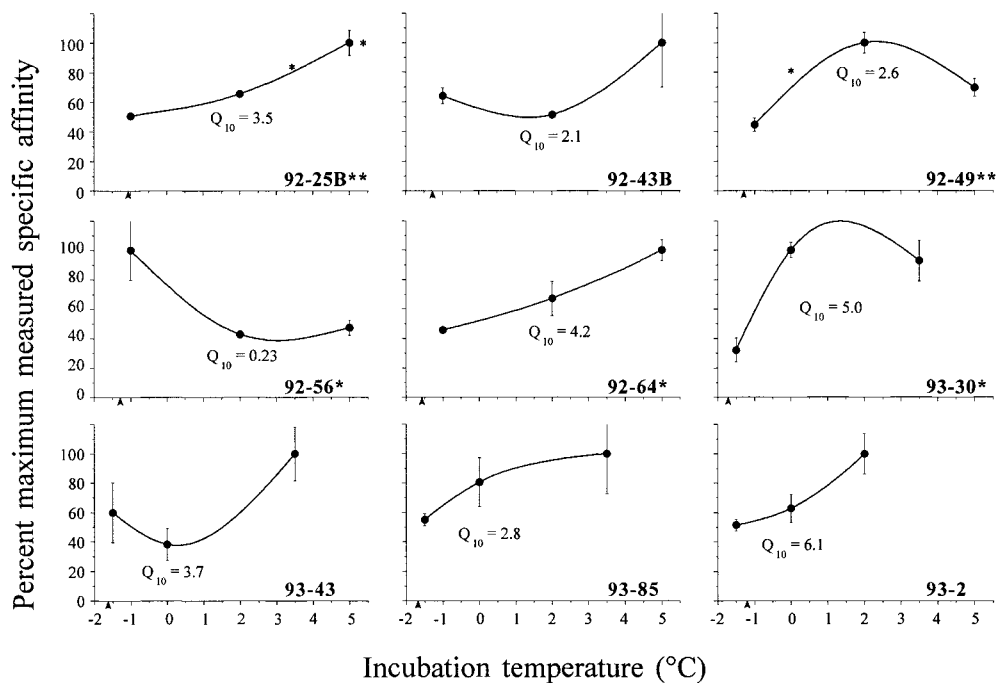


Fig. 6. Temperature dependence of a_s^o ($L\ g\text{-cells}^{-1}\ h^{-1}$) expressed as a percentage of the maximum a_s^o measured at each of the nine stations. Error bars indicate ± 1 SE (also scaled; see Table 4 for absolute values for a_s^o , SE, and n). Asterisks along and after each curve indicate significant overall temperature differences determined by the test for equivalence of slopes. Q_{10} values were calculated over the entire temperature range, providing an average. Arrowheads on the x-axis indicate in situ temperature at time of sampling.

lished growth studies and respiration rate measurements) that V_{max}^S would not vary much with temperature increases to only 5°C . These temperature effects appear to be related to in situ substrate concentrations, as shown by the relationship between Q_{10} and DCAA concentration (Fig. 5). As in situ substrate concentration increased, the temperature sensitivity of V_{max}^S decreased, indicating a shift to cold adaptation. Whether this shift occurred because of metabolic changes in otherwise similar microbial communities, or community succession from psychrotolerant to psychrophilic microorganisms, remains to be investigated. We found provisional support for both explanations: for the former in the observation of polynya Q_{10} values much greater than the diffusion coefficient Q_{10} of 1.4 (Jumars et al. 1993), for the latter, in the presence of an extremely psychrophilic community at Sta. 92-56.

Temperature effects on a_s^o were less significant than our literature-based hypotheses had predicted, with nearly half of the stations showing no significant increase due to short-term warming. Of the five stations that did exhibit significant temperature effects, two revealed reduced a_s^o at warmer temperatures, contrary to the current paradigm. These stations provided clear evidence for the dominance of extremely psychrophilic microbial communities. The remaining three stations that showed the expected increase in a_s^o with warming were all in the central or western region of the polynya, perhaps indicating the importance of hydrographic residence time on community structure (see below).

The observed relationship between Q_{10} for a_s^o and the C/N

ratio of POM suggests that cold-adapted populations associated with particles are dependent on a supply of N-rich organic matter. Q_{10} values for extracellular enzyme activity, particularly peptidase activity (releasing N-rich compounds), in the same polynya surface waters (Vetter and Deming 1994) are very similar to those we found for a_s^o and also include psychrophilic behavior. The temperature sensitivity of substrate uptake by microbial communities may thus be linked to an attached lifestyle (on particles) via extracellular enzyme activities. Direct tests of this prediction are underway in the nearby Northwater Polynya.

The absence of a significant correlation ($P > 0.05$) between Q_{10} values for V_{max}^S and a_s^o suggests a decoupling of these two processes or the mechanisms controlling the temperature sensitivity of each parameter. Some similarities emerged, however, when stations were grouped geographically. Similar response curves with consistently increasing values were observed for the two kinetic parameters at the time-series (TS) stations and Sta. 92-64 in the central, mostly open-water region where water mass residence times were longer (Top et al. 1997) and the cumulative radiative flux greater by the time of our sampling (Minnett et al. 1997) compared to other stations. In contrast, strongly psychrophilic behavior reflected in both a_s^o and V_{max}^S was observed at those stations in the far northeast corner of the study region more recently influenced by Arctic Ocean source waters (Wallace et al. 1995). A nearby northeastern station sampled earlier in the summer of the following year (93-85) showed

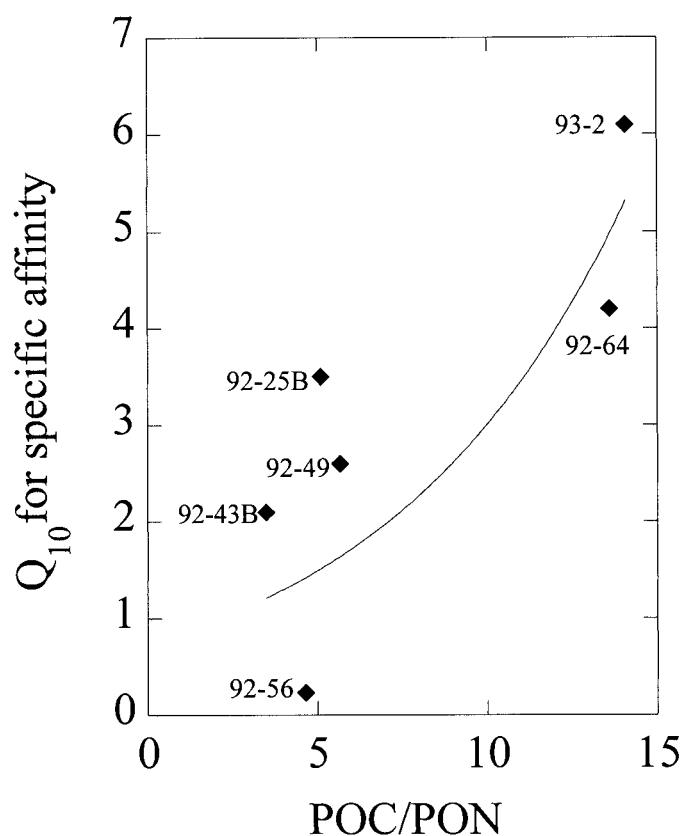


Fig. 7. Q_{10} values for a_s^o compared with the C/N (molar) ratio of POM: Q_{10} values show a weak but significant correlation ($P < 0.05$) with POC/PON (the exponential fit shown was only slightly better, $r^2 = 0.664$, than a linear fit, $r^2 = 0.661$, to the data).

no significant response to warming in either parameter, also in keeping with the presence of psychrophiles.

Comparisons between the rate of microbial community uptake of the AA mixture used in this study to the same concentration (10 nM) of a single AA (glutamic acid) or of glucose (Yager 1996; Yager and Deming in prep.) suggested that the AA mixture tended to be utilized more rapidly than glucose, which was used more rapidly than glutamic acid. Communities may show more rapid utilization of mixtures if different organisms in the community have different substrate preferences (niche theory), but membrane responses by pure cultures to mixed substrates also tend to be more rapid than responses to single substrates (*see* discussion by Button 1994). While the mechanistic kinetic interpretation for substrate mixtures may be more complicated than for single substrates, individual AAs are unlikely to be present in the marine environment in the absence of other AAs. By using a mixture of AAs that mimics the composition of phytoplankton exudate and pelagic bacteria, we expected the results to reflect in situ microbial community responses better than if we had used a single AA. Complex substrates had also been used in most of the previous experiments that led to the concept of an enhanced substrate requirement at low temperature.

Our approach of examining microbial substrate utilization only over short-term warming periods and a short range of

temperatures was also consistent with the goal to detect cold-adaptive characteristics of the in situ microbial community in the absence of a shift in community structure (Jumars et al. 1993).

About 19% of the variance in our results was not explained by temperature or substrate additions and may have been caused by unknown differential presence of grazers, because we did not prefilter the samples. Given our focus of temperature effects on substrate uptake, we opted not to prefilter because of the potentially dramatic and variable effect that filtration of large volumes of seawater can have on the DOM pool available to bacteria in the filtrate (Fuhrman and Bell 1985). Because our measures of kinetic parameters were averages for the entire microbial community, some of the remaining variation could also be explained by microbial diversity.

Conclusions

Polar seas are characterized by a highly seasonal food supply but perennially cold temperatures. In the NEW Polynya region, phytoplankton productivity and biomass have been described as a mosaic in terms of spatial and temporal heterogeneity (Smith et al. 1995). The most competitive heterotrophic bacterium in such an environment would be expected to have constitutive, high-affinity transport systems but metabolic rates sensitive to available substrate concentrations. On the basis of previous research by others, showing an enhanced substrate requirement by bacteria living at low temperatures, we had hypothesized that specific affinity would increase with warming and that the maximum specific uptake rate at saturating concentrations would be less sensitive to temperature. Instead, we have results from some stations that support these hypotheses and results from others that do not.

Most notably, pelagic microorganisms in the NEW Polynya exhibited high specific affinities for mixed AAs, even at subzero temperatures, indicating the capacity for responsiveness to pulses of N-rich substrate in this perennially cold environment. With respect to short-term temperature responses in the bacterioplankton, we observed heterogeneity. Extremely psychrophilic behavior occurred in the northeast quadrant, perhaps reflecting influence from the Arctic Ocean outflow and higher levels of available organic nitrogen. Temperature limitation was observed only in the central polynya regions, where residence times of water masses are longer and surface waters are warmer later in the season. Whether this geographical pattern of cold-adaptation represents metabolic changes in otherwise similar microbial communities or community succession in the polynya from psychrotolerant to more psychrophilic microorganisms remains to be investigated.

What is clear is that temperature–substrate interactions in perennially cold regions cannot be described by a single mode. Prediction for arctic ecosystems now becomes more challenging without a closer examination of the variation and controls on microbial community structure.

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