18. A. Kimchi, *et al.*, *FEBS Lett.* **134**, 212 (1981).
19. S. L. Lin, W. J. Pledger, I. Tamm, in *The Biology of the Interferon System*, W. E. Stewart II and H. Schellekens, Eds. (Elsevier Amsterdam, in press).
20. C. D. Stiles, R. R. Isberg, W. J. Pledger, H. N. Antoniades, C. D. Scher, *J. Cell. Physiol.* **99**, 395 (1979).
21. A. A. Creasey, J. C. Bartholomew, T. C. Merigan, *Exp. Cell Res.* **134**, 155 (1981).
22. E. Oleszak and A. D. Inglot, *J. Interferon Res.* **1**, 37 (1980).
23. L. M. Pfeffer, J. S. Murphy, I. Tamm, *Exp. Cell Res.* **121**, 111 (1979).
24. B. Herman and W. J. Pledger, *J. Cell Biol.* **100**, 1031 (1985).
25. R. Bablanian *et al.*, *Virology* **26**, 100 (1965).
26. I. Tamm, L. M. Pfeffer, J. S. Murphy, *Methods Enzymol.* **79B**, 461 (1981).
27. H. N. Antoniades, D. Stathakos, C. D. Scher, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2635 (1975).
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Temperature Regulation of Bacterial Activity During the Spring Bloom in Newfoundland Coastal Waters

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While the spring phytoplankton bloom in Newfoundland coastal waters is in progress during April and May, at water temperatures between −1° and +2°C, bacterial growth and respiratory rates remain low. Microbial community respiration is not measurable at −0.2°C. Particulate materials that would be utilized by microorganisms in 2 to 3 days at 20° to 25°C require 11 days at 4°C and 18 days at −0.2°C. Thus, photosynthesis is active but microbial utilization of the products is suppressed. High secondary production in cold water may result from the low rate of microbial decomposition, enabling herbivores to utilize much of the primary production.

HIGH SECONDARY PRODUCTIVITY in cold waters has been an enigma, because the rates of primary production do not seem to be large enough to support it (*1*). Cold-water food chains have been thought to be short and energetically efficient, but microbial pathways of energy flow are now known to occur in cold waters (*2*), in addition to the metazoan grazer-predator relationships (*3*). Dissolved organic materials and nonliving particulate materials are converted to living biomass by bacteria that are subsequently grazed by protozoa and other small eukaryotes. Since energy losses are 50 to 90% at each step in a food chain, a significant fraction of primary production is lost through energy conversions in this microbial loop. If, however, bacteria in cold water are less active metabolically than in warm water, the microbial loop will consume less energy and more primary biomass will be available to metazoans. Heterotrophic marine psychrophiles are inhibited by very low temperature (*4, 5*). For one bacterium isolated from water at 62°S, the increase in rate of the chemical reaction for each 10°C increase in temperature (Q_{10}), was measured; the extrapolated Q_{10} of respiration between +1° and 4°C was 142, whereas between 7° and 10°C it was 1.7. The growth rate declined from a maximum of 0.19 generation hour^{-1} at 4°C to 0.08 hour^{-1} at +1°C (*5*).

Phytoplankton photosynthesis also is suppressed by low temperature, but the decline in photosynthetic rate occurs at lower temperatures than does the decline in bacterial growth and metabolism (Fig. 1). Thus, between +1° and −1°C, photosynthesis is substantial but rates of bacterial growth and metabolism are low. This is potentially important where water temperature is in that critical range during the spring bloom period, when much of the annual primary production occurs (*6*). To examine these differential responses during the spring bloom, we incubated samples of natural seawater and appendicularian houses from Logy Bay, Newfoundland, at temperatures near 0°C and +4.2°C.

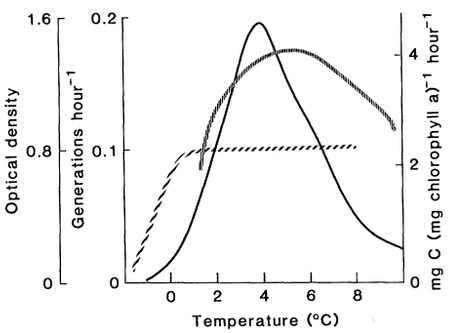

Fig. 1. Differential growth of phytoplankton and bacteria at very low temperatures. Growth of psychrophilic bacteria in culture at various temperatures (*4*), expressed as optical density of the culture (continuous line) and (*5*) expressed as generations hour^{-1} (line of short vertical marks), compared with potential production of phytoplankton, measured in the field (*11*), expressed as milligrams C (milligrams of chlorophyll *a*)$^{-1}$ hour^{-1} (dashed line).

Appendicularian houses are common, naturally occurring, macroparticulate matter on which bacteria grow. They support rates of photosynthesis and respiration much higher per unit volume than that in the surrounding water (*7*). Samples of water and living appendicularians, *Oikopleura vanhoeffeni*, were collected during April 1984 and 1985 by divers. Abandoned appendicularian houses were placed in glass vials with 23 ml of seawater from the collection site, and these together with control vials of seawater were held at controlled temperatures with natural illumination from laboratory windows (*8*). During April 1985, the change in oxygen concentration in control vials at −0.2°C was not significant (analysis of variance, F test, $P \geqq 0.50$) over 21 days of observation (Fig. 2). However, the presence of appendicularian houses resulted in a significant increase in oxygen concentration, $+2.64 \pm 2.02$ μM day^{-1} ($P \leqq 0.01$). At 4.2°C, oxygen concentration decreased at the same linear rate in both the vials with houses, -4.52 ± 2.76 μM day^{-1}, and the control vials, -4.50 ± 1.33 μM day^{-1} (analysis of covariance, t test, $P > 0.20$).

This experiment demonstrates that at ambient temperature of −0.2°C photosynthesis was greater than respiration, with some fraction of the primary producers attached to large particles, while at +4.2°C heterotrophic respiration exceeds photosynthesis.

At −0.2°C, the numbers of free-living bacteria were initially 1.2×10^4 ml^{-1}, increasing after a week to 10^6 ml^{-1} and remaining in the range of 2×10^6 to 7×10^6 ml^{-1} until week 3, when numbers of free bacteria were 1×10^5 to 5×10^5 ml^{-1}. Some bacteria were attached to particles, but on the order of 99% were free-living. Few flagellates were seen. At 4.2°C, however, bacteria attached to particles, in colonies too dense to be counted, formed the dominant bacterial biomass. Free-living bacteria were in the range 1×10^5 to 5×10^5 ml^{-1}

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throughout the 3 weeks. Small flagellates were numerous, on the order of 10^3 ml^{-1}. At −0.2°C the appendicularian houses and fecal pellets enclosed in them disintegrated into small fragments after 18 ± 3.4 days, whereas at 4.2°C disintegration was complete after 11 ± 2.2 days (±2 S.E.). In contrast, tunicate fecal particles collected at lower latitudes at 20° to 25°C disintegrate in 2 to 3 days (*9*).

During April 1984, the ratio of adenosine 5′-triphosphate (ATP) to chlorophyll *a* was <1 at all times, indicating that the biomass of attached organisms was dominated by autotrophs (*10*). Epifluorescence microscopy showed little bacterial growth on the house surfaces. The water in which the houses were held showed no significant increase in the bacterial production rate over that in seawater without houses (Table 1). Three observations corroborate the differential activity of autotrophic and heterotrophic microorganisms at very low temperature.

In both 1984 and 1985 the onset of the spring bloom of phytoplankton occurred about 1 April in Newfoundland coastal waters and continued into May. During this time, water temperature is −1° to +2°C, with little thermal stratification (*11*), confirming the provisional curve of photosynthetic potential at low temperatures developed by Li *et al.* (*12*). Bacterial numbers in coastal waters were consistently near 10^5 ml^{-1}, with most being free-living, single cocci. Rates of uptake of exogenous tritiated thymidine, a measure of bacterial production (Table 1), were comparable to those reported from the Antarctic at similar water temperatures (*3*). In contrast, off the coast of Florida, phytoplankton blooms producing 2 g of carbon per square meter per day are accompanied by populations of 10^6 bacteria ml^{-1} (*10*) and high rates of bacterial metabolism (*13*).

When we raised the temperature from −0.2° to 4.2°C, we also demonstrated a significant increase in community respiration, bacterial numbers, and formation of bacterial aggregates typical of rapidly growing populations (*14*). The increased abundance of heterotrophic flagellates indicated that the microbial loop of the food web was active. The presence of an appendicularian house in 23 ml of seawater did not significantly alter the community respiratory rate at 4.2°C, although this is a much higher density of houses than that occurring in coastal waters. To account for the linear rate of community respiration in the water in the vials over 21 days, dissolved organic materials must have been present in sufficient excess or were produced continuously by the microbial community. A water temperature of 4°C does not occur until June off the east coast of Newfoundland, and is limited to the upper 40 m of the water column (*11*).

Table 1. Production of bacteria in Newfoundland coastal waters and in water in which appendicularian houses were held in the laboratory at −1°C. Empty houses were abandoned by their occupants before being collected by divers. Occupied houses were those occupied by an appendicularian when collected by divers even though they were vacated before these observations began. Samples with houses and those from Logy Bay were processed within 1 hour of collection. Conception Bay samples were transported at ambient temperature (−1°C) and processed within 3 hours of collection.

Water source or sample type	No. of observations	Thymidine uptake (n*M* liter^{-1} day^{-1})
Logy Bay	5	17.0
Conception Bay	3	45.4
With houses		
Empty	3	78.3
Occupied	4	38.0

Our observations, as well as previous ones on antarctic bacteria and arctic phytoplankton (*4, 5, 12*), show that in the critical temperature range between −1.8° and +1°C, there is significantly less suppression of primary production than of bacterial growth and respiration. Moreover, Larsson and Hagström (*15*) found little bacterial production during the spring bloom in the Baltic Sea at temperatures near 0°C. This differential effect of temperature will be increased by thermal stratification of the water as the spring bloom continues. In the warmer surface layer, photosynthesis and bacterial growth will approach the optimum, but bacterial activity will still be suppressed in the bottom water, which remains below 0°C. Particulate organic matter falling into the lower layer will be utilized slowly by bacteria and will accumulate during the springtime period of intense photosynthesis. Examples of this thermal structure, with accumulation of phytoplankton near the bottom, have been reported from the Bering Sea and the Antarctic as well as Newfoundland (*16*).

These findings have important implications for the coastal marine food web and for metazoan consumers in particular. In the tropics and subtropics, even in coastal upwellings at 12° to 15°C, bacterial metabolism and growth are rapid, and organic production does not accumulate over long periods of time. The turnover of primary production is on the order of 1 to 3 days. Much energy does not reach the metazoans, because bacteria rapidly consume particular substrates and protozoans consume bacteria. In very high latitudes, where summer temperatures do not exceed +1°C, obligate psychrophiles may exhibit their highest rates of growth and metabolism at temperatures around 0°C (*17*). However, in Newfoundland and probably other temperate latitudes, bacterial production and respiration remain low during the spring bloom, and we postulate that more primary production finds its way to metazoan consumers, both benthic and planktonic invertebrates. This difference in the food chain in seasonally very cold water could significantly enhance the production of larger invertebrates and vertebrates, and it may be crucial in sustaining the major fisheries of the Grand Banks and the Bering Sea.

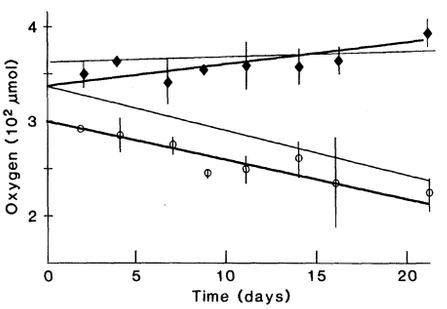

Fig. 2. The effect of temperature on changes in oxygen concentration in vials containing *Oikopleura vanhoeffeni* houses at −0.2°C (diamonds) and at +4.2°C (circles). Symbols indicate mean values at each observation period ±2 standard errors of the mean. The regression equations, c = (slope ± 2 S.E.)t + intercept, with n = number of points and s = SD of points about the line were as follows: at −0.2°C, control, $c = (0.17 \pm 0.35)t + 361$: $n = 9$, s = 6.85; house, $c = (2.06 \pm 0.54)t + 338$: $n = 21$, s = 15.3; at 4.2°C control, $c = (-4.3 \pm 0.50)t + 333$: $n = 9$, s = 9.78; house, $c = (-3.49 \pm 0.65)t + 295$: $n = 24$, s = 18.9. For controls (narrow lines) $n = 1$. For experimentals (heavy lines) $n = 3$, except on days 4, 9, and 11 at −0.2°C, $n = 2$.

REFERENCES AND NOTES

1. S. Z. El-Sayed and J. T. Turner, in *Polar Oceans*, M. J. Dunbar, Ed. (Arctic Institute of North America, Calgary, 1977), pp. 463–503; G. Hempel, in *Antarctic Nutrient Cycles and Food Webs*, W. R. Siegfried, P. R. Condy, R. M. Laws, Eds. (Springer-Verlag, Berlin, 1985), pp. 266–270.
2. J. A. Fuhrman and F. Azam, *Appl. Environ. Microbiol.* **39**, 1085 (1980); R. E. Hodson *et al.*, *Mar. Biol.* **61**, 89 (1981).
3. P. J. leB. Williams, *Kiel. Meeresforsch., Sonderh.* **5**, 1 (1981); F. Azam *et al.*, *Mar. Ecol. Prog. Ser.* **10**, 257 (1983).
4. R. Y. Morita, *Bact. Rev.* **39**, 144 (1975).
5. R. R. Christian and W. J. Wiebe, *Can. J. Microbiol.* **20**, 1341 (1974).
6. G. T. Evans and J. S. Parslow, *Biol. Oceanogr.* **3**, 327 (1985).
7. A. L. Alldredge, *J. Zool. London* **181**, 175 (1972); ——— and J. L. Cox, *J. Mar. Res.* **40**, 517 (1982); B. Prezelin and A. L. Alldredge, *Limnol. Oceanogr.* **28**, 1156 (1983).
8. During April 1985, single houses were held in 23-ml vials at −0.2° ± 0.08°C and +4.2° ± 0.08°C (mean ± 2 S.E.). The vials were filled with water that had been passed through a 260-µm nylon mesh to remove macrozooplankton. Three vials, each containing one house, and one seawater control vial were removed at intervals. The oxygen concentration of the water was measured, wet weight and chlorophyll content of two houses were measured,

and the third house with its surrounding water was examined for numbers of free-living and attached bacteria. In April 1984, one set of houses was examined periodically for changes in bacterial numbers and production, and chlorophyll *a* and ATP content. Oxygen concentration was measured with a Radiometer pO_2 electrode coupled to a PHM 71 amplifier fitted with a PHA934 oxygen module. In warmer water, an experiment of this kind would be negated by wall growth of bacteria, for example, bottle effect. The linear results in this experiment indicate that no significant bottle effect occurred. Chlorophyll *a* was measured by the method of C. S. Yentsch and D. W. Menzel [*Deep Sea Res.* **10**, 221 (1963)] as described by J. Strickland and T. R. Parsons [*Fish. Res. Board Can. Bull. 167* (1968)], using a Turner 110 Fluorometer. ATP of microorganisms was measured by the method of O. Holm-Hansen [*Limnol. Oceanogr.* **14**, 740 (1969)] as modified by D. M. Karl and O. Holm-Hansen [in *Handbook of Phycological Methods*, J. S. Cragie, Ed. (Cambridge Univ. Press, 1978), pp. 197–206], using a Science Applications, Inc., model 2000 ATP photometer. Bacteria were counted by the method of J. E. Hobbie, R. Daley, and J. P. Jasper [*Appl. Environ. Microbiol.* **33**, 1225 (1977)]. Bacterial production rates were measured by the method of J. A. Fuhrman and F. Azam (*3*). The [^{3}H-*methyl*]thymidine, 40 to 60 Ci/mmol, was obtained from New England Nuclear and counted in a Packard 300C scintillation counter.

9. L. R. Pomeroy and D. Deibel, *Limnol. Oceanogr.* **25**, 643 (1980); L. R. Pomeroy *et al.*, *Bull. Mar. Sci.* **35**, 426 (1984).
10. L. R. Pomeroy *et al.*, *Cont. Shelf Res.* **2**, 1 (1983).
11. D. Steele, *Technical Report 12* (Marine Sciences Research Laboratory, St. John's, Newfoundland, 1974); T. A. Kendaris, *Manuscript Report 1569* (Department of Fisheries and Oceans, St. John's, Newfoundland, 1980).
12. W. K. W. Li, J. C. Smith, T. Platt, *Mar. Ecol. Prog. Ser.* **17**, 237 (1984).
13. R. E. Hodson, A. E. Maccubbin, L. R. Pomeroy, *Mar. Biol.* **64**, 43 (1981).
14. B. A. Biddanda, *Mar. Ecol. Prog. Ser.* **20**, 241 (1985).
15. U. Larsson and Å. Hagström, *Mar. Biol.* **67**, 57 (1982).
16. V. Alexander and H. J. Niebauer, *Limnol. Oceanogr.* **26**, 1111 (1981); J. J. Walsh, E. T. Premuzic, T. E. Whitledge, in *Ecohydrodynamics*, J. C. J. Nihoul, Ed. (Elsevier, Amsterdam, 1981); C. P. McRoy, J. J. Goering, W. E. Shiels, in *Biological Oceanography of the Northern Pacific Ocean*, A. Y. Takenouti, Ed. (Idemitsu Shoten, Tokyo, 1972).
17. S. T. Kottmeier, S. M. Grossi, C. W. Sullivan, *Eos* **66**, 1323 (1985).
18. This work was supported by grant OCE-8110707 from NSF, a President's General Grant from Memorial University of Newfoundland, and an Operating Grant from the Natural Sciences and Engineering Research Council of Canada. We thank D. Idler and R. L. Haedrich for making available the facilities of the MSRL and NICOS for this study. We also thank members of the Diving Facility, MSRL, for assistance; J. Anderson, Department of Fisheries and Oceans Canada, for use of a Turner fluorometer; and R. Thompson for assistance with respirometry. We thank W. J. Wiebe, R. E. Hodson, R. Thompson, A. M. Wood, and three anonymous reviewers for critical review of drafts of this manuscript. MSRL contribution 606 and NICOS contribution 87.

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Functional Differences Between Two Classes of Sodium Channels in Developing Rat Skeletal Muscle

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Excitability is generated in developing skeletal muscle by the incorporation of sodium-selective ion channels into the surface membrane. Whole-cell and patch voltage-clamp recording from myotubes and their embryologic precursors, myoblasts, indicated that voltage-activated sodium current in myoblasts was more resistant to block by tetrodotoxin (TTX) than that in myotubes. Single-channel recording from both cell types showed two classes of sodium channels. One class had a lower single-channel conductance, activated at more hyperpolarized voltages, and was more resistant to TTX than the other. The proportion of TTX-resistant to TTX-sensitive sodium channels was higher in myoblasts than in myotubes. Thus, the difference in TTX sensitivity between myoblasts and myotubes can be explained by a difference in the proportion of the two classes of sodium channels. In addition, the lower conductance of TTX-resistant channels provides insight into the relationship between the TTX binding site and the external mouth of the sodium channel.

IN DEVELOPING SKELETAL MUSCLE, action potentials and the sodium currents that underlie them are initially quite insensitive to the specific sodium-channel blocker tetrodotoxin (TTX), but increase in their sensitivity to TTX as the cells mature (*1–6*). This change in TTX sensitivity with age could represent a gradual shift in the affinity of channels during development. Alternatively, if two populations of sodium channels with very different TTX affinities existed, an increase in the ratio of high-affinity to low-affinity channels could account for the results. Labeled-toxin binding and ion-flux studies support the explanation that there are two populations of sodium channels (*2, 4, 6*). However, two separate electrophysiological studies, based on direct measurement of functional sodium channels, support either the hypothesis of a gradual affinity shift (*3*) or the existence of two classes of channels (*5*). Here we show that functional forms of both TTX-sensitive and TTX-resistant channels coexist, both in early mononucleated myoblasts and in older multinucleated myotubes. The two classes of sodium channels retain their individual characteristics throughout development, while their relative proportions change. The amplitudes of the single-channel currents and the gating properties of the two classes are readily distinguishable. TTX-resistant channels carried less current at all voltages examined and could be activated at more negative membrane potentials.

The effects of TTX on macroscopic sodium currents were examined in myoblasts by whole-cell recording and in myotubes by averaging single-channel records from an outside-out patch (*7*). In both cell types the currents were activated by depolarizing steps. The addition of 312 n*M* TTX to the solution bathing a myoblast reduced the peak inward current by approximately 66 percent. (Fig. 1A). In a myotube patch, more than 95 percent of the inward current was abolished by 125 n*M* TTX (Fig. 1B), from which we calculate an equilibrium dissociation constant for toxin binding of less than 10 n*M* (assuming a single affinity binding site). In our experiments, the effect of TTX typically was greater on myotubes than on myoblasts, in agreement with previous reports (*2, 3, 6*). Other experiments showed that the shape of the dose-response relation of TTX inhibition of the sodium current in myoblasts was consistent with more than a single dissociation constant for block (*8*). At higher concentrations (>10 μ*M*) TTX reversibly abolished all inward current in both cell types; thus this current was passing through voltage-activated sodium channels.

Single-channel currents were obtained from both cell types with outside-out patches. Current records elicited by depolarizations to −40 mV revealed that single-channel currents tended to have either of two amplitudes (Fig. 2, upper panels). Large events were approximately 1.4 pA, and small events (arrowheads) were approximately 1.0 pA. Both classes of events were seen in seven out of eight patches from myotubes and in seven out of ten patches from myoblasts. In one myotube patch, only large events were seen and, in three patches from myoblasts, only small events were observed. The excised patches from myotubes typically had a higher density of functional channels than myoblasts. All single-channel events were completely blocked by 15 μ*M* TTX, which indicates that they were generated by sodium channels. Moderate concentrations of TTX (5 to 500 n*M*) reversibly reduced the

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