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Blue-Green Algal Inhibition of Diatom Growth: Transition from Mesotrophic to Eutrophic Community Structure

Abstract. *Cell-free filtrates of axenic or bacterized cultures of the dominant blue-green algae from a freshwater lake inhibited the growth of diatoms isolated from the same lake. Lake waters, collected during blue-green algal blooms, also inhibited diatom growth. In situ observations over a 5-year period indicate that diatom bloom populations vary inversely with the levels of the preceding blue-green algal populations. Blue-green algal dominance of eutrophic lakes is attributed to this allelopathy, and dilution is proposed as one cause for the limited occurrence of blue-green algal dominance in marine waters.*

The metabolic products of blue-green, prokaryotic algae play a role in determining annual bloom sequence in eutrophic freshwater lakes (*1*). These algae are also influential in some of the long-range, successional changes (oligotrophic-mesotrophic-eutrophic) in community dominance which occur as a lake ages. One of the most significant of these changes is the replacement of the diatom blooms of mesotrophic lakes by the offensive blue-green algal blooms of eutrophic lakes.

This change in dominance brings with it manifold undesirable effects. Blue-green algae not only produce unpleasant tastes, odors, colors, and floating clumps; they are also an unsatisfactory food for many organisms higher in the trophic structure. They are, in fact, detrimental, even toxic, to many organisms, including humans (*2*), which might coincidently ingest them during feeding or drinking activities. In contrast, as a diatom bloom depletes the lake's store of excess macronutrients (phosphorus, nitrogen, and carbon), it evidences no toxicity and produces neither visual nor olfactory offense; moreover, since diatom blooms occur in the spring or fall, human contact (swimming) is minimized. Diatoms are also an excellent food for zooplankton and fish. Furthermore, when diatom blooms subside, senescent cells fall into the hypolimnion, carrying much of their nutrient load out of the productive epilimnion. These nutrients are essentially unavailable for further algal growth until the fall turnover. On the other hand, planktonic blue-green algae die off rapidly, lysing and releasing most of their nutrients directly into the epilimnion. These nutrients are immediately recycled by bacterial, blue-green algal, or other growth.

In culture, diatoms are favored over blue-green algae in their responses to physical and chemical conditions which characterize the spring; that is, they are more efficient at harvesting nutrients and are more responsive to increased light. Nevertheless, in eutrophic lakes spring blue-green algal blooms displace the spring diatom blooms common to mesotrophic lakes. Therefore, light and nutrients alone do not exercise a definitive influence in situ. The bloom sequence of Linsley Pond, a eutrophic kettle lake in North Branford, Connecticut, demonstrates this.

In the first winter of my study (1971–72) the blue-green algal population of Linsley was consistently high (Fig. 1). In the following spring no diatom bloom occurred. Instead, blue-green algae dominated the plankton throughout the spring and summer. In the second winter (1972–73) a moderate blue-green algal bloom occurred. In the following spring a short-lived, but readily discernible, diatom bloom developed. This bloom consisted of various Fragilariaceae (*3*), culminating in a period of *Asterionella formosa* (800) (*4*) dominance. Blue-green algae dominated the plankton for the remainder of the summer. In the third winter (1973–74) no blue-green algal blooms occurred. The plankton of the following spring was dominated by a long-lived diatom bloom characterized by its diversity of com-

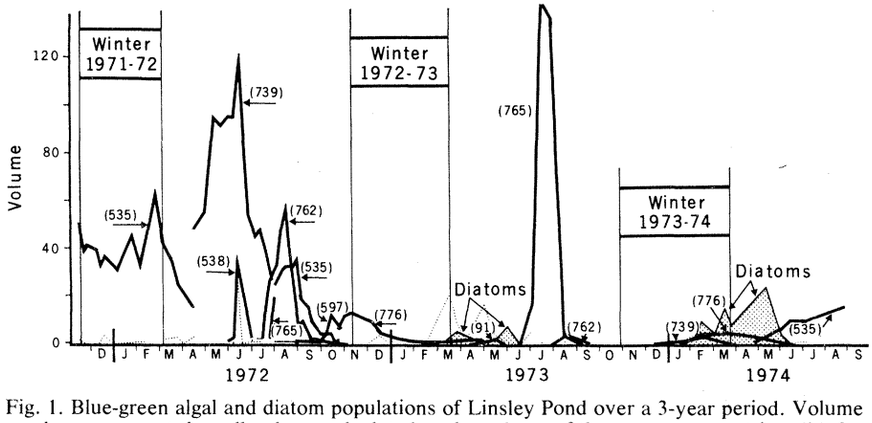

Fig. 1. Blue-green algal and diatom populations of Linsley Pond over a 3-year period. Volume numbers represent the cell volume calculated as the volume of the nearest geometric solid, for example, a sphere or a cylinder. The numbers represent the cell volumes (in cubic centimeters $\times 10^{-6}$ per milliliter of water) as calculated from cell counts (hemacytometer, Sedgewick Rafter, or membrane filter counts). Samples for counting were collected on a weekly (first year) or biweekly (subsequent years) schedule from the surface and from depths of 2.5 m, 5 m, 9 m, and 13.5 m (bottom). (Solid lines) Blue-green algae; (shaded areas) diatoms; (dotted lines) mixed flagellated forms. Numbers in parentheses represent Linsley Pond culture collection numbers. The corresponding Latin names are as follows: *Oscillatoria rubescens* (535), *Oscillatoria agardhii* (739), *Anabaena holsaticum* (538), *Anabaena elenkenii* (762), *Anabaena circinalis* (765), *Pseudanabaena galeata* (597), *Oscillatoria elegans* (776), and *Synechecoccus* sp. (91).

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ponent species (mainly Fragilariaceae with some Naviculaceae) (5). This consistent inverse correlation between winter populations of blue-green algae and the levels of spring diatom bloom indicates that under apparently favorable conditions the ability of an endogenous diatom species to develop bloom populations in spring is limited by the occurrence of a winter blue-green algal bloom.

To determine if this limiting of diatoms could be attributed to a waterborne substance rather than to unknown physical conditions or to some facet of direct competition, I bioassayed waters collected from the pond for their capacity to inhibit diatom growth (6). Bioassay organisms were diatoms (7) isolated from Linsley and maintained in unialgal cultures, axenic or bacterized. The results indicate a consistent, autoclave-labile inhibition of diatoms (Table 1a).

To assure that the source of inhibition was a substance produced by the blue-green algae rather than by some other unrecognized organism or group of organisms (8), I bioassayed cell-free filtrates of blue-green algal cultures. The eight dominant blue-green algae of the 3-year study period (see Fig. 1) and *Aphanizomenon flos-aquae* (766) (9) were isolated from Linsley and maintained in unialgal cultures, axenic or bacterized. Cell-free filtrates (9) (0.45-μm filter) of each blue-green alga were tested against the bioassay diatoms from Linsley. Each filtrate demonstrated autoclave-labile inhibition of most, or all, Linsley bioassay diatoms (Table 1b).

The presence or absence of bacteria did not qualitatively affect the test results. There was, however, a quantitative effect in that the diatom inhibition was diminished in the following order: (i) axenic producer blue-green alga versus axenic bioassay diatom (strongest effects), (ii) axenic producer versus bacterized diatom, (iii) bacterized producer versus axenic diatom, and (iv) bacterized producer versus bacterized diatom (weakest effects). This ordering indicates biodegradability, which is consistent with the premise that inhibition is due to a substance rather than to a physical condition in the lake or in cultures.

Unnaturally dense populations in culture are often criticized, and without population control even clearly demonstrated allelopathy would be rejected by many as an explanation of ecologically significant in situ events. If a culture population were 100 times the density of the in situ population to which it were being compared, the quantity of metabolic substance produced in the culture could reasonably be interpreted as 100 times the quantity produced in situ. This would require that filtrates be diluted 100 times prior to testing in order to substantiate a claim of similar in situ activity. During this study I controlled the populations in blue-green algal producer cultures by avoiding high-nutrient, artificial media and used instead a growth medium consisting of equal portions of charcoal-treated lake water from before and after the fall turnover with macronutrients added (1½ percent ESI) (enrichment seawater I) (10). This procedure produced population densities in culture similar to those in situ.

There is a second reason for the control of concentration or dilution in these studies. Dilution of the filtrates to 33 percent of normal strength eliminates diatom inhibition. This may explain the contrast in the responses of freshwater and marine planktonic communities to excess nutrients. Freshwaters respond to excess nutrients with blue-green algal blooms, whereas marine waters respond with diatom blooms. On the basis of data from this study, it appears that allelopathy is essential to the capacity of blue-green algae to overcome the advantages of diatoms. I suggest that the absence of obvious allelopathy in marine waters is due, at least in part, to the mechanical dispersal of substances produced by blue-green algae. Such dispersal would take place to the point where allelopathic substances are diluted below a critical effective concentration and cannot, therefore, overcome the advantages of diatoms (11).

Detailed study of filtrates of axenic *Anabaena holsaticum* (538) indicates that its inhibitory metabolites are inactivated by autoclaving for 20 minutes at 121°C, partially inactivated by heating for 20 minutes at 90°C, and not substantially affected by heating for 20 minutes at 60°C. Furthermore, adding a tris buffer to the filtrates before autoclaving preserves the inhibition. This result suggests that the rise in pH (6) as CO_2 is driven out of the water, rather than the high temperature during autoclaving, inactivates the inhibitory substance.

Separation of *A. holsaticum* (538) filtrates on ultrafilters (Amicon) indicates the presence of a complex of autoclave-labile, metabolic products, which in

Table 1. Results of the bioassays.

Date of water sample or filtrate-producing organism	Number of				
	Replicate tests	Diatom varieties tested	Varieties inhibited	Varieties not affected	Varieties enhanced
a. Bioassay of pond water against pond diatoms (from culture)					
7 January 1973	2	3	3	0	0
18 March 1973	2	18	17	0	1*
13 May 1973	2	8	8	0	0
2 June 1973	2	9	9	0	0
13 August 1973	2	7	7	0	0
14 February 1974	2	8	8	0	0
13 June 1974	2	13	13	0	0
b. Bioassay of filtrates of blue-green algal cultures against pond diatoms (from culture)					
Oscillatoria rubescens (535)	6	8	5	3	0
Oscillatoria agardhii (739)	3	6	6	0	0
Anabaena holsaticum (538)	175	29	29	0	0
Pseudanabaena galeata (597)	4	8	7	1	0
Oscillatoria elegans (776)	2	6	5	1	0
Synechecoccus sp. (91)	4	9	9	0	0
Aphanizomenon flos-aquae (766)	4	7	4	3	0
Anabaena elenkenii (762)	2	3	2	0	1†
Nostoc muscorum, non-Linsley	2	8	4	4	0
Nostoc sp., non-Linsley	2	8	3	5	0

**Asterionella formosa* (800) reacted positively to water collected on 18 March 1973. This diatom dominated an in situ bloom during March through June 1973. It also proved to be the most resistant of the diatoms tested to the inhibitory effects of blue-green algae. †*Synedra* sp. (299), like *Asterionella formosa* (800), a member of the family Fragilariaceae, responded positively to filtrates of *Anabaena elenkenii* (762). The Fragilariaceae in general were more resistant than other families of diatoms to the inhibitory effects of blue-green algal filtrates.

combination inhibit diatom growth. This complex contains at least one stimulatory and two inhibitory substances. (i) Inhibitor 1 is peach-colored, has a molecular weight (*12*) between 10,000 and 12,000, has a size between 30 and 50 Å (*13*), and is oily to the touch. (ii) Enhancer 1 is vivid mid-yellow, has a molecular weight between 1,000 and 10,000, has a size between 10 and 29 Å, and is possibly a steroid (*14*); it is masked by inhibitor 1 in full-strength filtrates but not when filtrates are diluted. (iii) Inhibitor 2 is colorless, has a molecular weight less than 500, and is less than 10 Å in size (*15*).

The inhibitory substance is nonvolatile and dialyzable. It retains its activity after extraction with ether, dialysis, or separation by ultrafiltration. It is partially eliminated by freezing. Twofold and fivefold concentrations produce increased inhibition and rapid death, respectively; dilutions to 0.5 times the natural concentration retain activity.

Although determination of the significance of blue-green algal allelopathy to bloom patterning in other lakes awaits further investigation, a minimal basis for generalization can be developed. Filtrates of Linsley blue-green algae were bioassayed against non-Linsley diatoms, and filtrates of axenic, non-Linsley *Nostoc* sp. were bioassayed against Linsley diatoms. In both cases the pattern of inhibition was incomplete. This finding contrasts sharply with the ubiquitous inhibition evidenced when only Linsley organisms were used (*16*). It should serve to caution investigators against using organisms isolated from different lakes because they are given the same taxonomic label as organisms in situ or because they are conveniently available from a culture collection (*17*). Such experiments, intended to parallel and illuminate events in situ, would produce results which only partially reflect events in situ. The degree of correlation of laboratory and in situ results would logically depend upon the degree of physiological, not taxonomic, similarity between organisms.

Allelopathy offers an explanation for the most offensive characteristic of accelerated eutrophication, the rapid takeover of the plankton by blue-green algae. It could also explain the contrasting responses of freshwater and marine ecosystems to excess nutrients. Finally, it suggests an opportunity to ameliorate the negative impact of heavy, prolonged, blue-green algal dominance in eutrophic lakes by intentionally eliminating winter blue-green algal blooms. In this way lake waters would purge themselves naturally of the allelopathic substances left behind by blue-green algae, thus permitting the return of diatoms to the spring bloom. Ultimately, such manipulation of natural populations can lead to biological control of eutrophication.

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2. D. Schwimmer and M. Schwimmer, in *Algae and Man*, D. Jackson, Ed. (Plenum, New York, 1964), p. 368.
3. Fragilariaceae may be less susceptible than other diatoms to blue-green algal allelopathy.
4. Numbers in parentheses after species names are identification numbers for culture collection.
5. Minimal in situ monitoring during years 4 and 5 of the study indicated bloom patterns similar to those of years 3 and 2, respectively.
6. Waters were passed through a 0.45-μm Millipore filter. Half of each sample was autoclaved. Approximately 1 week was required for reequilibration of dissolved CO_2. At inoculation, *p*H values were within 0.1 unit for each pair of filtered-only and filtered and autoclaved portions. Macronutrient (1½ percent ESI) was added to each test sample [L. Provasoli, "Cultures and collections of algae," *U.S.-Japanese Conference, Hakone, 1966* (Japanese Society of Plant Physiologists, Tokyo, 1968)].
7. Bioassayed diatoms included axenic *Nitschia frustulum* (224), axenic *Nitschia* sp. (352), axenic *Synedra famnilica* (202), axenic *Synedra* sp. (299), axenic *Cyclotella* sp. (211), bacterized *Asterionella formosa* (800), bacterized *Fragilaria* sp. (99), and bacterized *Tabellaria* sp. (764). For several tests a greater variety of test diatoms was used.
8. Review articles list varied examples of extracellular metabolite-based inhibitions between organisms [R. Hartman, *Pymatuning Symp. Ecol.* **2**, 38 (1960); R. Pourriot, *Année Biol.* **7-8**, 337 (1966)].
9. A common producer of nuisance blooms in freshwater, *Aphanizomenon flos-aquae* (766), was observed in quantity each July and August. It was never dominant.
10. For additional comment on the growth medium, see (*1*). A "filtrate" is a large-volume culture of a producer blue-green alga. It is harvested by passage through a 0.45-μm filter during early stationary growth.
11. Common marine diatoms, *Skeletonema costatum*, *Thalassiosira fluviatilis*, and *Cyclotella nana*, were bioassayed against filtrates of freshwater blue-green algae. The growth medium was a combination of half filtrate and half double-strength DC medium [L. Provasoli, J. McLaughlin, M. Droop, *Arch. Mikrobiol.* **25**, 392 (1957)]. All the diatoms evidenced a sensitivity to filtrates similar to that of the Linsley bioassay diatoms.
12. Molecular weight is the nominal retention weight, based on 90 percent retention by ultrafilters, of protein molecules of the listed molecular weights.
13. Size is based on the pore size of the ultrafilter or dialysis membrane.
14. Amicon informs users that their UM2 filter binds steroids selectively. Enhancer 1 is bound by UM2 filters.
15. Inhibitor 2 may be an artifact of the bioassay procedures; pending additional characterization or physical separation, it is suspect.
16. Of the four non-Linsley freshwater diatoms tested, none evidenced consistent inhibition in waters collected from Linsley or in filtrates of Linsley blue-green algae. Only three out of eight Linsley bioassay diatoms evidenced consistent inhibition in filtrates of the non-Linsley *Nostoc* sp.; five were neutral or produced results too subtle to be definitive.
17. This restriction is more important in freshwater studies than in marine studies. The surface/volume ratios of lake far exceed those of the oceans; therefore, lake waters more closely reflect the unique chemistry and climate of their locales. Also, lake waters, and their plankton, tend to be physically isolated from each other.
18. I thank L. Provasoli and G. E. Hutchinson for advice; R. Patrick, S. Golubic, and F. Drouet for assistance with taxonomy; and Haskins Laboratories, Inc., Yale University, and the Department of Environmental Science, Rutgers University, for support. This work was supported in part by Environmental Protection Agency research grant RA 801387.

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Nitrogen Fixation and Delayed Leaf Senescence in Soybeans

Abstract. *Delayed leaf senescence has been found in a soybean population which maintains its chlorophyll and ribulosebisphosphate carboxylase activity in leaves and nitrogen fixation (acetylene reduction) activity in root nodules throughout seed maturation. Incorporation of delayed leaf senescence into an agronomically desirable genetic background may help to increase seed yield and symbiotic nitrogen fixation during seed development.*

Soybeans [*Glycine max* (L.) Merr.], one of the three major cash crops in the United States, must assimilate large quantities of nitrogen (6.0 kg of N per 100 kg of seed) to produce protein-rich seeds. A recent analysis suggests that soybeans are "self-destructive" in the sense that proteins required for photosynthesis are degraded in the leaf to supply amino acids to developing seeds (*1*). The role of photosynthesis in maintaining symbiotic nitrogen reduction by *Rhizobium* bacteria in legume root nodules has long been known (*2*). The enzyme ribulosebisphosphate carboxylase comprises about 25 percent of the protein in soybean leaves and is primarily responsible for photosynthetic carbon dioxide fixation in soybean leaves. Loss of ribulosebisphosphate carboxylase activity during senescence has been well documented in several plants (*3*). It has been suggested that an increase in nitrogen input during rapid seed development may be achieved by extending the exponential phase of nitrogen fixation (*4*) or by increasing the proportion of photosynthate allocated to support nitrogen fixation (*5*). Another way to increase nitrogen fixation would be to maintain photosynthetically active leaves throughout reproductive growth. This report provides data which demonstrate that certain soybean genotypes have the

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