EFFECTS OF *AUREOCOCCUS ANOPHAGEFFERENS* ("BROWN TIDE") ON THE LATERAL CILIA OF 5 SPECIES OF BIVALVE MOLLUSCS

CHRISTY DRAPER,[1] LOUIS GAINEY, [1] SANDRA SHUMWAY,[2,3] AND LYNDA SHAPIRO[3]
[1] Department of Biological Sciences, University of Southern Maine, Portland, ME 04103; [2] Dept. of Marine Resources, W. Boothbay Harbor, ME 04575; [3] Bigelow Laboratory for Ocean Sciences, W. Boothbay Harbor, ME 04575

ABSTRACT

Aureococcus anophagefferens had no effect on the lateral ciliary activity of *Mya arenaria*, *Geukensia demissa*, and *Argopecten irradians*. *Aureococcus* caused a significant decrease in the lateral ciliary activity in isolated gills of *Mercenaria mercenaria*, and *Mytilus edulis*. The lateral cilia of the species that were unaffected by *Aureococcus* were also unaffected by the neurotransmitter dopamine, while the lateral cilia of the species inhibited by *Aureococcus* were also inhibited by dopamine.

INTRODUCTION

During the summers of 1985 and 1986, several concurrent blooms of a previously undescribed chrysophycean alga were reported from Long Island embayments, Narragansett Bay, Rhode Island and Barnegat Bay, New Jersey [1,2,3,4]. The organism was subsequently identified as a chrysophycean alga, *Aureococcus anophagefferens* [2,5]. This species ranges in size from 1.5-2.0 µm in diameter and occurred in concentrations as high as 10^6 cells ml^{-1} which resulted in discoloration of the waters or "brown tide" [1,3].

The effects of these "brown tides" on shellfish populations were immediately evident and, in some cases, catastrophic. Populations of the mussel, *Mytilus edulis*, experienced mortalities of 95% during the bloom and the bay scallop fishery was virtually eliminated by two successive "brown tides" during the summers of 1985 and 1986 in New York; however, the mechanisms responsible for these mortalities remained unclear [6,7].

The efficiency with which bivalve molluscs can retain particles filtered from seawater is known to vary between species of shellfish and is, in most cases, directly related to the size of the algal cells with small cells generally retained less efficiently than larger cells [8,9]. The very small size of *A. anophagefferens* led investigators to determine the effects of this alga on feeding rates in several species of bivalve molluscs. The gills of scallops, *Argopecten irradians*, and mussels, *Mytilus edulis*, captured cells with only 36 and 59% efficiency respectively [7]. Furthermore, feeding rates in both species were significantly depressed when fed cultured *Aureococcus* at bloom densities. Reduced feeding rates have been observed in *M. edulis* and the quahog, *Mercenaria mercenaria*, in the presence of *A. anophagefferens*; reductions in clearance rates by the mussels were independent of particle size and substances dissolved in bloom waters had no effect on clearance rates [10].

Although the small size and high density of *Aureococcus* had detrimental effects on bay scallops, these effects were not enough to account for the starvation experienced by bivalves in the field; these results might be ascribed to toxic effects following more prolonged exposure to bivalves to *Aureococcus* cells [7]. Other authors have recently suggested that chronic toxicity of *A. anophagefferens* cells at high densities might be responsible for the detrimental effects observed in

Toxic Marine Phytoplankton
Edna Granelli et al., Editors

bivalves [10].

In the present study, the effects of exposure to both pure cultures of *A. anophagefferens* on ciliary activity in several species of bivalve molluscs have been examined using isolated gills. Activity of the lateral cilia has been monitored in an effort to assess possible toxic effects of the alga on the feeding mechanisms of bivalve molluscs.

MATERIALS AND METHODS

Original cultures of *Aureococcus anophagefferens* were supplied by E. M. Cosper (MSRC, State University of New York, Stony Brook). The bivalves used in the experiments were obtained from the Maine Department of Marine Resources, Boothbay Harbor, ME. with the exception of *Argopecten irradians* which were supplied by S. Tettlebach (Southampton College, Southampton, N.Y.). Animals were kept at 10~ C on a 12 hr light/dark cycle prior to use. The bivalve gills were removed and placed in bowls with 10 ml of artificial sea water; the gills were used after the appearance of metachronal waves on the lateral cilia, usually within 1 to 12 hrs depending upon the species. All of the gills were observed with a compound microscope; a strobe was used to determine the frequency of ciliary beating. Gills were first observed in artificial sea water; for the treatments, the artificial sea water was replaced with bloom concentrations of brown tide, (approximately 10^6 cells/ml). For each species of bivalve, 15 treatments and 8 controls were used. Readings were taken every hour for 6 hrs and again at 24 hrs. All experiments were performed at 10°C.

Two way repeated measures analysis of variance was used, with time, treatment, and animal nested within treatment as factors. All tests were at the .05 level of significance. Errors are 1 standard error.

RESULTS AND DISCUSSION

Aureococcus had no significant effect on the activity of lateral cilia of the following species of bivalve molluscs: *Mya arenaria*, *Geukensia demissa*, and *Argopecten irradians*. However, *Aureococcus* inhibited the lateral cilia of both *Mercenaria mercenaria*, and *Mytilus edulis*. When *Mercenaria* gills were exposed to *Aureococcus*, the percent original ciliary rate began to decline within an hour of exposure, and reached a minimum of 32% (± 15) 6 hr after exposure. Twenty four hours after exposure the mean ciliary rate had returned to 85% (± 6.5) (Fig. 1). When *Mytilus* gills were exposed to *Aureococcus*, the percent original ciliary rate began to decline within 1 hr and reached a minimum of 48% (± 10) 3 hr after exposure. Twenty four hours after exposure, the mean ciliary rate had returned to 74% (± 15) (Fig. 1). When *Mytilus* gills were exposed to water from which *Aureococcus* had been removed by filtration, there was no effect upon lateral ciliary activity (Fig. 2).

Aureococcus had no effect on lateral ciliary activity in either *Mya arenaria*, *Argopecten irradians*, or *Geukensia demissa*. However, *Aureococcus* inhibited lateral ciliary activity of *Mytilus* and *Mercenaria*. Exogenously applied dopamine had no effect on lateral ciliary activity in *Geukensia demissa* [*11*]. We have found similar results for both *Mya* and *Argopecten* (unpublished results). Dopamine is known to inhibit lateral ciliary acitivity of *Mytilus* [12]; moreover, dopamine as well as the enzymes for its synthesis and degradation are present in the gill [13]. We have also found that exogenously applied dopamine inhibits the lateral ciliary activity of *Mercenaria*. However, water from which *Aureococcus* had been removed by filtration had no effect on clearance rates of *Mytilus*, ruling out the possibility that a brown tide exudate that mimics dopamine is responsible for ciliary inhibition. In addition, dopamine inhibition occurs within minutes of application, not several hours, as seen with *Aureococcus* inhibition. The gills of

Mytilus are known to secrete both chymotrypsin and alpha amylase [14] and this enzyme activity could account for the time lag for inhibition if the gills are digesting part of the cell wall of *Aureococcus* and releasing a dopamine agonist. Further studies with dopamine antagonists and enzyme inhibitors will test this hypothesis. The discrepancy between our results on isolated gills of *Argopecten* and those of Bricelj and Kuentsner [7], who found reduced clearance rates in intact scallops, can be explained by assuming that the compound released from *Aureococcus* has its effects on the nervous system of the animal rather than on the gills, which are unaffected by dopamine.

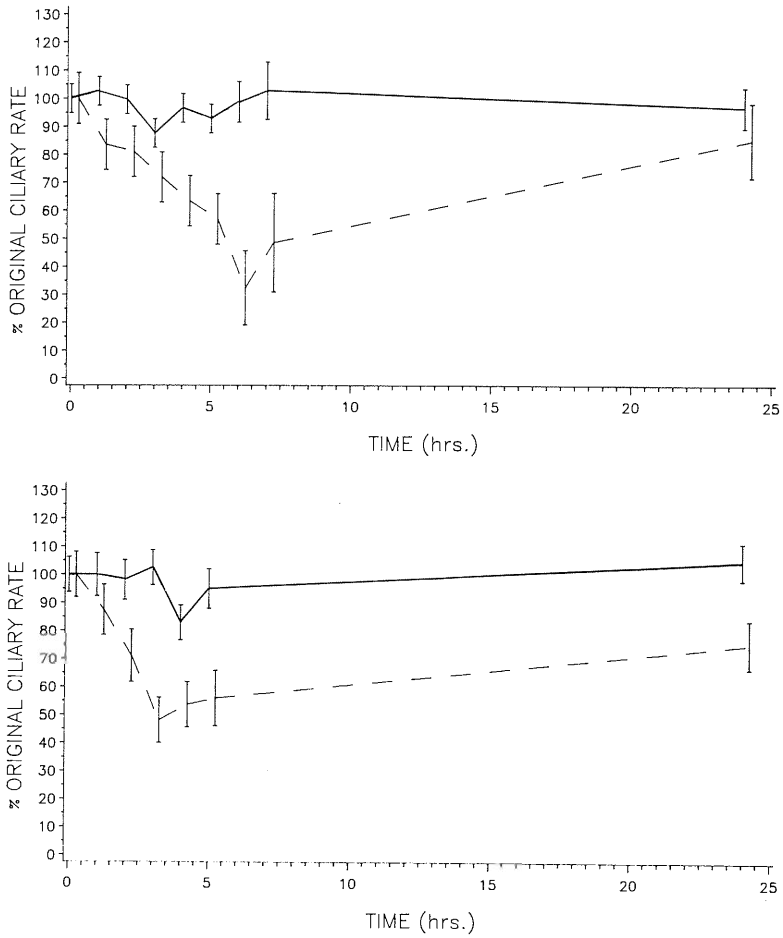

FIG. 1. Percent original ciliary rate of *Mercenaria mercenaria* (top), and *Mytilus edulis* (bottom) exposed to *Aureococcus*. Error bars are ± 1 standard error. Solid line: Control; Dashed line: Exposed to *Aureococcus*.

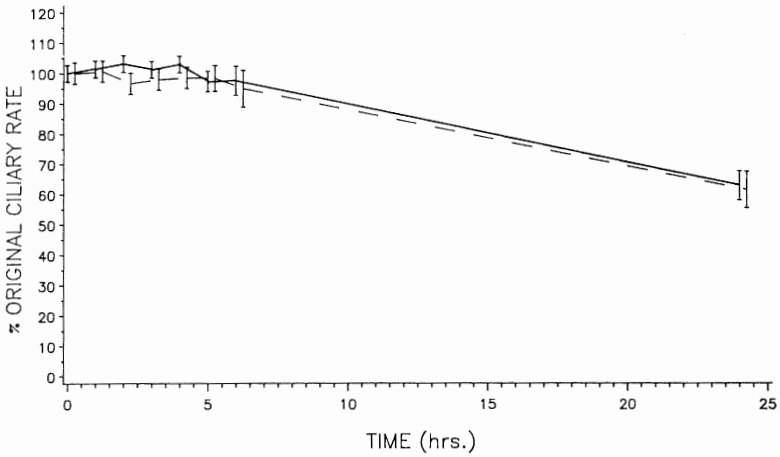

FIG. 2. Percent original ciliary rate of *Mytilus edulis* exposed to water from which *Aureococcus* had been removed with a .8 micron filter. Error bars are ± 1 standard error. Solid line: Control; Dashed line: Exposed to *Aureococcus* "water".

REFERENCES

1. E. M. Cosper, W. C. Dennison, E. J. Carpenter, V. M. Bricelj, J. G. Mitchell, S. H. Kuenstner, D. Colflesh, and M. Dewey, Estuaries *10*, 284-290 (1987).
2. J. McN. Seiburth, P. W. Johnson, and P. E. Hargreaves in: Proc. Emergency Conference on "Brown Tide." (State Dept., New York State, Albany, N.Y., 1986).
3. G. A. Tracey, P. W. Johnson, R. W. Steele, P. E. Hargreaves, and J. McN. Sieburth, J. Shellfish Res. 7, 671-675 (1988).
4. P. Olsen, in: Proc. Emergency Conference on "Brown Tide." (State Dept. New York State, Albany, N.Y., 1986).
5. J. McN. Sieburth, P. W. Johnson, and P.E. Hargreaves, J. Phycol. *24*, 416-425 (1988).
6. G. A. Tracey, Trans. Amer. Geophys. Union *66*, 1303 (1985).
7. V. M. Bricelj, and S. H. Kuenstner in: Notes on Coastal and Estuarine Studies. E. M. Cosper et al, eds. Springer-Verlag, N.Y., (1989).
8. F. Mohlenberg, and H. U. Riisgard, Ophelia *17*, 239-246 (1978).
9. B. L. Bayne, and R. C. Newell, in: The Mollusca v. IV, A.S.M. Saleuddin and K. M. Wilbur, eds. Academic Press, N.Y. (1983) pp. 407-515.
10. G. A. Tracey, Mar. Ecol. Prog. Ser. *50*, 73-81 (1988).
11. E. J. Catapane, Comp. Biochem. Physiol. *75C*, 403-405 (1983).
12. A Paparo, and E. Aiello, Comp. Gen. Pharmacol. *1*, 241-250 (1970).
13. E. J. Catapane, G. B. Stefano, and E. Aiello. J. exp. Biol. *83*, 315-323 (1979).
14. E. Pequignat, Mar. Biol. *19*, 227-244 (1973).