Present evidence thus suggests that the production of icosahedral VLPs in many multicellular algae may be restricted to a short-lived stage in the life cycle. Continued research on such VLP infections will be required to explain why this occurs. The possibility that a latent virus infection may be involved has recently been discussed in *Cylindrocapsa* (6).

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1. Baker, J. R. J. & Evans, L. V. 1973. The ship fouling alga *Ectocarpus*. I. Ultrastructure and cytochemistry of plurilocular reproductive stages. *Protoplasma* 77:1–13.
2. Bouck, G. B. 1965. Fine structure and organelle associations in the brown algae. *J. Cell Biol.* 26:523–37.
3. Chapman, R. L. & Lang, N. J. 1973. Virus-like particles and nuclear inclusions in the red alga *Porphyridium purpureum* (Bory) Drew et Ross. *J. Phycol.* 9:117–22.
4. Clitheroe, S. B. & Evans, L. V. 1974. Virus-like particles in the brown alga *Ectocarpus*. *J. Ultrastruct. Res.* 49:211–7.
5. Gibbs, A., Skotnicki, A. H., Gardiner, J. E., Walker, E. S. & Hollings, M. 1975. A tobamovirus of a green alga. *Virology* 64:571–4.
6. Hoffman, L. R. & Stanker, L. H. 1976. Virus-like particles in the green alga *Cylindrocapsa*. *Can. J. Bot.* 54:2827–41.
7. Lee, R. E. 1971. Systemic viral material in the cells of the fresh water red alga *Sirodotia tenuissima* (Holden) Skuja. *J. Cell Sci.* 8:623–31.
8. Manton, I. & Leadbeater, B. S. C. 1974. Fine-structural observations on six species of *Chrysochromulina* from wild Danish marine nanoplankton, including a description of *C. campanulifera* sp. nov. and a preliminary summary of the nanoplankton as a whole. *Dansk Videns. Sel. Biol. Skrifter* 20(5):1–26.
9. Markey, D. P. 1974. A possible virus infection in the brown alga *Pylaiella littoralis*. *Protoplasma* 80:223–32.
10. Mattox, K. R., Steward, K. D. & Floyd, G. L. 1972. Probable virus infections in four genera of green algae. *Can. J. Microbiol.* 18:1620–1.
11. Moestrup, Ø. & Thomsen, H. A. 1974. An ultrastructural study of the flagellate *Pyramimonas orientalis* with particular emphasis on golgi apparatus activity and the flagellar apparatus. *Protoplasma* 81:247–69.
12. Pienaar, R. N. 1975. Viral-like infections in three species of marine nanoplankton. *Proceedings of the Electron Microscopical Society South Africa* 5:31–2.
13. ——— 1976. Virus-like particles in three species of phytoplankton from San Juan Island, Washington. *Phycologia* 15:185–90.
14. Pearson, B. R. & Norris, R. E. 1974. Intranuclear virus-like particles in the marine alga *Platymonas* sp. (Chlorophyta, Prasinophyceae). *Phycologia* 13:5–9.
15. Pickett-Heaps, J. D. 1972. A possible virus infection in the green alga *Oedogonium*. *J. Phycol.* 8:44–7.
16. Pocock, M. A. 1960. *Hydrodictyon*: a comparative biological study. *J. S. Afr. Bot.* 26:167–319.
17. Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruc. Res.* 26:31–43.
18. Tomas, R. N. & Cox, E. R. 1973. Observations on the symbiosis of *Peridinium balticum* and its intracellular alga. I. Ultrastructure. *J. Phycol.* 9:304–23.
19. Toth, R. & Wilce, R. T. 1972. Virus-like particles in the marine alga *Chorda tomentosa* Lyngbye (Phaeophyceae). *J. Phycol.* 8:126–30.

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NOTE

ACUTE TOXICITY OF SOME BLUEGREEN ALGAE TO THE PROTOZOAN *PARAMECIUM CAUDATUM*[1]

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ABSTRACT

Four species of bluegreen algae were tested for possible effect on the protozoan Paramecium caudatum *Ehrenberg. Toxicity was demonstrated using lyophilized cells of* Fischerella epiphytica *Ghose and* Gloeotrichia echinulata *(Smith) Richter.* Nostoc linckia *(Roth) Bornet & Thuret failed to show any effects when lyophilized but became toxic when sonified.* Anabaena flos-aquae *(Lyngb.) Bréb. was nontoxic in all tests.* G. echinulata *was lethal at 0.1 mg·ml*$^{-1}$ *which is comparable to the toxic concentration of* Aphanizomenon flos-aquae *(L.) Ralfs reported for microcrustaceans.*

Key index words: Anabaena; Aphanizomenon; *bioassay; bloom algae; bluegreen algae;* Fischerella; Gloeotrichia; Nostoc; Paramecium; *toxic algae; toxicity, protozoan; toxicity, zooplankton; water bloom algae*

Many species of bluegreen algae are known to produce toxins. Those species most often reported include *Anabaena flos-aquae* (Lyngb.) Bréb., *Aphanizomenon flos-aquae* (L.) Ralfs, and *Microcystis aeruginosa* Kütz. em. Elenkin (4). Primary concern in

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the study of algal toxicity has focused on the effect of bluegreen algae on economically important species that include fish, waterfowl, and domestic animals. Few studies have been conducted to elucidate the possible toxicosis mediated against freshwater invertebrates. Stangenberg (10) reports that freeze-thawed extracts of *M. aeruginosa* were toxic to the cladoceran *Daphnia longispina* O. F. Müller and the ostracod *Eucypris virens* (Jurine), whereas Gentile and Maloney (2) showed *Ap. flos-aquae* toxic to cladocerans *Bosmina longirostris* (O. F. Müller) and *D. catawba* Coker as well as to fish (*Fundulus heteroclitus*, *Cyprinodon variegatus*, *Notemigonus crysoleucas*). Finally, in a comprehensive study of bluegreen algal toxicity toward the ostracod *Cyprinotus incongruens* Ramdohr, Mills and Wyatt (6) found 25% of tested algae toxic. Although there is evidence that some bluegreen algae are toxic to microcrustaceans, only a few reports exist on the effect of bluegreen algae on protozoans. Since these organisms are potential predators of bluegreen algae and are also a natural component of the aquatic environment, tests were conducted to determine the effect of four species of bluegreen algae on the ciliate *Paramecium caudatum* Ehrbg.

Anabaena flos-aquae (NRC 44), *Nostoc linckia* (Roth) Bornet & Thuret (NT 69-43), *Gloeotrichia echinulata* (Smith) Richter (UTEX 1303), and *Fischerella epiphytica* Ghose (NT 69-32) were obtained from the personal collection of Dr. J. T. Wyatt, U.S. Army Environmental Hygiene Agency. Numbers in parentheses indicate his original source. All were grown in Gorham's ASM-1 medium (5) as modified by Gentile and Maloney (2). Eighteen liters of sterile medium were placed in each 20 l pyrex carboy and inoculated with 1 g (wet wt) of alga. The carboys were illuminated with cool-white fluorescent light at an intensity of 1,400 lx and incubated at 25 C in a walk-in incubator. Cotton-filtered air (2 l·min^{-1}) was bubbled in to provide CO_2, N_2 and agitation. After 30 days the algae were harvested and lyophilized. *Nostoc linckia*, *Fischerella epiphytica*, and *Gloeotrichia echinulata* were collected by centrifugation at 3,000 × *g* 5 min. *Anabaena flos-aquae* was harvested using a continuous flow centrifuge. All were washed twice to remove metabolites and recentrifuged. Upon final collection, the algae were lyophilized and stored desiccated at −60 C. Algal suspensions for toxicity testing were prepared by adding 1 mg lyophilized alga to 1 ml Peter's osmotic solution (7) (total ions ca. 1 mM). Sonified suspensions were prepared by sonifying the algae in Peter's solution 5 min (0 C) at setting 5 on a Branson sonifier (Stamford, Connecticut). This resulted in lysis of ca. 50% of the cells and destruction of the filaments. Dilutions of 10^{-1} were made with Peter's solution where appropriate.

Paramecium caudatum PW_2 was grown on the axenic medium of Soldo et al. (9). A 5 day culture (mid-log) was washed free of the medium by allowing the paramecia to migrate upward through a column (13 × 510 mm) of sterile Peter's solution. Ten washed organisms were randomly distributed to each well of a spotplate containing 1 ml test suspension. Toxicity tests were conducted at 25 C in triplicate resulting in 30 *P. caudatum* for each algal species and treatment.

The test animals were monitored using a Bausch and Lomb StereoZoom 7 Microscope. Death of *P. caudatum* resulted in the lysis of the organism; therefore, counts of the remaining organisms were taken as live counts. Tests of lyophilized material were followed for 48 h, after which the controls began showing die-off.

TABLE 1. *Survival of* Paramecium caudatum PW_2 *in presence of lyophilized and sonified bluegreen algae.*

Test	Dose (mg·ml^{-1})	Treatment[a]	Time (h) 0	2	8	12	24	48
Control—Peter's soln. (8)	—	—	30	30	30	30	30	30
Anabaena flos-aquae	1.0	L	30	30	30	30	30	30
	1.0	S	30	30	30	30	30	30
	0.1	L	30	30	30	30	30	30
Fischerella epiphytica	1.0	L	30	30	—	—	—	—
	1.0	S	30	0	—	—	—	—
	0.1	L	30	30	30	30	28	28
Gloeotrichia echinulata	1.0	L	30	0	—	—	—	—
	1.0	S	30	0	—	—	—	—
	0.1	L	30	0	—	—	—	—
	0.01	L	30	30	30	30	30	27[b]
	0.001	L	30	30	30	30	30	30[c]
Nostoc linckia	1.0	L	30	30	30	30	30	30
	1.0	S	30	9[d]	0	—	—	—
	0.1	L	30	30	30	30	30	30

[a] L = lyophilized; S = lyophilized and sonified.
[b] All surviving paramecium lethargic.
[c] Two-thirds surviving paramecium lethargic.
[d] Evidence of ballooning.

The lyophilized algae showed varying toxicities to *P. caudatum* (Table 1). *Anabaena flos-aquae* and *Nostoc linckia* were nontoxic at all concentrations. *Fischerella epiphytica* was toxic within 8 h at the highest concentration but failed to show toxicity when diluted. *Gloeotrichia echinulata* was the most toxic, causing death or reduced activity at progressively lower concentrations. *Nostoc linckia* became toxic only after sonification. Prior to death, *P. caudatum* ballooned to double its normal size and ceased swimming movement although ciliary movement did not totally subside. After death the membrane remained as a large empty "ghost." This observation was unique to this algal treatment and species.

Anabaena flos-aquae NRC 44 has been shown toxic to mice in laboratory experiments, Gorham et al. (5) reporting an MLD of 640 mg·kg^{-1} of lyophilized material. However, the results from our experiments with the same strain showed no effect when tested on rats at similar concentrations (unpubl.). In addition, no apparent effect on *P. caudatum* was observed. Our findings are in agreement with those of Mills and Wyatt (6), who also failed to show toxicity with *A. flos-aquae* NRC 44 against the ostracod *Cyprinotus incongruens*. Carmichael (pers. comm.) suggested that this strain loses toxicity in culture which may be responsible for the negative results. He and his co-workers recently demonstrated that different clones of *A. flos-aquae* may produce several toxins (1) and their mode of action results in blockage of electrical impulse transmission across the myoneural junction. This leads us to believe that neurotoxin producing strains may be nontoxic to protozoans which is confirmed by Gorham (pers. comm.).

Nostoc linckia was toxic to mice and gerbils (8) at

doses of 600 and 200 mg·kg^{-1}, respectively, but had not previously been tested against aquatic organisms. Our results indicate that whole cells of *N. linckia* failed to produce toxicity but become toxic upon sonification. This is probably the result of an endotoxin which is released at cell lysis. Under natural conditions one might therefore expect it would only be toxic if ingested or rapidly lysed in large quantity. Since *N. linckia* is not a common "bloom" alga, ingestion is a more likely route of intoxication. The swelling of *P. caudatum* may indicate a change in the membrane permeability reminiscent of the swelling observed during uncoupling of oxidative phosphorylation in mitochondria (unpubl.).

Gloeotrichia echinulata is toxic to vertebrates (3) and *Cyprinotus incongruens* in live culture (6). *Gloeotrichia* showed the highest level of toxicity of the four tested. At the lower concentrations death was not noted, but swimming activity ceased and ciliary movement was greatly reduced. The toxic concentration (0.1 mg·ml^{-1}), compares favorably with the findings of Gentile and Maloney (2) for *Bosmina longirostris* using *Aphanizomenon flos-aquae*. They report survival time of ca. 1 h at this concentration, and we have shown death for *P. caudatum* within 2 h using *G. echinulata*.

Fischerella epiphytica was toxic to *P. caudatum* with or without sonification and has shown extremely rapid toxicity to *C. incongruens*.

In general species which are toxic to microcrustaceans are also toxic to *P. caudatum*. The concentrations necessary to demonstrate toxicity to *P. caudatum* vary with the species of alga. This might be attributed to several as yet unproven causes, which include: a) production of toxins which are distinct from one another operating either at different sites or at the same site with different efficiencies; b) different relative amounts of the same toxin among the species; or, c) differences in availability of the toxin(s) as different species may bind the toxin more strongly.

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1. Carmichael, W. W. & Gorham, P. R. 1976. Anatoxins from clones of *Anabaena flos-aquae* isolated from lakes of Western Canada. International Symposium on Experimental Use of Algal Cultures in Limnology. Sandefjord, Norway, 26–28 October. 21 pp.
2. Gentile, J. H. & Maloney, T. E. 1969. Toxicity and environmental requirements of a strain of *Aphanizomenon flos-aquae* (L.) Ralfs. *Can. J. Microbiol.* 15:165–73.
3. Gillam, W. G. 1925. The effect on livestock of water contaminated with freshwater algae. *J. Am. Vet. Med. Assoc.* 67:780–4.
4. Gorham, P. R. 1964. Toxic algae. *In* Jackson, D. F. [Ed.] *Algae and Man*, New York, Plenum Press, 307–36.
5. Gorham, P. R., McLachlan, J., Hammer, U. T. & Kim, W. K. 1964. Isolation and culture of toxic strains of *Anabaena flos-aquae* (Lyngb.) de Brèb. *Int. Ver. Theor. Angew. Limnol. Verh.* 15:796–804.
6. Mills, J. G. & Wyatt, J. T. 1974. Ostracod reactions to nontoxic and toxic algae. *Oecologia Berlin* 17:171–7.
7. Peters, R. A. 1921. The substances needed for the growth for the pure culture of *Colpidium colpoda*. *J. Physiol.* 55:1–32.
8. Phillips, L., Pav, D. I., Mutton, A. F. & Wyatt, J. T. 1973. A preliminary report on the toxicity of the bluegreen alga, *Nostoc linckia*. *J. Tenn. Acad. Sci.* 48:54.
9. Soldo, A. T., Godoy, G. A. & van Wagtendonk, W. J. 1966. Growth of particle bearing and particle free *Paramecium aurelia* in axenic culture. *J. Protozool.* 13:494–7.
10. Stangenberg, M. 1968. Toxic effects of *Microcystis aeruginosa* Kg. extracts on *Daphnia longispina* O. F. Müller and *Eucypris virens* Jurine. *Hydrobiologia* 32:81–7.

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NOTE

ULTRASTRUCTURE OF *NOCTILUCA MILIARIS* (PYRROPHYTA) WITH GREEN FLAGELLATE SYMBIONTS[1,2]

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ABSTRACT

The ultrastructure of the Noctiluca miliaris *Suriray from Southeast Asian waters which contains the green flagellate,* Pedinomonas noctilucae *(Subrahmanyan) Sweeney, is in all major respects similar to that of the European strain. New details of the thecal vesicles, pellicle and underlying microtubules are presented. The possibility that the lipid vesicles are identical with the strongly phase-retarding bodies in the surface cytoplasm, some of which are "microsources" of bioluminescence, is suggested.*

Key index words: bioluminescence; dinoflagellate; Noctiluca; Pedinomonas; *phytoplankton; symbiont; theca*

Noctiluca miliaris Suriray is a large holozoic marine dinoflagellate found worldwide, even in the Chuk-

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[2] This paper is dedicated to Dr. Luigi Provasoli who once said he wished to culture every living thing in the ocean, from flagellate to whale, and in a large measure he has succeeded.