Rapid Enhancement of Heterotrophic but not Photosynthetic Activities in Arctic Microbial Plankton at Mesobiotic Temperatures

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Summary. Marine microbial plankton from eastern Canadian Arctic waters have the remarkable ability of rapidly accumulating nutrients (amino acids, glucose, phosphate) at mesobiotic temperatures (≈30 °C) after an acclimation period of a few days. Curves of the nutrient uptake response to temperature established before and after an 8 h acclimation period at 30 °C showed that maximum uptake rates attained much higher values and were established at much higher temperatures after the acclimation period. This enhancement was inhibited by chloramphenicol (but not cycloheximide) and occurred even when the possibility of bacterial enhancement due to animal and plant death was reduced by prior removal of organisms using a screen of 1 μm nominal pore diameter. The enhancement at high temperatures was evidenced only in heterotrophic but not in photosynthetic activities and was concomitant with marked increases in the apparent activity of microbial DNA synthesis and bacterial cell density. The enhancement may have been due to a rapid and opportunistic growth of presumed non-psychrophilic members of the bacterioplankton.

Introduction

In waters that undergo seasonal variations in temperature, there is generally a selection of bacteria according to thermal types by water temperature (Sieburth 1967; Nedwell and Floodgate 1971; Ferroni and Kaminski 1980; Romanenko 1982; Gow and Mills 1984). On the other hand, the polar seas are persistently cold and are therefore a natural habitat for psychrophilic bacteria (Morita 1975). However, based on studies such as those of McDonald et al. (1963); Wiebe and Hendricks (1974), and Kaneko et al. (1979), Baross and Morita (1978) emphasized that "it is an oversimplification to say that only psychrophiles occur in stable cold environments, because most of the microorganisms isolated from permanently cold areas are psychrotrophic". In fact, mesophilic and thermophilic bacteria have also been isolated from arctic waters (McBee and McBee 1956). Notwithstanding a polar flora comprised of various thermal types, it might be expected that much of the heterotrophic activity in the polar seas will be due to psychrophiles since, in cultures at least, they grow faster than psychrophots at low temperatures (Harder and Veldkamp 1971; Morita and Burton 1970).

During a recent cruise to the eastern Canadian subarctic and arctic where water temperature ranged from about −1.5 to 8.0 °C, we established temperature response curves for the uptake of tritiated organic substrates by size-fractionated microbial plankton using a temperature gradient incubator. The wide temperature range and the fine resolution within this range afforded by the incubator allowed us to detect multiple optima in the temperature response curves. In many instances, peaks in activity occurred above 15 °C, a widely-accepted temperature above which peaks in activity are attributed to non-psychrophiles (Morita 1975). We report here that at mesobiotic temperatures (20° to 45 °C, Aragno 1981), arctic microbial plankton exhibit a rapid (h) enhancement in heterotrophic, but not photosynthetic, activity and that there is an associated increase in bacterial cell numbers.

Materials and Methods

Experiments were conducted from July to September, 1983 on board CS Hudson (cruise 83-023) at numerous sites in the eastern Canadian subarctic (Labrador Sea, Davis Strait) and arctic (Baffin Bay, Lancaster Sound, Jones Sound). Seawater was collected with a pump sampler system as in previous work (Li 1984).

The uptake rate of various radiolabelled substrates was measured as a function of temperature (temperature response curve) with a temperature gradient incubator (Model 675, Scientific Industries Inc., Bohemia, N.Y.). Seawater was measured into a glass bottle and received either D-[6-3H(N)]-glucose (31.1 Ci mmol⁻¹, NET-100A, New England Nuclear Corp. [NEN]; final activity of about 0.05 μCi ml⁻¹ equivalent to 1.6 nM added glucose), a [3H]-labelled-L-amino acid mixture
(23.0 Ci mmol⁻¹, NET-250, NEN; final activity of about 0.05 μCi ml⁻¹ equivalent to 2.2 nM added amino acids). [³²P orthophosphoric acid (carrier-free, NEZ 112, NEN; final activity of about 0.2 μCi ml⁻¹), or [methyl-³¹P]-dioxothymidine 5'-triphosphate, tetrasodium salt (75.7 Ci mmol⁻¹, NET-221X, NEN; final activity of about 0.02 μCi ml⁻¹ equivalent to 0.26 nM added TTP). Subsamples (15.0 ml) of radiolabelled seawater were measured (Dissenette, Brinkman Instruments, Inc., Westbury, N.Y.) into each of 30 glass tubes in the temperature gradient block. The temperature gradient maintained within the block was usually from about -2 to 38°C - the gradient consisted of 30 temperatures.

Additionally, the incubator was modified to allow simultaneous measurement of light-dependent [¹⁴C] bicarbonate uptake (photosynthesis) as a function of temperature. A pair of cool-white fluorescent lamps were mounted about 5 cm above the recessed compartments on the top of the temperature block. These lights provided about 65 to 90 W m⁻² incident PAR, a range over which the irradiance is known to be saturating for the rate of photosynthesis in arctic phytoplankton (Platt et al. 1982; Harrison and Platt 1983). Subsamples (1.0 ml) of seawater radiolabelled with sodium [¹⁴C] bicarbonate (47.1 mCi mmol⁻¹, NEC-086H, NEN; final activity of about 5 to 50 μCi ml⁻¹) were measured (Eppendorf Repeater 4780, Brinkman Instruments, Inc.) into each of 40 optically-clear, plastic incubation chambers (No. 4804 Tissue Culture Chamber/Slide, Lab-Tek Products, Naperville, Ill.) placed end-to-end in the recessed compartments of the temperature block. Radiolabelled samples were incubated in the temperature gradient for 2 h.

The development of enhanced heterotrophic activity and growth at 30°C was investigated in time course experiments involving sudden transitions of temperature (temperature shift experiments). Seawater was measured into darkened polycarbonate bottles (Naige) and the time course of substrate uptake was initiated by introduction of the various radioisotopes at the activity levels used in the temperature gradient response experiments. Samples were shifted to different temperatures by allocating subsamples to different polycarbonate bottles equilibrated to the new temperatures in water-baths (0.0, 14.0, and 30.0°C). The time course of the response was followed by frequent subsampling (20.0 ml) over 24 h. All glassware and plasticware used in sample-handling was acid-washed, rinsed with deionized, distilled water and rinsed with seawater sample before use.

In all experiments, radiolabelled plankton were collected by vacuum filtration onto Nuclepore membranes. Rapid post-incubation processing of samples was made possible by using three filtration manifolds, each capable of handling 12 samples simultaneously (1225 Sampling Manifold, Millipore Corp., Bedford, Mass.). Each sample was divided into two portions which were then filtered separately onto 1.0 μm and 0.2 μm Nuclepore membranes. We (Li et al. 1983; Li 1984) have previously used this protocol to separate picoplankton from microplankton. We operationally define microplankton (an abbreviation for the nanoplankton and microplankton terminology of Sieburth et al. 1978) as those cells retained on 1.0 μm filters and picoplankton as those which pass 1.0 μm filters but are retained on 0.2 μm filters. In this parallel filtration protocol, activity due to picoplankton is calculated as the difference between activities retained on 0.2 μm and 1.0 μm filters.

Filters on which plankton labelled with [³¹H] TTP were collected were extracted with 5.0 ml of 5% trichloroacetic acid (TCA) in an ice-bath for 30 min. One mg of deoxyribonucleic acid was added to each sample to promote precipitation of TCA-insoluble materials (Karl et al. 1981). The samples were then filtered onto another 0.2 μm Nuclepore membrane, washed with 5.0 ml of 5% TCA and rinsed with ethanol. Details of subsequent steps in the protocol employed to process filters in preparation for liquid scintillation counting have been published (Li 1984).

Bacteria were enumerated in triplicate samples preserved with filtered (Milllex-GS, Millipore Corp.) formaldehyde (2% final concentration). Cells were stained for 10 min with 4'-6-diamidino-2-phenylindole (DAPI) (Porter and Feig 1980; Roberts and Sephton 1981) at a final concentration of 1 μg ml⁻¹. For each of the three replicates, triplicate subsamples were filtered onto 0.2 μm Nuclepore membranes counterstained with Irgalan Black dye (2 g l⁻¹). The filters were inspected at a magnification of 1250 x under a Wild Leitz Orthoplan microscope. Typically, we counted a least 450 cells from each filter. Solution blanks were checked frequently throughout the cell enumeration procedure.

**Results**

**Heterotrophic Activity versus Temperature**

We present examples of temperature response curves for microbial uptake of [³¹H] glucose (Figs. 1 and 2), [³¹H]-labelled amino acids (Figs. 3 and 4A), and [³²PO₄⁻³] (Fig. 5A). First, in each case, there was significant activity due to both pico- and microplankton at in situ temperatures. Second, in many cases, multiple peaks in activity were evident and some of these were at temperatures above 15°C.

The temperature response changed markedly when radiolabel uptake was initiated after an 8 h period during which the seawater sample was held at 30°C in a water bath. First, the uptake of both [³¹H]-labelled amino acids (Fig. 4B) and [³²PO₄⁻³] (Fig. 5B) were already completely eliminated in situ temperatures. Second, the peaks in activity occurred at 30°C (except in the case of [³²PO₄⁻³]-uptake by microplankton: an original peak at 20°C remained but the other original peak at 11°C gave way to a new peak at 35°C). Third, the uptake rates in the

![Fig. 1.](attachment:image.png)
Fig. 2. $[^3]$H glucose uptake rate as a function of temperature in an arctic microbial assemblage. Symbols as described in Fig. 1. The sample was collected on Sept. 3, 1983, from 15 m at 74°45’ N, 70°48’ W where bacterial density was $7.14 \pm 0.96 \times 10^5$ cell ml$^{-1}$.

Fig. 3. $[^3]$H-labelled amino acids uptake rate as a function of temperature in a subarctic microbial assemblage. Symbols as described in Fig. 1. The sample was collected on Aug. 4, 1983, from 5 m at 53°21.7’ N, 54°25.7’ W where bacterial density was $5.37 \pm 1.06 \times 10^5$ cell ml$^{-1}$.

Fig. 4A, B. $[^3]$H-labelled amino acids uptake rate as a function of temperature in an arctic microbial assemblage. Symbols as described in Fig. 1. A The sample was collected on Sept. 6, 1983, from 25 m at 72°11.8’ N, 65°42’ W where bacterial density was $8.19 \pm 2.56 \times 10^5$ cell ml$^{-1}$ and temperature was $-1.4^\circ$C. B The same sample held at 30°C for 8 h before incubation in temperature gradient with radioisotope. Note the different ordinate scales in (A) and (B); the inset compares >0.2 μm plankton activity on the same scale.

Mesobiotic temperature range were very much higher: the insets in Figs. 4B and 5B compare responses of microbial plankton (>0.2 μm), on the same ordinate scale, before and after the 8 h holding period at 30°C. We also measured the rate of incorporation (over 2 h) of $[^3]$H TTP into TCA-insoluble material in plankton that had been held at 30°C for 8 h (Fig. 6). Unfortunately, we do not have the temperature response of $[^3]$H TTP incorporation in the same sample not heat-treated. Nevertheless, Fig. 6 shows almost no incorporation at low temperatures; rates of incorporation were highest around 30° to 32°C for both picoplankton and microplankton. These results therefore suggest that at mesobiotic temperatures, there is active synthesis of DNA. The presumption of subsequent cell division was shown to be true in an experiment to be described below (Table 1).

**Photosynthetic Activity versus Temperature**

We present examples of temperature response curves for microbial uptake of $^{14}$HCO$_3^-$ in the light (Figs. 7–9A).
Table 1. Bacterial cell numbers after 25 h incubation under seven different temperature schedules

<table>
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<th>Schedule no.</th>
<th>Temperature (°C) at time t (h)</th>
<th>$N_{25}$ b</th>
<th>$N_{25}/N_0$ c</th>
</tr>
</thead>
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<td>t = 2</td>
<td>t = 10</td>
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</tr>
<tr>
<td>7</td>
<td>0</td>
<td>30</td>
<td>14</td>
</tr>
</tbody>
</table>

a Sample was collected on Sept. 13, 1983 from 10 m at 76°04.5’N, 82°12’W where water temperature was −1.0°C.  
b $10^6$ cells ml$^{-1}$ (± SD) at t = 25 h  
c Initial no. of cells, $N_0 = 3.43 ± 0.75 \times 10^5$ cells ml$^{-1}$

Due to mechanical problems with the incubator, the lowest temperatures during measurements of photosynthesis were always a few degrees above in situ temperatures. However, previous work in these waters (Li et al. 1984) included measurements at in situ temperatures and it was found that light-saturated rates of photosynthesis obey Arrhenius kinetics in the suboptimal temperature range. In the temperature range of at least 5°C above ambient to 5°C below ambient, the apparent Arrhenius activation energy for photosynthesis was 16.9 ± 0.85 kcal mol$^{-1}$ for arctic phytoplankton and 16.0 ± 0.64 kcal mol$^{-1}$ for subarctic phytoplankton (Li et al. 1984). The present data show photosynthetic rate optima at about 20°C for subarctic phytoplankton (Fig. 7) and at about 12°C (Fig. 9A) to 15°C (Fig. 8) for arctic phytoplankton. The contribution of picoplankton to overall photosynthesis varied from negligible (Fig. 9A), to significant within a narrow temperature range (Fig. 8), to substantial over the entire range of temperature tested (Fig. 7).

The temperature response changed markedly when radiolabel uptake was initiated after an 8 h period during which the seawater sample was held at 30°C in a water bath (Fig. 9B). First, the uptake of H$^3$CO$_2$ was apparently almost completely eliminated at low temperatures. Second, the peaks in activity presumably occurred at or above 30°C. Third, most significantly, the uptake rates were very low: the inset in Fig. 9B compares responses of the phytoplankton (>0.2 μm), on the same ordinate scale, before and after the 8 h holding period at 30°C.

**Characterization of Heterotrophic Enhancement at 30°C**

The time course for the development of enhanced heterotrophic activity at 30°C was studied in temperature shift experiments. Radiolabelled plankton samples were incubated at 0°C for 2 h and then shifted to 30°C. Immediately, there was a cessation in the accumulation of H$^3$H-labelled amino acids in picoplankton (Fig. 10A) and mi-
Fig. 7. Rate of $^{14}$CO$_3^-$ uptake in the light as a function of temperature in an arctic microbial assemblage. Symbols as described in Fig. 1. The sample was collected on Aug. 10, 1983, from 10 m at 64°09.4' N, 57°12.6' W.

Fig. 8. Rate of $^{14}$CO$_3^-$ uptake in the light as a function of temperature in an arctic microbial assemblage. Symbols as described in Fig. 1. The sample was collected on Aug. 23, 1983, from 27 m at 74°07' N, 81°54' W.

Fig. 9. A, B. Rate of $^{14}$CO$_3^-$ uptake in the light as a function of temperature in an arctic microbial assemblage. Symbols as described in Fig. 1. A The sample was collected on Sept. 10, 1983, from 27 m at 72°03.6' N, 68°18.4' W where temperature was -1.1°C. B The same sample held at 30°C for 8 h before incubation in the temperature gradient with the isotope. Note the different ordinate scales in (A) and (B); the inset compares >0.2 μm plankton activity on the same scale.

croplankton (Fig. 10B). of $^3$H glucose in picoplankton (Fig. 11A) and microplankton (Fig. 11B), and of $^{32}$PO$_4^{3-}$ in picoplankton (Fig. 12A). The accumulation of $^{32}$PO$_4^{3-}$ in microplankton (Fig. 12B) increased. After 6–12 h at 30°C, there was rapid accumulation of radioactivity in all cases (Figs. 10A, B; 11A, B; 12A, B), in accord with the results from the temperature gradient experiments performed on plankton collected at 30°C for 8 h (Figs. 4B, 5B).

In the time course experiments, a second temperature shift was imposed 8 h after the initial shift from 0°C to 30°C: subsamples were secondarily shifted from 30°C to either 14°C or 0°C. At 0°C, no further accumulation of radioactivity was evident in most cases (Figs. 10A, B; 11B; 12B). At 14°C, there was substantial accumulation in all cases (Figs. 10A, B; 11A, B; 12A, B).
Fig. 10. Accumulation of \(^3\)H-labelled amino acids in (A) picoplankton and (B) microplankton during temperature shifts. The first shift was from 0° (○) to 30°C (○) at 2 h. The second shift was from 30° to either 0° or 14°C (△) at 10 h; one subsample did not experience a second shift and was maintained at 30°C. The sample was collected on Aug. 22, 1983, from 12 m at 76°04.8'N, 82°09.4'W where bacterial density was 3.42 ± 0.54 × 10^9 cells ml^-1 and temperature was 0.5°C.

Fig. 11. Accumulation of \(^3\)H glucose in (A) picoplankton and (B) microplankton during temperature shifts. Symbols and explanations of shift protocol described in Fig. 10. The sample was collected on Aug. 30, 1983, from 20 m at 76°12'N, 82°15' W where bacterial density was 5.69 ± 1.37 × 10^9 cells ml^-1 and temperature was -1.1°C.

secondary downward shift were also in general accord with the results from the temperature gradient experiments (Figs. 4B, 5B).

On another occasion (Sept. 6, 1983; 72°11.8’N, 65°42.0’W; 25 m sample; in situ temperature = -1.4°C), we found that even if the secondary downward shift to 0°C occurred as soon as 1 h (rather than the customary 8 h) after the initial upward shift from 0° to 30°C, there was no further measurable accumulation of \(^3\)H-labelled amino acids in either pico- or microplankton. In other words, the effect of heat shock appeared to be irreversible after 1 h (shorter shock periods have not been tested).

In other experiments, we found that the enhancement of heterotrophic activity at 30°C could be eliminated by treatment with 100 µM chloramphenicol (an inhibitor of prokaryotic protein synthesis) but not with 100 µM cycloheximide (an inhibitor of eukaryotic protein synthesis). This was true for accumulation of \(^3\)H-labelled amino acids by picoplankton (Fig. 13A) and microplankton (Fig. 13B) as well as for accumulation of \(^3\)H glucose by picoplankton (Fig. 14A) and microplankton (Fig. 14B). These results strongly suggest that only prokaryotes (bacteria) were involved in the enhancement phenomenon and that there were insignificant contributions from eukaryotes (e.g. algae) to measured heterotrophic activity, even in the microplankton. It is interesting to further note that in this one seawater sample, the length of time which passed before rapid activity became evident at 30°C was about 6 h for \(^3\)H-labelled amino acids but at least 12 h for \(^3\)H glucose.

In yet another experiment, enhancement of heterotrophic activity at 30°C was obtained even when the possibility of bacterial stimulation due to animal and plant death was reduced. The experiment was performed by removing microplankton from the sample using a 1 µm Nuclepore membrane before radiolabelling and incubation at 30°C. Figure 15A shows that in the absence of microplankton, the activity in picoplankton was still enhanced by incubation at 30°C. The time course of the de-
The accumulation of $^{32}$PO$_4^{2-}$ in (A) pico-plankton and (B) microplankton during temperature shifts. Symbols and explanations of shift protocol described in Fig. 10. The sample was collected on Sept. 13, 1983, from 10 m at 76°04.5' N, 82°12' W where bacterial density was $3.43 \pm 0.75 \times 10^5$ cells ml$^{-1}$ and temperature was $-1.0^\circ C$.

![Graph showing accumulation of $^{32}$PO$_4^{2-}$](image1)

Fig. 12. Accumulation of $^{32}$PO$_4^{2-}$ in (A) pico-plankton and (B) microplankton during temperature shifts. Symbols and explanations of shift protocol described in Fig. 10. The sample was collected on Sept. 13, 1983, from 10 m at 76°04.5' N, 82°12' W where bacterial density was $3.43 \pm 0.75 \times 10^5$ cells ml$^{-1}$ and temperature was $-1.0^\circ C$.

![Graph showing accumulation of $^{32}$PO$_4^{2-}$](image2)

Fig. 13. Effects of chloramphenicol (100 μM) and cycloheximide (100 μM) on the accumulation of $^3$H-labelled amino acids in (A) pico-plankton and (B) microplankton during a temperature shift. At 2 h, the sample at 0°C (○) was divided into four subsamples: the first was maintained at 0°C (●), the second was shifted to 30°C (△), the third received chloramphenicol and was shifted to 30°C (△), the fourth received cycloheximide and was shifted to 30°C (▽). The sample was collected on Sept. 3, 1983, from 15 m at 74°45' N, 70°48' W where bacterial density was $7.15 \pm 0.96 \times 10^5$ cell ml$^{-1}$ and temperature was $-1.4^\circ C$.

![Graph showing effects of chloramphenicol and cycloheximide](image3)

The development of enhanced activity was quite similar to that of a parallel incubation of the same seawater sample which did contain microplankton (Fig. 15B).

In a final experiment, we enumerated bacterial cells before and after a 25 h incubation under 7 different schedules involving single and double temperature shifts (Table 1). There was an 18% increase in the number of cells in the 0°C control incubation (schedule 1): this corresponded to a doubling time of 4.4 days. In contrast, when the sample was shifted to 30°C and held at that temperature for 23 h, there was an eightfold increase in cell numbers (schedule 3). We know that there was a lag period before cell division because 8 h after the shift to 30°C, the change in cell number from the initial value was insignificant ($3.43 \pm 0.75 \times 10^5$ to $3.66 \pm 0.71 \times 10^5$ cells ml$^{-1}$). Therefore, the rate of cell increase from 8 – 23 h after the temperature shift was very high: the minimum estimate was 0.20 doublings h$^{-1}$ (doubling time = 5 h). At 14°C (schedule 2), the minimum estimate of growth rate was 1.36 doublings d$^{-1}$ (doubling time = 18 h).

**Discussion**

We have presented evidence suggesting that in arctic marine waters, there are bacteria whose heterotrophic activity and growth are rapidly enhanced at mesotrophic temperatures. However, we have no direct evidence that the bacteria active and growing at high temperatures were not the same ones that were active and growing at low temperatures.

Oppenheimer and Droste-Hansen (1960) suggested that “a single species of bacteria may have optimal activity at more than one temperature by utilizing different metabolic pathways. The more or less abrupt changes which occur in (the physical properties of) water will influence the selection and nature of the metabolic reactions”. On this basis, they speculated that polar mi-
Fig. 14. Effects of chloramphenicol (100 μM) and cycloheximide (100 μM) on the accumulation of [3H] glucose in (A) picoplankton and (B) microplankton during a temperature shift. Symbols and explanations of shift and inhibition protocols described in Fig. 13. The sample was the same as that used in the Fig. 13 experiment.

Fig. 15A, B. Accumulation of [3H]-labelled amino acids at 0° (solid symbols) and 30°C (open symbols). A The sample was filtered through a 1μm Nuclepore membrane before incubation: picoplankton activity at 0° (●) and at 30°C (○). B The sample was size-fractionated after incubation: picoplankton activity at 0° (●) and at 30°C (○); microplankton activity at 0° (■) and at 30°C (□). The sample was collected on Sept. 14, 1983, from 10 m at 76°04.5'N, 82°12'W where bacterial density was 4.12 ± 0.62 x 10^3 cells ml^-1 and temperature was -1.0°C.

Microorganisms may also be active in tropical environments. However, earlier (Zobell and Conn 1940) and subsequent studies (Moriya 1975) have shown that many psychrophilic bacteria isolated from cold marine waters are quickly rendered nonviable at mesobiotic temperatures. We surmise that this would have happened to the psychrophiles in our plankton samples. On the other hand, Sieburth (1964) found that a “psychrophilic” Arthrobacter could grow at 30°C to 34°C by changing pleiomorphically from a gram-negative myeloid form to a gram-positive coryneform stage. This change was reversible. In our experiments, we found that a heat shock of 30°C for 1 h had a detrimental effect on heterotrophic activity that was irreversible, at least over the subsequent 23 h observation period. Similarly, Hodson et al. (1981) reported that a heat shock of 21°C for 1 h irreversibly inhibited uptake of [3H] ATP by antarctic microbial plankton over a subsequent 4 h observation period. We suspect that the enhancement of bacterial activity and growth was the result of an opportunistic growth of non-psychrophiles.

It is difficult to imagine many situations in cold polar regions where an opportunity for such an enhancement might occur. One such possibility is in the digestive tract of marine mammals or birds. In this connection, it is worthwhile noting that phytoplankton were not enhanced by mesobiotic temperatures in our experiments (Fig. 9); photosynthesis would not occur in the darkness of digestive tracts. The H^14CO_3^- activity measured after 8 h at 30°C was probably due to anaplerotic uptake (Kornberg 1966) by bacteria present in numbers probably much greater than before (Table 1).

The existence of non-psychrophiles in cold marine environments is usually established by assessing colony formation on enriched solid media at various temperatures (McBee and McBee 1956; Sieburth 1964, 1967; Nedwell and Floodgate 1971; Kaneko et al. 1977, 1979; Ruger 1982; Gow and Mills 1984) — a procedure that necessarily favours the development of non-oligotrophs (Buck 1979; Van Es and Meyer-Reil 1982) and the consideration of events on the time scale of days to weeks. Recognizing that nutritional conditions can have an effect on
temperature-influenced competition in psychrophilic bacteria (Harder and Veldkamp 1971) and that significant measurable events take place quickly after a temperature shift (Ng et al. 1962; Chaloner-Larsson and Yamazaki 1977; Lemaux et al. 1978; Yamamori et al. 1978; Hofle 1979; Ryals et al. 1982), our experiments considered events on a time scale of hours and at conditions of nutrition approximating the natural situation. Our usual addition of [3H] glucose resulted in an enrichment of only 1.6 nM. Previous experiments in these waters (Li 1983 and unpublished data) indicate a modal value \( n = 54 \) of about 30 nM glucose for \( K_+ S_0 \) (Wright and Hobbie 1966), i.e. the sum of the “half-saturation constant” and the ambient glucose concentration. Our enrichment would have contributed an apparently negligible bias in the natural turnover rate of glucose in these waters (Li 1983).

The experiment demonstrating bacterial enhancement at 30°C in spite of the prior removal of microplankton from the seawater (Fig. 15A) suggested that any direct nutrient enrichment arising from animal and plant death was unimportant. Inadvertent nutrient enrichment conceivably could have arisen if the pre-incubation filtration of animals and plants disrupted cell integrity. However, this would not have been sufficient to account for the bacterial enhancement at 30°C as evidenced by the results of the control experiment wherein the plankton were filtered after incubation (Fig. 15B). However, we could not discount the possibility of nutrient enrichment arising from “thermally induced leakage” (Morita 1975) of cellular material from psychrophilic bacteria in the picoplankton.

The dependence of heterotrophic activity on temperature in short-term (h) incubations has been investigated in other cold marine environments such as the aphotic Pacific ocean (Takahashi and Ichimura 1971; Maia et al. 1977; Fuhrman and Azam 1983), the Beaufort Sea (Griffiths et al. 1977), and in Antarctic waters (Gillespie et al. 1976; Morita et al. 1977; Holm-Hansen et al. 1977; Hodson et al. 1981). In almost all cases including our own, maximum activity was recorded at temperatures significantly higher than those in situ. This is to be expected since even psychrophiles achieve their fastest growth at temperatures of about 5° to 15°C (Morita 1975). On the other hand, there have been no any strong indications of multiple activity peaks of the sort illustrated in Figs. 1 – 3 and 5A. To some extent, this might have been due to a consideration of too narrow a temperature range in some cases or an inadequate resolution of the activity curve between widely-spaced temperatures in other cases. In his study of heterotrophic activity in a freshwater system, Romanenko (1982) was able to detect multiple temperature optima using a “polythermostat”.

The activity-temperature curve is used to describe the thermal characteristics of the microbial assemblage in situ and to assess the degree to which the assemblage is adapted to its environmental temperature (e.g. Allen and Brock 1968; Fuhrman and Azam 1983). For this purpose, it is essential that the incubations be short enough so that only the primary temperature response is recorded. We are convinced that without some indication of their dependence on time, activity-temperature curves are very difficult to interpret. First, we have shown that for a given plankton sample the time course for the development of rapid enhancement can differ depending on whether the response is accumulation of 3H-labelled amino acids (Fig. 13) or [3H] glucose (Fig. 14). Second, for a given sample, such a time course can also differ between size fractions of the plankton (Fig. 12 and 15B). Third, for a given substrate, such a time course can also differ among plankton samples (cf. Fig. 10 vs. 13 vs. 15B and Fig. 11 bs. 14). It is therefore not possible to discount the possibility that temperature-activity curves, especially those with multiple optima, represent both primary and secondary responses to temperature.

In summary, our experiments have repeatedly and convincingly demonstrated a rapid (= h) enhancement of heterotrophic activity in the natural microbial flora of eastern Canadian arctic waters. The microbes responsible are believed to be bacteria, both living freely in the water and associated with particles. The enhancement depends on protein synthesis (i.e. chlorophenicol-sensitive) and is associated with an apparent active synthesis of DNA (i.e. incorporation of 3H TTP into acid-insoluble materials) and an increase in the number of bacterial cells. The enhancement does not depend on possible nutrient enrichment from heat-killed animals and plants.

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