

The Physiological Effect of *Aureococcus anophagefferens* (“brown tide”) on the Lateral Cilia of Bivalve Mollusks*

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Abstract. The “brown tide” alga, *Aureococcus anophagefferens*, had no effect on the activity of the lateral cilia of isolated gills of the bivalve mollusks *Argopecten irradians*, *Geukensia demissa*, and *Mya arenaria*. *A. anophagefferens* caused a significant decrease in the activity of lateral cilia of five other bivalve mollusks, *Crassostrea virginica*, *Ostrea edulis*, *Mercenaria mercenaria*, *Modiolus modiolus*, and *Mytilus edulis*. Exposure of isolated gills of *M. edulis* to the water from which *A. anophagefferens* had been removed, or to polystyrene beads at the same concentration as *A. anophagefferens*, had no effect upon the activity of the lateral cilia. Thus, the inhibition of the lateral cilia is not caused by a compound excreted into the water, nor is it the result of the high density of cells. The response of lateral cilia to dopamine was identical to the response to *A. anophagefferens*; lateral cilia that were inhibited by dopamine were also inhibited by *A. anophagefferens*. Pretreatment of the gills of *M. edulis* with the dopamine antagonist ergometrine blocked the inhibition of the lateral cilia by both dopamine and *A. anophagefferens*. A water-soluble inhibitory compound was released from *A. anophagefferens* by exposing the cells to amylase, and then removing the cells by filtration. The effect of this inhibitory compound was also blocked by ergometrine. We propose that the isolated gills are digesting the extracellular coat of *A. anophagefferens* releasing a water soluble dopamine-mimetic compound that causes inhibition of lateral cilia.

Introduction

Monospecific blooms of a small, previously undescribed, coccoid chrysophycean alga (approximately 2 μm in diameter) began to plague coastal embayments of the northeastern United States during the summer of 1985, with recurrent blooms in some areas continuing through 1990. Concurrent blooms were reported in Gardiners Bay, Great South Bay, and Peconic Bay, Long Island, New York; Narragansett Bay, Rhode Island; and Barnegat Bay, New Jersey (Tracey, 1985; Olsen, 1986; Sieburth *et al.*, 1986; Cospér *et al.*, 1987). Concentrations as high as 10^6 cells ml^{-1} were recorded and resulted in discoloration of the waters and thus the term “brown tide” (Cospér *et al.*, 1987; Sieburth *et al.*, 1988). The organism has since been identified as *Aureococcus anophagefferens* (Sieburth *et al.*, 1986, 1988).

The effects of these “brown tides” on shellfish populations were immediately evident and, in some cases, catastrophic (Shumway, 1990). Bricelj *et al.* (1987) reported recruitment failure and starvation of post-reproductive adult bay scallops, *Argopecten irradians*, in eastern Long Island embayments. Mussels, *Mytilus edulis*, suffered reproductive failure and mortalities ranging from 30 to 100% of natural and transplanted animals in Narragansett Bay, Rhode Island (Tracey, 1988). The mechanisms responsible for these mortalities remain unclear (Tracey, 1985; Bricelj and Kuenstner, 1989). Bricelj and Kuenstner (1989) suggested that the adverse effects of *A. anophagefferens* on bivalve mollusks may be a result of small size, high density, poor digestibility, toxicity, or deficiency in essential micronutrients.

The efficiency with which bivalve mollusks can retain particles filtered from seawater varies between species of

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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 (Mohlenberg and Riisgard, 1978; Bayne and Newell, 1983). The very small size of *A. anophagefferens* led investigators to determine the effects of this alga on feeding rates in several species of bivalve mollusks. Scallops, *A. irradians*, and mussels, *M. edulis*, retained filtered cells with only 36 and 59% efficiency, respectively (Bricelj and Kuenstner, 1989), compared to 100% efficiency when fed cells with a diameter greater than 5 μm (Bayne and Newell, 1983). Furthermore, feeding rates in both species were significantly depressed when fed cultured *A. anophagefferens* at bloom densities. Reduced clearance 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 (Tracey, 1988; Tracey *et al.*, 1990). While specific cell toxins have not been identified from *A. anophagefferens*, there is morphological and physiological evidence to suggest that toxicity is related to a component of the cell surface. (Tracey *et al.*, 1988, 1990; Draper *et al.*, 1990).

Although the small size and high density of *A. anophagefferens* had detrimental effects on bay scallops, these effects were not enough to account for the starvation experienced by scallops in the field; these results might be ascribed to toxic effects following more prolonged exposure to bivalves to *Aureococcus* cells (Bricelj and Kuenstner, 1989). Other authors have also suggested that chronic toxicity of *A. anophagefferens* cells at high densities might be responsible for the detrimental effects observed in bivalve mollusks (Tracey *et al.*, 1988). There is strong evidence that chronic toxicity at high densities, rather than small size, indigestibility, or poor nutritional quality is responsible for the detrimental effects seen in bivalves (Tracey, 1988; Bricelj and Kuenstner, 1989; Bricelj *et al.*, 1989; Gallager *et al.*, 1989; Draper *et al.*, 1990). Recent studies indicate that direct contact with *Aureococcus* cells is necessary to inhibit grazing (Tracey, 1988; Gallager *et al.*, 1989; Ward and Targett, 1989; Draper *et al.*, 1990).

The control of lateral ciliary activity in bivalve mollusks, in particular *Mytilus edulis*, has been studied extensively during the last 70 years, and there is considerable evidence that dopamine is an inhibitory neurotransmitter involved in regulation of lateral cilia (for a review of early work, see Aiello, 1960). Exogenously applied dopamine inhibits the lateral cilia of *M. edulis* and *Crassostrea virginica* but not *Geukensia demissa* (Paparo and Aiello, 1970; Catapane, 1983; Paparo, 1985). In addition, high frequency stimulation of the cerebro-visceral nerve connective of *M. edulis* resulted in inhibition of the lateral

cilia; this response was abolished by pretreating the gill or visceral ganglion with the antagonist ergometrine, which also blocked the effects of exogenously applied dopamine (Catapane *et al.*, 1978, 1979). Dopamine has been localized in the branchial nerve fibers of *M. edulis*, and these fibers appear to make synaptic contact with the lateral ciliary cells (Stefano and Aiello, 1975; Paparo, 1972). Paparo and Finch (1972) showed that isolated gills metabolized DOPA and tyrosine to dopamine.

Calcium has also been shown to regulate the activity of lateral cilia. For example, the lateral cilia of isolated gills and triton-extracted cells will beat in the absence of extracellular calcium, while an increase in the concentration of intracellular calcium will cause inhibition of the lateral cilia (Walter and Satir, 1978; Paparo, 1980; Murakami, 1983; Stommel and Stevens, 1985). Paparo and Murphy (1975) proposed that the inhibitory effects of dopamine on the lateral cilia are mediated by an influx of calcium, and studies on mammalian systems support this hypothesis (Ahlijanian and Cooper, 1988; Felder *et al.*, 1989; Mahan *et al.*, 1990).

In the present study, we examined the effects of *Aureococcus anophagefferens* and dopamine on the activity of the lateral cilia of eight species of bivalve mollusks. In addition, we examined the effects of polystyrene microspheres, the culture media from which *A. anophagefferens* had been removed by filtration, and the culture media from which *A. anophagefferens* treated with amylase had been removed by filtration on the activity of the lateral cilia of *Mytilus edulis*. Preliminary results of this study were presented at the Fourth International Conference on Toxic Marine Phytoplankton (Draper *et al.*, 1990) and to the American Society of Zoologists (Gainey and Shumway, 1989).

Materials and Methods

The original non-axenic culture of *Aureococcus anophagefferens* was supplied by E. M. Cosper (MSRC, State University of New York, Stony Brook). The culture was maintained at 18°C in an f/2 medium with .01 mM disodium glycerophosphate added and sodium silicate omitted (Guillard, 1975). Cells were harvested during the late exponential growth phase as toxicity varies in relation to environmental growth conditions (Tracey *et al.*, 1990). Adult specimens of bivalves were collected at the following localities: *Mytilus edulis*, Falmouth; *Mya arenaria*, Boothbay Harbor; *Modiolus modiolus*, Damariscotta River; *Crassostrea virginica* and *Ostrea edulis*, Mook Sea Farms, Damariscotta (all in Maine); *Argopecten irradians*, Shinnecock Bay, Southampton, New York; *Geukensia demissa*, Sakonnet River, Tiverton, Rhode Island; and *Mercenaria mercenaria*, Wachapreague, Virginia. With the exception of *Mytilus edulis*, all animals were main-

tained at the Maine Department of Marine Resources Laboratory, Boothbay Harbor, in running seawater at temperatures ranging from 9 to 12°C and a salinity of 33 ‰. Prior to use in experiments, animals were held at 10°C, 30 ‰ salinity on a 12 h light-dark cycle at the University of Southern Maine, Portland, for periods ranging from 1 week to a month.

Gills were dissected distal to the visceral ganglion and were then separated into demibranchs. The isolated demibranchs were pinned to strips of rubber band that had been glued to the bottom of petri dishes. The dishes were filled with 10 ml of artificial seawater with an ionic composition as follows: CaCl₂ = 10 mM, KCl = 9.8 mM, MgCl₂ = 53 mM, NaBr = .57 mM, NaCl = 400 mM, NaHCO₃ = 2.5 mM, Na₂SO₄ = 28 mM (ASW, Welsh *et al.*, 1968). The gills were used after the appearance of metachronal waves on the lateral cilia, usually within 1 to 12 h after dissection, depending upon the species.

The gills were observed at a magnification of 100× with a compound microscope with the substage illuminator replaced with a mirror. The rate of lateral ciliary beating was determined using a Pasco Sf-9211 strobe driven by a Grass SD-9 stimulator. The gills were initially viewed at a flashing rate of 60 Hz, which was above the frequency of flicker fusion for our eyes; the flashing rate of the strobe was then decreased until the metachronal waves appeared motionless in the shape of a horseshoe. At harmonics above the actual ciliary rate, the wavelength of the metachronal waves appeared shorter than the true wavelength and the waves appeared to flicker (Catapane *et al.*, 1978, pers. obs.). Harmonics below the actual ciliary rate were avoided by always starting the strobe at a higher frequency than the actual rate.

Gills were first observed in artificial sea water; for the treatments, the artificial sea water was replaced with *A. anophagefferens* at concentrations of approximately 10⁶ cells/ml. Readings were taken every hour for at least 5 h and again at 24 h for *Geukensia demissa*, *Mya arenaria*, *Mercenaria mercenaria*, and *Mytilus edulis*. Due to the fragility of the gills in *Argopecten irradians*, *Crassostrea virginica*, *Ostrea edulis*, and *Modiolus modiolus*, it was not possible to take readings at 24 h. All experiments were performed at 10°C.

Effects of dissolved exudates

Suspensions of *A. anophagefferens* were filtered through a 0.8 μm filter using the minimal vacuum possible to maintain flow through the filter; the filtered cells were viewed microscopically to insure that cell lysis did not occur. This filtrate was added to isolated gills of *Mytilus edulis* and ciliary rates were measured as described previously.

Effects of particulates

Isolated gills of *M. edulis* were exposed to 2.9 μm diameter polystyrene microspheres at a concentration of 7.85 × 10⁶ spheres/ml. The microspheres were obtained from Polysciences, Inc., Warrington, Pennsylvania.

Effects of dopamine

The effects of dopamine on the lateral cilia of *Mytilus edulis*, *Geukensia demissa*, and *Crassostrea virginica* have been described previously (Paparo and Aiello, 1970; Catapane, 1983; Paparo, 1985). We tested the effects of dopamine on these bivalves as well as the others used in this study. Isolated gills were exposed to 10⁻⁴ M dopamine in ASW; this concentration is an order of magnitude greater than necessary to cause complete lateral ciliary arrest in *Mytilus edulis* (Catapane, 1983). The presence or absence of metachronal waves was noted immediately after the addition of dopamine and every 2 min thereafter for 20 min. If the lateral cilia ceased beating within this period, the response to dopamine was considered positive.

Effect of ergometrine

Catapane *et al.* (1978) demonstrated that application of 10⁻⁵ M ergometrine (ergonovine) to the isolated gills of *Mytilus edulis* had no effect on the rate of previously beating lateral cilia. Catapane *et al.* (1979) also demonstrated that application of 10⁻⁵ M ergometrine to the visceral ganglion blocked the lateral ciliary inhibition brought about by the addition of 10⁻⁴ M dopamine to the visceral ganglion if the ergometrine was added 10 min prior to the addition of dopamine. To determine if ergometrine would also block the lateral ciliary inhibition brought about by exposure of the isolated gills of *M. edulis* to 10⁻⁴ M dopamine, we exposed isolated gills to 10⁻⁵ M ergometrine. We then added fresh 10⁻⁴ M dopamine in ASW hourly for 4 h and again at 24 h. Both ergometrine and dopamine were purchased from Sigma Chemical Co.

Effects of amylase

Cultures of *A. anophagefferens* were exposed to a saturated solution of alpha amylase (Type I-A, Sigma Chemical Co, 4 mg/ml) for at least 16 h. The *Aureococcus* amylase mixture was then filtered through an 0.8 μm filter as described previously. Isolated gills of *M. edulis* were exposed to amylase at the same concentration to test the effects of amylase on lateral ciliary activity.

Ciliary rates were expressed as a percentage of the initial rate of each isolated gill preparation. The effects of various experimental treatments were evaluated using an F test, with the mean square for experimental treatment as a numerator and the mean square for animal nested within treatment, where animal was a class variable that repre-

sented repeated measures on the same piece of gill, as an error term. Superscripts and subscripts for F values represent degrees of freedom for the numerator and denominator. The Type III mean squares used for this test were generated using the model: percent initial rate = treatment time animal (treatment). The statistical procedures were performed using the general linear model program (Proc GLM) in version 5 of the Statistical Analysis System (SAS). Tests were considered significant at probabilities less than 0.05. Errors and error bars are ± 1 standard error. Although readings were taken on experimental and control groups at the same time intervals, the groups have been offset by 15 min in all figures for the sake of clarity.

Results

Basal ciliary rates

The mean lateral ciliary rate of the eight species of bivalves prior to exposure to *Aureococcus anophagefferens* ranged from a low of 17 Hz in *Geukensia demissa* to a high of 25 Hz in *Crassostrea virginica* (Table I).

The response of bivalve gills to *A. anophagefferens*

Aureococcus anophagefferens had no significant effect on the activity of the lateral cilia of the following species of bivalve mollusks: *Argopecten irradians* ($F_{20}^1 = .07$, $P = .79$), *Geukensia demissa* ($F_{24}^1 = .98$, $P = .33$), and *Mya arenaria* ($F_{16}^1 = .00$, $P = .97$, Fig. 1).

A. anophagefferens inhibited the lateral cilia in the following species of bivalves: *Crassostrea virginica* ($F_{30}^1 = 36.44$, $P = .0001$), *Ostrea edulis* ($F_{30}^1 = 35.73$, $P = .0001$), *Mercenaria mercenaria* ($F_{21}^1 = 7.30$, $P = .01$), *Modiolus modiolus* ($F_{22}^1 = 36.44$, $P = .001$), and *Mytilus edulis* ($F_{22}^1 = 13.91$, $P = .001$, Fig. 1). All of these species exhibited depressed lateral ciliary activity within 1 h after exposure to *A. anophagefferens*, with the exception of *M. modiolus*, where the mean rate did not begin to decline until 3 h after exposure.

The time until maximal ciliary inhibition, and the magnitude of the inhibition, was species specific. For example, in *M. edulis* the mean rate declined to 48% (± 10.2 , $n = 16$) of the initial rate 3 h after exposure. In *C. virginica*, the mean rate declined to only 75% (± 3.4 , $n = 24$) of the initial rate 4 h after exposure, while in *M. modiolus* the mean rate declined to 89% (± 0.86 , $n = 18$) of the initial rate 5 h after exposure. In both *O. edulis* and *M. mercenaria*, the mean rates were 78% (± 1.4 , $n = 24$) and 32% (± 15 , $n = 15$) of the initial rates respectively, 6 h after exposure.

In *M. edulis* and *M. mercenaria*, the gills were hardy enough to carry out observations for longer periods of time than was possible for some of the other species. Twenty-four hours after exposure, the mean, ciliary rate

Table I

Mean lateral ciliary rates (beats/s), rounded to the nearest integer, of bivalves used in this study

| Species | Mean rate (beats/s) | Standard error | n |
|------------------------------|---------------------|----------------|----|
| <i>Argopecten irradians</i> | 24 | 1.6 | 22 |
| <i>Geukensia demissa</i> | 17 | .47 | 26 |
| <i>Mya arenaria</i> | 20 | 1.42 | 18 |
| <i>Crassostrea virginica</i> | 25 | .12 | 32 |
| <i>Ostrea edulis</i> | 24 | .29 | 32 |
| <i>Mercenaria mercenaria</i> | 20 | .45 | 23 |
| <i>Modiolus modiolus</i> | 20 | .08 | 24 |
| <i>Mytilus edulis</i> | 19 | .74 | 25 |

of *M. mercenaria* had risen to 85% (± 6.5 , $n = 15$), while the controls had a mean rate of 96% (± 3.7 , $n = 8$) of the initial rate. Twenty-four hours after exposure, the mean rate of *M. edulis* had risen to 94% (± 15 , $n = 16$), while the controls had a mean rate of 108% (± 7.3 , $n = 9$) of the initial rate.

The nature of ciliary inhibition in *Mytilus edulis*

To determine if ciliary inhibition was caused by an exudate of *A. anophagefferens*, we exposed isolated gills of *M. edulis* to seawater from which *A. anophagefferens* had been removed by filtration. This water had no effect upon lateral ciliary activity ($F_{16}^1 = .56$, $P = .46$, Fig. 2).

To determine if ciliary inhibition was caused by the presence of high concentrations of particulate matter, we exposed isolated gills of *M. edulis* to polystyrene microspheres. The microspheres had no effect upon lateral ciliary activity ($F_{15}^1 = .69$, $P = .42$, Fig. 3).

All of the species tested that showed lateral ciliary inhibition upon exposure to *A. anophagefferens* also showed lateral ciliary inhibition upon exposure to 10^{-4} M dopamine; moreover, those species that did not have lateral ciliary inhibition upon exposure to *A. anophagefferens* were not inhibited by exposure to dopamine (Table II).

To determine if the inhibition of lateral cilia caused by exposure to *A. anophagefferens* was caused by dopamine, or a dopamine agonist, we first exposed isolated gills to 10^{-5} M ergometrine and then to fresh 10^{-4} M dopamine hourly for 4 h and again at 24 h to test whether ergometrine would block the inhibition brought about by dopamine. There was no difference in the rates of these gills after these treatments ($F_{20}^1 = .03$, $P = .99$), i.e., the single dose of ergometrine blocked the effects of dopamine for 24 h. We then exposed isolated gills of *M. edulis* to 10^{-5} M ergometrine for 10 min prior to exposure to *A. anophagefferens*. Gills pretreated with ergometrine showed no inhibition when exposed to *A. anophagefferens*, in

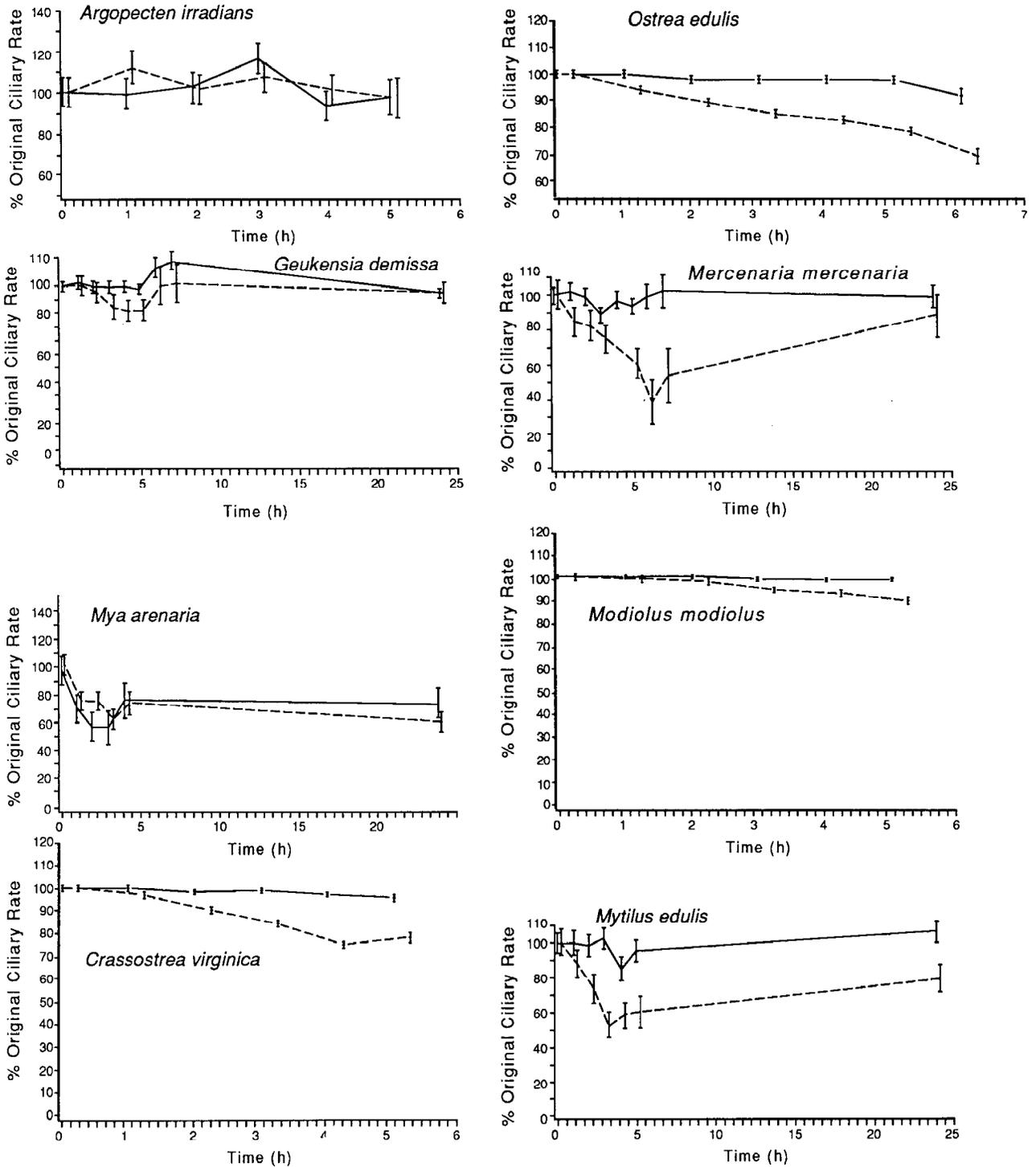


Figure 1. The effect of *Aureococcus anophagefferens* (brown tide) on the lateral cilia of eight species of bivalve mollusks. Solid line: control (c); dashed line: brown tide (BT). Number of gills: *Argopecten*: c = 9, BT = 13; *Geukensia*: c = 9, BT = 17; *Mya*: c = 6, BT = 12; *Crassostrea* and *Ostrea*: c = 8, BT = 24; *Mercenaria*: c = 8, BT = 15; *Modiolus*: c = 6, BT = 18; *Mytilus*: c = 9, BT = 16. Error bars are ± 1 standard error. Although readings were taken at the same times for both controls and treatments, they have been offset for the sake of clarity in this and subsequent figures.

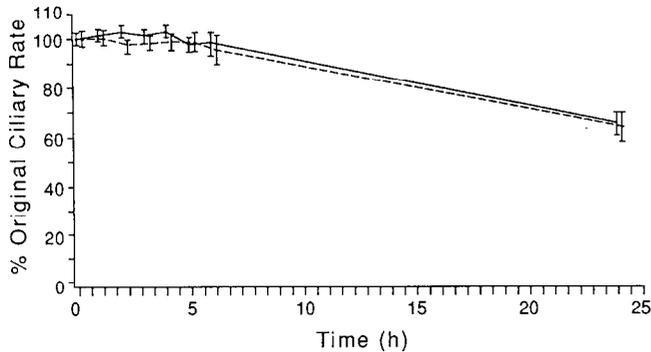


Figure 2. The effect of water, from which *Aureococcus anophagefferens* had been removed by filtration, on the lateral cilia of *Mytilus edulis*. Solid line: control (n = 6); dashed line: treatment (n = 12). Error bars are ± 1 standard error.

contrast to gills exposed to *A. anophagefferens* without a pretreatment of ergometrine ($F_{14}^1 = 16.49$, $P = .001$, Fig. 4).

We tested the hypothesis that the isolated gills were digesting the extracellular polysaccharide coat of *A. anophagefferens*, which was described by Sieburth *et al.* (1988), by exposing cultures of *A. anophagefferens* to amylase. Exposure of isolated gills to seawater containing amylase had no effect upon lateral ciliary activity ($F_{15}^1 = .12$, $P = .74$). Exposure of isolated gills of *M. edulis* to filtered seawater which had contained *A. anophagefferens* treated with amylase resulted in decreased lateral ciliary activity ($F_{16}^1 = 7.19$, $P = .02$, Fig. 5). There was no difference in the activity of lateral cilia of isolated gills exposed to the enzymatically treated *A. anophagefferens* filtrate or to untreated *A. anophagefferens* ($F_{14}^1 = .02$, $P = .88$).

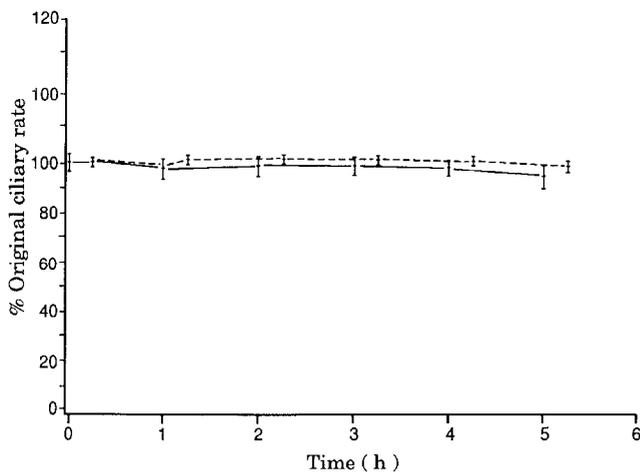


Figure 3. The effect of polystyrene microspheres on the lateral cilia of *Mytilus edulis*. Solid line: control (n = 8); dashed line: treatment (n = 11). Error bars are ± 1 standard error.

Table II

Summary of responses of lateral cilia to *Aureococcus anophagefferens* and exogenously applied 10^{-4} M dopamine

| Species | Response to <i>Aureococcus</i> | Response to dopamine |
|------------------------------|--------------------------------|----------------------|
| <i>Argopectin irradians</i> | — | — |
| <i>Geukensia demissa</i> | — | — |
| <i>Mya arenaria</i> | — | — |
| <i>Crassostrea virginica</i> | inh | inh |
| <i>Ostrea edulis</i> | inh | inh |
| <i>Mercenaria mercenaria</i> | inh | inh |
| <i>Modiolus modiolus</i> | inh | inh |
| <i>Mytilus edulis</i> | inh | inh |

— = no response, inh = inhibition.

To test if the inhibition was caused by the same type of compound in the experiment with the enzymatically treated *A. anophagefferens* filtrate and the experiments with intact *A. anophagefferens*, we exposed isolated gills to ergometrine, as described previously, then exposed the gills to the water from *A. anophagefferens* treated with amylase. The ergometrine blocked the inhibition of the lateral cilia brought about by the *A. anophagefferens* amylase filtrate ($F_{12}^1 = 5.39$, $P = .04$, Fig. 6). In addition, there was no difference in the rates of untreated gills, gills exposed to ergometrine and the *A. anophagefferens* amylase filtrate, and gills exposed to ergometrine and intact *A. anophagefferens* ($F_5^2 = 1.51$, $P = .31$).

Discussion

The bivalves whose lateral cilia were inhibited by *A. anophagefferens* were the same species whose lateral cilia were inhibited by dopamine. There are three possibilities

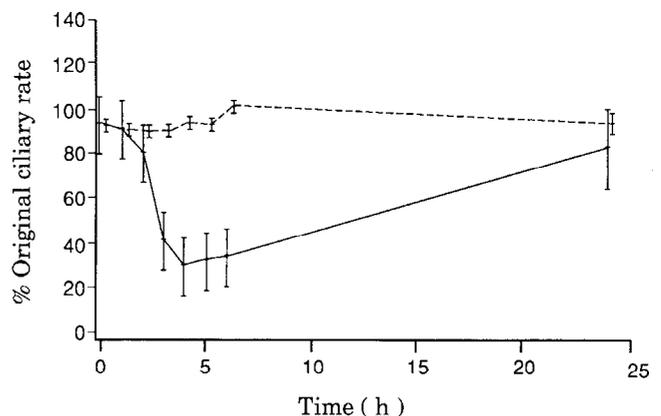


Figure 4. The effect of exposing the gills of *M. edulis* to 10^{-5} M ergometrine and then to *Aureococcus anophagefferens*. Solid line: control (exposed to *A. anophagefferens*, n = 8); dashed line: treatment (n = 10). Error bars are ± 1 standard error.

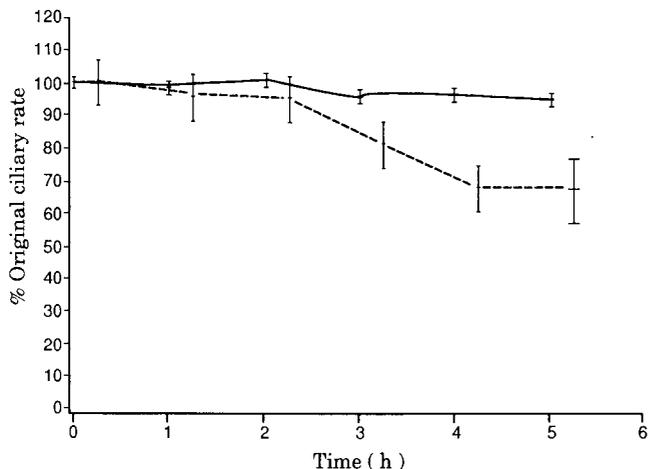


Figure 5. The effect of exposing *Aureococcus anophagefferens* to amylase, filtering the water, and exposing the gills of *Mytilus edulis* to the filtrate. Solid line: control (n = 9); dashed line: treatment (n = 10). Error bars are ± 1 standard error.

that could explain this correlation. First, *A. anophagefferens* could release dopamine upon contact with the bivalve gill. We think that this is unlikely because the oxidation products of dopamine are readily visible in seawater (pers. obs.) and we have not observed this characteristic discoloration in any of our experiments with either intact *A. anophagefferens*, or with the water from enzymatically treated *A. anophagefferens*. Moreover, the inhibitory effects of exogenously applied dopamine typically appear within 2 to 3 min and disappear within 20 to 30 min after application (Catapano *et al.*, 1978; pers. obs.), while the effects of the water from enzymatically treated *A. anophagefferens* did not appear for several hours and persisted for at least 5 h. Second, the inhibition could be caused by a dopamine-mimetic compound. Third, the inhibition could be the result of the stimulation of branchial nerve fibers in the gill. These fibers innervate the lateral ciliated cells and contain dopamine (Paparo, 1972; Stefano and Aiello, 1975). Our experiments cannot distinguish between these latter two possibilities because the dopamine antagonist ergometrine would block the inhibition of the lateral cilia brought about by exposure to *A. anophagefferens* in either case. We think that it is unlikely that the effects of *A. anophagefferens* are brought about by a direct influx of calcium into the lateral ciliated cells. If this were the case, inhibition of the lateral cilia would probably have occurred in all of the species tested, and the effects would not have been blocked by ergometrine.

Tracey (1988) found decreased clearance rates in both *Mytilus* and *Mercenaria* exposed to *A. anophagefferens*, and our results on isolated gills are parallel to his. Bricelj and Kuenstner (1989) found that *A. anophagefferens* inhibited clearance rates in juvenile *Argopecten irradians*; however, we found no inhibition of isolated gills of adult

A. irradians. Several possibilities exist for this discrepancy. Sensitivity to *A. anophagefferens* could be age dependent, or the *A. anophagefferens* dopamine-mimetic could interact directly with the visceral ganglia in intact scallops to bring about lateral ciliary inhibition via nerves innervating the gills rather than acting directly on the gills, because the lateral cilia of isolated scallop gills are unaffected by dopamine.

Physical contact between a bivalve and *A. anophagefferens* is apparently necessary for inhibition. Tracey (1988) found no inhibition of clearance rates when *M. edulis* were exposed to Narragansett Bay water from which *A. anophagefferens* had been removed by filtration. Ward and Targett (1989) found similar results with *M. edulis* exposed to filtrates of cultured *A. anophagefferens*. We have found similar results with isolated gills. Taken together, these results indicate that *A. anophagefferens* is not excreting an inhibitory compound into the water. In addition, Ward and Targett (1989) found that exposing *M. edulis* to lysed cell filtrates of *A. anophagefferens* had no effect on clearance rates, indicating that the inhibitory compound is not intracellular in origin.

The inhibition of ciliary activity of isolated gills, and decreased clearance in whole animals, is not caused by the high density of small particulates present during a brown tide. Tracey (1988) found no inhibition of clearance in *M. edulis* exposed to high densities of kaolin clay particles, and we have found similar results with isolated gills exposed to polystyrene microspheres.

Pequignat (1973) found that the gills of *M. edulis* secrete amylase, and Sieburth *et al.* (1988) described *A. anophagefferens* as having "... a diffuse, sometimes copious layer of exocellular organic material which is probably

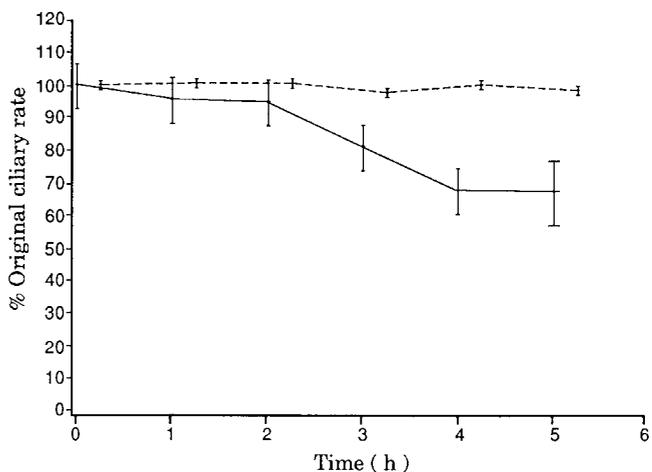


Figure 6. The effect of pretreating the gills of *Mytilus edulis* with 10^{-5} M ergometrine and then exposing the gills to the filtrate of *A. anophagefferens* treated with amylase. Solid line: filtrate of *A. anophagefferens* treated with amylase (n = 10); dashed line: treatment (n = 4). See text for further control comparisons.

polysaccharide. . . ." Our results demonstrate that the inhibitory compound is released upon exposure of *A. anophagefferens* to amylase. That this inhibitory compound is similar to that released upon exposure of intact *A. anophagefferens* to bivalve gills is indicated by the blockage of inhibition by pre-treatment of the gills with ergometrine in both instances.

The time for inhibition of clearance rates in bivalves exposed to *A. anophagefferens* is much faster than the time for inhibition of the lateral cilia of isolated gills. Tracey (1988) found inhibition of clearance rates after only 20 min of exposure to *A. anophagefferens*, whereas we found that the time for inhibition was much slower in isolated gills. These differences should, in fact, be expected for the following reason. The ingestion of *A. anophagefferens* by whole animals would result in the faster digestion of the extracellular coat in comparison to the digestion by isolated gills because digestion would occur not only on the gills but also in the digestive system of a whole animal.

In summary, we propose that the inhibition of clearance in bivalve mollusks exposed to *A. anophagefferens* is brought about by the digestion of the extracellular coat, releasing a dopamine-mimetic compound, which brings about the arrest of the lateral cilia in certain species.

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