Reports

Microscale Patchiness of Nutrients in Plankton Communities

Abstract. *Autoradiography was used to identify the presence of nutrient patches produced by zooplankton. Algal cells which encounter patches of phosphorus-33 released by swimming animals accumulate more label than cells that do not enter the patches. Differential labeling of algae does not occur when turbulence in the fluid is increased by stirring. Nutrient patchiness at the scale of millimeters or less in nature probably influences the course of competition and coexistence among the phytoplankton.*

Phytoplankton in lakes and oceans probably experience nutrient environments that are variable at small scales. Differences in competitive success among species of algae may depend in part on their relative abilities to profit from brief encounters with nutrient-rich patches produced by zooplankton. This view complements those about species structure and population dynamics of phytoplankton communities that have been extrapolated from results of continuous algal culture (*1*). Suggestions that competition in nature is mimicked in culture systems (*2*) rely on the assumption that environmental concentrations are homogeneous, like those of well-mixed cultures.

Because inorganic phosphorus in fresh waters and inorganic nitrogen in ocean waters are often undetectable by conventional means (*3*), it may seem reasonable that populations succeed through their relative abilities to obtain limiting nutrients from constant near-zero concentrations as they do in continuous algal cultures. Heterogeneity in supplies of limiting nutrients, however, can theoretically lead to changes in the biotic fabric of plankton communities, and some laboratory studies have shown this (*4*).

A crucial question is whether or not the requisite patchiness occurs in nature. The answer depends in part on the identity and importance of patch-generating processes. That swimming zooplankton might create nutrient micropatches of crucial importance to oceanic phytoplankton has been suggested (*5*) and subsequently attacked on the ground that dispersion is too rapid at small scales for the proposed mechanisms to work (*6*). Some investigators state that microzones could not supply the nutrient requirements of phytoplankton (*7*). Our work on lakes Washington and Ontario has shown that nutrients released from crustacean zooplankton through egestion and excretion provide the major share of those used by phytoplankton during summer months (*8*). We now report that phytoplankton obtain some nutrients from micropatches.

To determine whether algae benefit from zones of nutrient enrichment near animals, we prepared adult females (2 mm long) of the freshwater cladoceran *Daphnia pulex* by feeding them heavily labeled cells of the green alga *Ankistrodesmus falcatus* for 2 days to achieve body burdens of about 5 μCi of ^{33}P. The animals were well rinsed in cell-free nonradioactive medium, and groups of ten were transferred by pipette to two experimental vessels, each of which contained 10^5 cells per milliliter of *Chlamydomonas reinhardti* in 300 ml. The *Chlamydomonas* had been suspended for 2 days before the experiments in culture medium free of added phosphorus so that their cellular reserves of phosphorus were reduced (*9*).

One experimental vessel was mixed with a magnetic stirring bar (300 rev/min) separated from the animals by Nitex netting to avoid injury to them. The second vessel was not stirred. Although they were not injured, the animals that were stirred released phosphorus somewhat faster than those in the unstirred treatment, as judged by liquid scintillation counts and autoradiographic analyses. In low light (0.169 μE m^{-2} sec^{-1}, 400 to 700 nm) the animals swam throughout the volume of both vessels.

A third vessel containing algae but no animals was mixed vigorously (670 rev/min), and at intervals of 2 minutes we injected 10 μl of sterile water containing 0.01 μCi of ^{33}P and 0.1 nmole of PO_4. We calculated these additions to match expected release rates from the animals, which were determined from preliminary experiments. Dye studies had shown that the mixing was sufficient to disperse added substances almost instantly.

After 30 minutes the contents of the vessels were poured through Nitex sieves, which retained the animals, into beakers containing 1 ml of acid Lugol's preservative. The duration of the experiment was set to ensure, on the basis of theoretical expectations and preliminary experiments, that fewer than 20 percent of the algal cells in the unstirred vessel would encounter nutrient patches produced by the animals. The interval was also short enough so that no egestion of intact cells of *Chlamydomonas* could be detected. Samples of 1 ml were drawn immediately through 0.45-μm Millipore filters which were then prepared for track autoradiography. We used Kodak NTB-3 emulsion and recorded tracks as five or more silver grains along a trajectory. The tracks record the paths taken by individual β particles emitted from decaying ^{33}P nuclei.

Frequency distributions of observed β tracks per cell (Table 1) suggest that all cells were equally labeled in the two mixed vessels (treatments 1 and 3) and that the amount of ^{33}P per cell was variable in the unstirred vessel (treat-

Table 1. Frequency distributions of β tracks per cell (observed) and Poisson distributions with identical means (predicted) (*10*). Treatment 1, stirred vessel with *Daphnia* as ^{33}P source; treatment 2, unstirred vessel with *Daphnia* as ^{33}P source; treatment 3, stirred vessel without *Daphnia* and with ^{33}P added at intervals; 300 cells were counted for each treatment.

Tracks per cell	Distribution (number of cells)					
	Treatment 1		Treatment 2		Treatment 3	
	Observed	Predicted	Observed	Predicted	Observed	Predicted
0	180	179.0	248	233.6	183	180.8
1	91	92.5	32	58.4	89	91.6
2	23	23.9	17	7.3	22	23.2
3	6	4.1	3	0.6	5	3.9
4	0	0.5	0	0.04	1	0.5
Significance level						
	.63		1.1×10^{-7}		.81	

0036-8075/82/0514-0729$01.00/0

ment 2). Because the tracks represent individual events of radionuclide decay, a Poisson distribution of tracks per cell is expected if all the cells contain an identical amount of radioisotope. Treatments 1 and 3 follow the Poisson model. The observed distribution of β tracks in treatment 2, however, differs indisputably from a Poisson distribution ($P = 10^{-7}$). There are more cells with no tracks as well as with two or three tracks than can be matched by a Poisson distribution (*10*). This finding suggests that the algal cells became labeled differentially while they were in the presence of the radioactive animals. Most cells were weakly labeled but a few apparently encountered patches enriched with $^{33}PO_4$ released by the swimming animals. Without artificial stirring the patches persisted long enough for the algal cells to exploit them. When the solution was stirred, all cells were labeled equally, indicating that the patches can be dispersed fast enough by artificial stirring to make the isotope equally available to all cells.

The results from the track autoradiography were corroborated by independent experiments in which a thin emulsion layer of Kodak NTB-2 nuclear emulsion was used and grain distributions constructed. In this case the model distribution tested is not Poisson but Neyman's type A (*11*). The distribution of tracks or grains matched that of the respective model for stirred treatments but was more skewed than the model in unstirred treatments.

The unstirred treatments we used are more representative of natural communities than the stirred treatments. When turbulent energy spectra, length scales of diffusivity, and the characteristics of fluid flow around microzooplankton are considered the prominent mechanism of dispersion in lakes and the open ocean at scales of a millimeter or less arises from molecular processes (*12*). Microcinematography has shown that the fluid environment around feeding *Daphnia* and both marine and freshwater copepods is viscous (*13*). Our results show that nutrient patches produced by zooplankton exist long enough for algae which encounter them to absorb more nutrient than do algae outside the patches. Using estimates of swimming speed and phosphorus release for *Daphnia* (*14*), we calculated characteristics of the nutrient micropatches produced by the animals. As judged by their uptake physiology, the *Chlamydomonas* can augment their cell quota by as much as 12 percent per encounter. Such circumstances place a premium on the maximal rates at which cells can sequester the enriched nutrient rather than on the efficiency with which they can gather the nutrient when ambient values are low. An important force structuring species composition in phytoplankton communities when planktonic herbivores are abundant may not be merely differential mortality imposed by the herbivores but also differential abilities of the algae to exploit short-lived nutrient patches that are rare but dependable.

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9. At the end of 2 days in phosphorus-deficient medium the *Chlamydomonas* contained 2.4×10^{-9} μmole of phosphorus per cell and maximum uptake rates had increased to 9×10^{-11} μmole of phosphorus per cell per minute. The cells were 6 μm in diameter.
10. Chi-square goodness of fit tests were used to test our observed frequencies against the Poisson distribution with a mean equal to that estimated from the observations and 3 degrees of freedom. The difficulty of discerning many individual β tracks derived from any single cell of *Chlamydomonas* required that exposure times be kept short enough so that probabilities of finding more than four tracks per cell would be negligible.
11. N. L. Johnson and S. Kotz, *Discrete Distributions* (Houghton Mifflin, Boston, 1969), p. 216. Stirred treatments conformed to Neyman's distribution ($P > .85$), but the unstirred treatment did not ($P < .012$).
12. Observed power spectra for physical and biological properties demonstrate that the effects of large-scale physical forces dissipate effectively during the cascade to scales on the order of meters [T. M. Powell, P. J. Richerson, T. M. Dillon, B. A. Agee, B. J. Dozier, D. A. Godden, L. O. Myrup, *Science* **189**, 1088 (1975); K. L. Denman and T. Platt, *J. Mar. Res.* **34**, 593 (1976)]. Temperature microstructure in open waters suggests that diffusive fluxes at scales of centimeters are low [M. C. Gregg, C. S. Cox, P. W. Hacker, *J. Phys. Oceanogr.* **3**, 458 (1973), M. C. Gregg, *ibid.* **10**, 915 (1980)]. Thus energy at large scales is not expected to result in turbulence on scales of millimeters. Turbulence is also not likely to be generated at that scale by swimming microzooplankton because flow will be laminar, as suggested by the low Reynolds numbers (< 100) [W. C. Kerfoot, D. C. Kellog, J. R. Strickler, in *Evolution and Ecology of Zooplankton Communities*, W. C. Kerfoot, Ed. (University Press of New England, Hanover, N.H., 1980), p. 10] and by drag characteristics [J. T. Lehman, *Limnol. Oceanogr.* **22**, 170 (1977)].
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15. Supported in part by NSF grant DEB79-22143 to J.T.L. This report is Great Lakes Environmental Research Laboratory Contribution 282.

22 December 1981; revised 11 February 1982

Residual Calcium Ions Depress Activation of Calcium-Dependent Current

Abstract. *Calcium ions enter and accumulate during depolarization of some cells, activating a potassium current, $I_{K(Ca)}$, that depends on the cytoplasmic concentration of calcium ions, $[Ca]_i$. However, elevation of $[Ca]_i$ can depress $I_{K(Ca)}$ elicited by a subsequent membrane depolarization. The depression of $I_{K(Ca)}$ is ascribed here to a $[Ca]_i$-mediated inactivation of the voltage-gated calcium conductance, which causes a net reduction in calcium ions available for the activation of $I_{K(Ca)}$. This suggests that other processes dependent on gated calcium entry may also be depressed by small background elevations in cytosolic free calcium ions.*

One regulatory function of calcium ions (Ca^{2+}) entering the cell during membrane excitation is the activation of a calcium-dependent potassium channel (*1*), which carries the current $I_{K(Ca)}$. This outward current is important in a variety of neural, sensory, and neuromuscular phenomena (*2*). It helps prevent prolonged depolarization of the membrane by the calcium current, I_{Ca}, which inactivates rather slowly and incompletely (*3, 4*), and has been implicated in cyclic pacemaker activity (*5, 6*) as well as certain neuropathologies (*7*).

At a given membrane voltage, $I_{K(Ca)}$ is activated in proportion to the intracellular concentration of free Ca^{2+} (*5, 6*). However, $I_{K(Ca)}$ elicited by a depolarizing test pulse (P_2) is reduced in amplitude if the test pulse occurs after the concen-

0036-8075/82/0514-0730S01.00/0