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The effects of the toxic dinoflagellate *Protogonyaulax tamarensis* on the feeding and behaviour of bivalve molluscs*

Sandra E. Shumway[1,2] and Terry L. Cucci[1]

[1]*Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine, U.S.A.; and* [2]*Department of Marine Resources, West Boothbay Harbor, Maine, U.S.A.*

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A series of experiments was carried out to assess the effects of the toxic dinoflagellate *Protogonyaulax tamarensis* on shell-valve activity, rates of particle clearance, preingestive, and postingestive particle selection in seven species of bivalve molluscs from three geographic localities: Maine, Rhode Island, and Spain. The responses observed were species-specific and varied with collection locality. Responses included shell-valve closure and/or siphon retraction (*Mya arenaria, Mytilus edulis, Geukensia demissa*), reduced rates of particle clearance (*M. arenaria, G. demissa*), increased rates of particle clearance (*Ostrea edulis*), production of mucus (*M. edulis* from Spain and Rhode Island, *Placopecten magellanicus, G. demissa*). Mortalities were noted in *M. edulis* from both Spain and Rhode Island. Two species (*Modiolus modiolus and Spisula solidissma*) exhibited no effects of the toxic dinoflagellates. Data are also presented for particle selection, clearance and filtration rates for the seven species. It is suggested that species which are periodically exposed to dinoflagellate blooms may have evolved mechanisms permitting them to exploit the toxic organisms as food with no ill effects.

Key words: Toxic dinoflagellate; Bivalve mollusc; Filtration/clearance rate; Behavior

INTRODUCTION

Toxic marine dinoflagellate blooms have long been recognized to have a significant impact on the utilization of shellfish resources (LoCicero, 1975; Taylor and Seliger, 1979; Anderson et al., 1985). Many commercially important, filter-feeding bivalve molluscs accumulate the toxins in their tissues rendering them vectors of paralytic shellfish poisoning (PSP) and unfit for human consumption. PSP is a

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Correspondence to: S.E. Shumway, Dept. of Marine Resources, West Boothbay Harbor, ME 04575, U.S.A.

* Bigelow Laboratory for Ocean Sciences contribution 87003.

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serious, sometimes fatal illness induced by consuming shellfish that have ingested large quantities of toxic dinoflagellates and has been extensively reviewed (see Russell, 1965, 1984; Halstead, 1965; and Schantz, 1975).

In the western Atlantic, the predominant toxic dinoflagellate (causative organism in PSP) is *Protogonyaulax (= Gonyaulax) tamarensis*. Blooms occur annually on the eastern coasts of Canada and the northeastern U.S.A. between May/June and September/October. Filter-feeding bivalve molluscs accumulate the dinoflagellates, usually in their digestive glands. During this time, extensive monitoring of commercially harvested shellfish takes place and the closure of many productive shellfish areas becomes necessary. The ensuing bans on shellfish harvesting result in substantial financial losses to the fishermen as well as decreased supplies of shellfish to the consumers.

While the toxic dinoflagellates and the threat to public health caused by their presence have been widely studied, very little attention has been given to the effects of these organisms on their molluscan hosts. It has, in fact, been generally assumed that the dinoflagellates have little or no effect(s) on the general well-being of these animals (Prakash et al., 1971; Quayle, 1969).

The ability of shellfish to accumulate toxins may be largely dependent upon filtration rates and the density and distribution of the toxic dinoflagellates as well as the individual species' capabilities for selective ingestion and/or absorption (Gilfillan and Hanson, 1975; Shumway et al., 1985a). A number of authors have shown that various species of dinoflagellates have an adverse effect on, or are actively selected for/against by filter-feeding molluscs; e.g., *Mytilus californianus* has been shown to selectively ingest both toxic and nontoxic dinoflagellates in natural sea water (Buley, 1936; Fox and Coe, 1943), although these cells may pass through the gut unchanged and in viable condition within the fecal material. It has been reported that *M. californianus* shows a vigorous uptake of toxins when exposed to *Protogonyaulax catenella* (Dupuy and Sparks, 1968). In contrast, *Mytilus edulis* exposed to the toxic dinoflagellate *Gyrodinium aureolum* Hulbert showed a reduction in clearance rates associated with direct toxic effects from the dinoflagellates and not by the toxins released into the water (Widdows et al., 1979).

Unlike *M. edulis*, other species of bivalve molluscs have exhibited more negative reactions to the presence of toxic dinoflagellates. Shell-valve closure (Dupuy and Sparks, 1968; Smith, 1958; Ballantine and Morton, 1956; Sievers, 1969; Ray and Aldrich, 1967; Shumway et al., 1985b), reduced pumping/filtering rates (Dupuy and Sparks, 1968; Ray and Aldrich, 1967; Helm et al., 1974; Gilfillan and Hanson, 1975), and selection against these toxic dinoflagellates by formation of pseudofeces (Dupuy and Sparks, 1968; Smith, 1958) are major responses of bivalve molluscs when exposed to toxic dinoflagellates. In some areas, mass mortalities of bivalve molluscs have been reported as a result of dinoflagellate blooms (Horstman, 1981; Adams et al., 1968; Ingham et al., 1968; Ceurvels and Der Hovanesian, 1972). Further, toxicity levels are known to vary between species at a given locality, e.g. *M.*

edulis was found to be six times more toxic than *M. arenaria* during an outbreak of PSP (Gilfillan and Hanson, 1975). It has also been observed that it takes up to 10 days longer for *M. arenaria* to become toxic than for *M. edulis* from the same location (J.W. Hurst, pers. comm.), probably due to the retraction of the siphons in the presence of *P. tamarensis* demonstrated by Shumway et al. (1985b).

In spite of these data, the statement that *P. tamarensis* has no effect on the host organisms is still common. This statement needs not only clarification but verification. Our previous studies on selective feeding (Cucci et al., 1985; Shumway et al., 1985a) and on the effects of *P. tamarensis* on the behaviour and physiology of bivalve molluscs (Shumway et al., 1985b) have demonstrated that these animals display an array of adaptations and responses to various food sources and that these responses cannot be generalized for the group as a whole.

The use of animal behaviour as a monitoring tool has been discussed at length by Olla et al. (1980a, b). Environmental monitoring involves the detection and measurement of abnormal effects of some foreign substance on resident populations of animals. Since behaviour tends to be adaptive, it represents activity patterns acquired through natural selection during the evolutionary history of an animal. The acquired adaptations may render the animals capable of coping with, or completely avoiding, naturally occurring environmental perturbations. Induced short-term changes in the environment may, however, induce changes in behaviour that are nonadaptive. These behavioural changes, those which vary from established norms, may provide an added indicator of the level of environmental stress invoked and may prove useful in monitoring programs.

The present study is part of an integrated study of the effects of *P. tamarensis* on the behavior and physiology of a number of commercially important bivalve molluscs. Our objectives in the present investigation are to determine, in the presence of the toxic dinoflagellate *P. tamarensis*, (a) behavioral responses, (b) preingestive vs. postingestive particle selection, (c) clearance rates, (d) preferential selection for or against *P. tamarensis*, and (e) the possible role of *P. tamarensis* as a food source in a number of commercially important bivalve molluscs.

MATERIALS AND METHODS

Specimens of the following bivalve molluscs were collected at various localities in Maine: *Crassostrea virginica* (Gmelin) (Damariscotta River, Walpole), *Mya arenaria* L. (Long Cove, Searsport), *Mytilus edulis* L. (Boothbay Harbor), *Ostrea edulis* L. (Dodge Cove, Damariscotta River), *Placopecten magellanicus* (Gmelin) (lower Damariscotta River), and *Spisula solidissima* Dillwyn (Rockland). In addition, specimens of *M. edulis* and *Geukensia demissa* were collected from the Sakonnet River, Tiverton, Rhode Island. A further sample of *M. edulis* was brought from La Coruna, Spain. These imported animals were assumed to have had no prior exposure to *Protogonyaulax tamarensis*. Animals were transported to the laboratory

immediately and scrubbed to remove all epiphytes. The animals were maintained in sand-filtered, running sea water from Boothbay Harbor at 15°C prior to use in experiments. The sand filter removed all particles larger than 20 μm, i.e., *P. tamarensis*. No supplementary food was given to the animals.

Feeding experiments

Before each experiment, all animals were purged in filtered sea water (0.7-μm Gelman glass fiber) for 24 h and the water changed at least once. All experiments were carried out at the same time of day (early a.m.) at 12°C. Individual animals were placed in bell jars containing the algal mixture (see below) which were gently aerated. Control vessels were left without animals to correct for algal cell division during experiments. Experiments lasted for 1 h and samples were taken for flow cytometric analyses at 30- and 60-min intervals. Any pseudofeces produced during the experiments were collected and also analyzed. At the end of 1 h, the animals were removed from the feeding media and placed in filtered sea water (same as above) for feces collection (which usually occurred after 4 h). Feces and pseudofeces were collected with glass Pasteur pipettes and all samples were observed under a fluorescence microscope prior to flow cytometric measurements.

The above procedure was carried out on Day 1 of the experiment when the animals were given an algal mixture only. The animals were then purged in filtered sea water overnight and used on the following day when they were fed a similar algal mixture to that of Day 1 with *Protogonyaulax* added. The experimental procedure was identical to that already described.

Algal cultures were supplied from the Culture Center for Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, and consisted of the following: the dinoflagellate *Prorocentrum minimum* (Pavillard) Schiller (clone Exuv) which is 5–6.15 × 8.75–12.5 × 12.5–15 μm in size, the cryptomonad flagellate *Chroomonas salina* (Wislouch) Butcher (clone 3C) which is 6.25–7.5 × 8.75–12.5 μm in size, *Thalassiosira pseudonana* (Hustedt) Hasle et Heimdal (clone 3H) which is 3–4 μm in size and *P. tamarensis* var. *excavata* (Lebour) Taylor (clone GT429) which is 30–45 μm in size. Cultures were grown in f/2 media at 15°C with a 14:10 photoperiod. The three algal clones, 3C, 3H, and Exuv, were mixed just prior to the experiment to obtain equal cell densities with a final cell concentration of 10^5 cells · ml^{-1} in each of the test jars. GT429, when used, was added to the mixed culture at a cell density of 5 × 10^5 cells · l^{-1} to simulate bloom conditions.

Cells were analyzed on a Coulter Epics V Flow Cytometer/Sorter by utilizing differences in their fluorescing intensities from the photosynthesizing pigments of chlorophyll (Exuv, 3C, GT429, and 3H) and phycoerythrin (3C). The instrument has a single argon ion 5-W laser with an excitation wavelength of 514 nm and a power of 1 000 mW. Fluorescence derived from each particle is split by a 590-nm dichroic mirror and is received by two photomultiplier tubes located at 90° to the intersection of the laser beam and sample stream. One receives a wavelength spectral

TABLE I

Comparison of three methods to determine cell density (cells · ml^{-1}) of the algal clone 3C (*C. salina*) after being grazed upon by *M. edulis* for 60 min.

Individual (*M. edulis*)	Sample time (min)	Coulter Counts ($\times 10^4$)	Bead method ($\times 10^4$)	Time method ($\times 10^4$)
A	0	11.90	12.31	11.90
	15	9.16	9.07	11.39
	30	5.62	6.97	6.52
	60	2.82	2.64	2.75
B	0	12.00	20.63*	12.00
	15	4.44	4.10	4.13
	30	1.51	1.45	1.29
	60	0.61	0.11	0.10

* Error probably caused by insufficient mixing of beads.

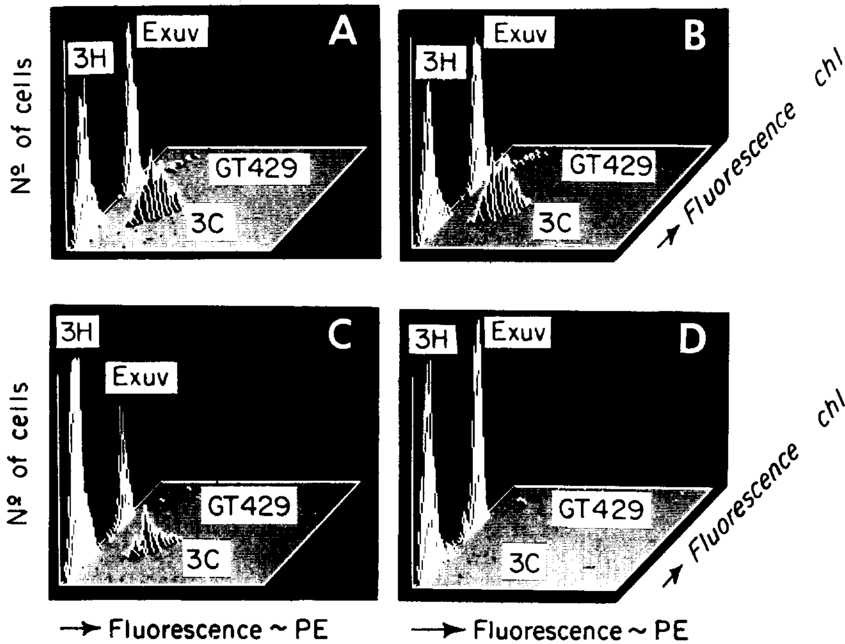

Fig. 1. Bivariate histogram plots of number of cells (Z) and X = fluorescence (approximating phycoerythrin, PE) vs. Y = fluorescence (approximating chlorophyll) comparing relative cell numbers within an algal mixture of 3H, 3C, Exuv, and GT429 due to grazing by *O. edulis* at time 0 min (A), 60 min (B) (cells removed uniformly) and within the pseudofeces (C) and feces (D) after 60 min. Note all algal species present in initial culture (A) and after grazing (B). GT429 at low levels in pseudofeces and high levels of 3H, i.e., preferential rejection (C). Complete absence of 3C in the feces (D) indicates preferential digestion in gut.

region 630 nm such as would result from chlorophyll emission and the other receives shorter wavelengths (540–560 nm) such as would result from phycoerythrin emission. The events (number of cells) registered met gate criteria on chlorophyll fluorescence, therefore only algal cells were analyzed. [See Yentsch et al. (1983) for a complete description of flow cytometry methodology.] A total of 2 000 cells was analyzed for each sample, with the total being partitioned among the three clones (four when GT429 was added to the mixture). Since all samples were run at a constant flow rate throughout an entire experiment, we were able to calculate the clearance rate of each individual by the differences in the amount of time required to analyze 2 000 cells (initial analysis times averaged $\approx$300 s; after 60 min, average analysis timed ranged from 500 to $>$1 000 s). Table I shows a comparison of three methods (Coulter Counter, Model ZM; uniform bead counting on flow cytometer, and the time method utilized here) used to determine actual cell density of the algal clone 3C. Actual cell concentrations were calculated according to the following formula:

$$\frac{\text{counts/sec@}T_n}{\text{counts/sec@}T_o} \times \text{ initial cell conc. (cells} \cdot \text{ml}^{-1})$$

where T_n and T_o are times (s) taken to count 2 000 events after experimental grazing and in control vessels, respectively. Analysis of covariance showed no significant differences between any of the three methods at $\alpha = 0.20$.

A typical bivariate histogram plot is shown in Fig. 1 where the number of cells analyzed is plotted with increasing fluorescence approximating phycoerythrin (X-axis) and increasing fluorescence approximating chlorophyll (Y-axis).

Estimates of clearance rates were obtained from the following equation (Coughlan, 1969):

$$V_w = [M \cdot (n \cdot t)^{-1} \cdot \log_e(\text{conc.}_o \cdot \text{conc.}_t{}^{-1})] \qquad (1)$$

where V_w = filtration rate (ml $\cdot$ h^{-1}), M = volume of suspension (ml), n = number of individuals (in this case, n–1), conc.$_o$ = concentration of particles at time 0 and conc.$_t$ = concentration of particles at time t.

Problems associated with estimating the clearance rate of particles from a fixed volume of medium are partially minimized by utilization of this equation and by using a relatively large volume of suspension medium. In addition, we are able to record a reduction in cell concentration over a relatively short period of time and hence can estimate clearance rate without a major decrease in residual cell concentration.

Behavior/activity experiments

Shell-valve activity was monitored with stress gauges as described previously (Djangmah et al., 1979). In each case, the animal was cemented, using dental cement, to a piece of slate by one valve. This prevented movement within the ex-

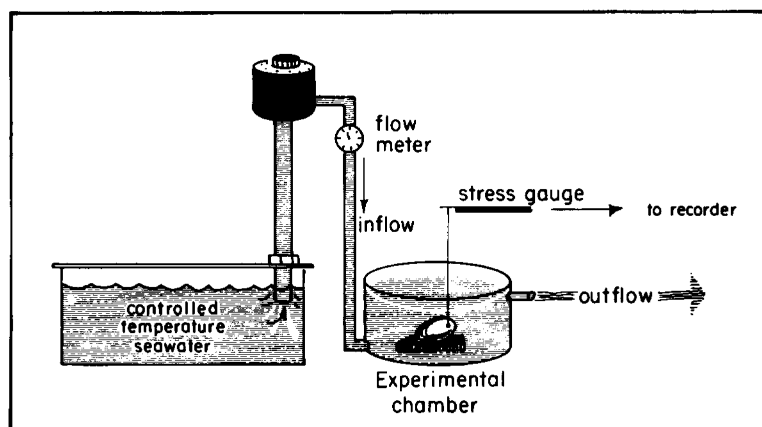

Fig. 2. Experimental system used to monitor shell-valve activity in bivalve molluscs exposed to the toxic dinoflagellate *P. tamarensis*.

TABLE II

The decline in cell counts (cells · ml^{-1}) per liter of sea water after 1 h. Values calculated from the difference between cell counts at time 0 and after 60 min (calculated regression lines for cell concentration (Y) vs. time in minutes (X) where $Y = a + bX$) and corrected for volume. Total cell densities at time 0 were 1.05×10^4 and 1.0×10^4 cells · ml^{-1} for all experiments with and without GT429, respectively.

Species	Dry tissue (g)	Cell concentrations after 60 min (cells·ml^{-1} × 10^3)				Total cell concentration after 60 min (cells·ml^{-1} × 10^3)	Reduction in cell per g tissue·h^{-1} (cells·ml^{-1} × 10^3)
		3H	3C	Exuv	GT429		
Ostrea	0.161 ± 0.035	1.92	2.02	1.84	-	5.78	26.21
Ostrea	0.161 ± 0.035	2.65	0.46	1.03	0.20	4.34	38.26
Placopecten	3.311 ± 0.359	1.15	2.05	1.80	-	5.00	1.51
Placopecten	3.311 ± 0.359	1.67	1.86	1.48	0.51	5.52	1.50
Mya	0.694 ± 0.106	1.05	1.53	1.18	-	3.76	8.99
Mya	0.694 ± 0.106	2.05	1.97	1.73	0.25	6.00	6.48
Mytilus (local)	1.461 ± 0.151	0.34	0.47	0.17	-	0.98	6.17
Mytilus (local)	1.461 ± 0.151	0.15	0.30	0.12	0.03	0.60	6.78
Mytilus (Sakonnet)	4.373 ± 0.735	0.90	0.84	0.79	-	2.53	1.70
Mytilus (Sakonnet)	4.373 ± 0.735	0.75	0.90	0.78	0.13	2.56	1.81
Spisula	0.570 ± 0.080	1.48	1.56	1.54	-	4.58	9.51
Spisula	0.570 ± 0.080	1.60	1.70	1.67	0.22	5.19	9.32
Crassostrea	1.540 ± 0.356	0.62	0.68	0.38	-	1.68	5.40
Crassostrea	1.540 ± 0.356	0.70	1.17	0.82	0.09	2.78	5.01

perimental chamber. The other valve was connected to a stress gauge by a fine nylon thread. The stress gauge was connected, via a Wheatstone Bridge circuit, to an Omniscribe chart recorder. While the animal's shell valves were closed, a base line trace was obtained. All animals were allowed at least 24 h to recover from the temporary aerial exposure experienced while being cemented in place. A 'normal' activity pattern was monitored over at least a 24-h period before the addition of either the control culture (Exuv) or the toxic dinoflagellate *P. tamarensis* (GT429). These cultures were introduced via a temperature controlled, flow-through system (Fig. 2). Time of introduction of the cultures was noted on the recordings and subsequent opening and closing movements of the shell valves were recorded. At least six individuals of each species were monitored. Animals were also monitored visually and siphon activity was recorded. Samples were taken of the water in the experimental chamber at hourly intervals after introduction of cultures to monitor feeding activity.

RESULTS AND DISCUSSION

The concentrations ($cells \cdot ml^{-1}$) of the four cell types referred to as 3H, 3C, Exuv, and GT429 (see Methods and Materials) at time 0 and after 60 min are sum-

TABLE III

The clearance rate and minimum values for V_w and V'_w (irrigation rate and weight · specific irrigation rates, respectively) estimated assuming 100% retention efficiency of 3H, 3C, Exuv, and GT429. Calculated from Table II, where $V_w = [M \cdot (n \cdot t)^{-1} \cdot \log_e(conc_0 \cdot conc_t^{-1}]$ (see Equation 1). Experiments with GT429 introduced into the food source are indicated by*.

Species	Dry wt (g)	Initial ration ($cells \cdot ml^{-1} \times 10^4$)	Clearance rate ($cells\ l^{-1} \cdot h^{-1} \times 10^3$)	V_w ($l \cdot h^{-1}$)	V'_w ($ml \cdot h^{-2} \cdot g^{-1}$)
Ostrea	0.161	1.0	4220[b]	548[b]	3403[b]
*Ostrea**	0.161	1.05	6160	884	5488
Placopecten	3.311	1.0	5000	3465	1047
Placopecten	3.311	1.05	4980	3215	971
Mya	0.694	1.0	6240[b]	978[b]	1409[b]
*Mya**	0.694	1.05	4500	280	403
Mytilus (local)	1.461	1.0	9020[b]	4646[b]	3180[b]
Mytilus (local)*	1.461	1.05	9900	5724	3918
Mytilus (Sakonnet)	4.373	1.0	7470	2749	629
Mytilus (Sakonnet)*	4.373	1.05	7940	2823	645
Spisula	0.570	1.0	5420	781	1370
*Spisula**	0.570	1.05	5310	705	1236
Crassostrea	1.540	1.0	8320[a]	3567[a]	2316[a]
*Crassostrea**	1.540	1.05	7720	2658	1726

[a] Rate is significantly different from animals exposed to GT429 ($P \leq 0.05$).
[b] Rate is significantly different from animals exposed to GT429 ($P \leq 0.01$).

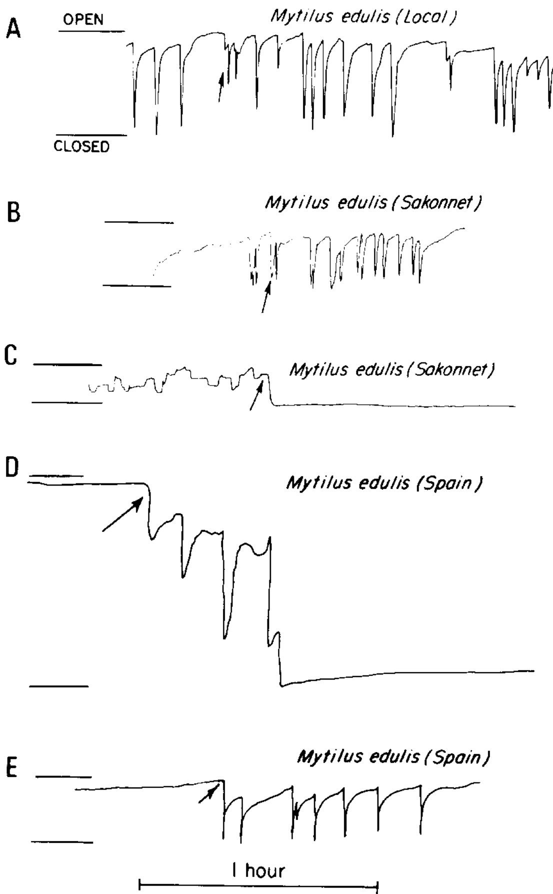

Fig. 3. Actual recordings of shell-valve activity of mussels from three localities after exposure to the toxic dinoflagellate *P. tamarensis*. Arrows indicate introduction of GT429.

marized in Table II. Each point is a mean of six vessels, each containing one bivalve mollusc. From these data, the clearance rates and minimum values for $\dot{V}_w$ (irrigation rates) were estimated (Table III) assuming 100% retention efficiency of the algal species used in the experiments. All filtration rates reported are in general agreement with those reported for these and other species of bivalve molluscs [see Newell (1979) and Bayne and Newell (1984) for reviews].

The results of the selective feeding experiments are summarized in Figs. 4, 6, 7, and 8. The results reported for selective feeding by animals under initial conditions (no GT429 present) do not differ from those reported previously (Shumway et al., 1985a). The two species not previously studied, *M. edulis* and *S. solidissima*, both showed differential absorption of food particles in the gut (postingestive selection).

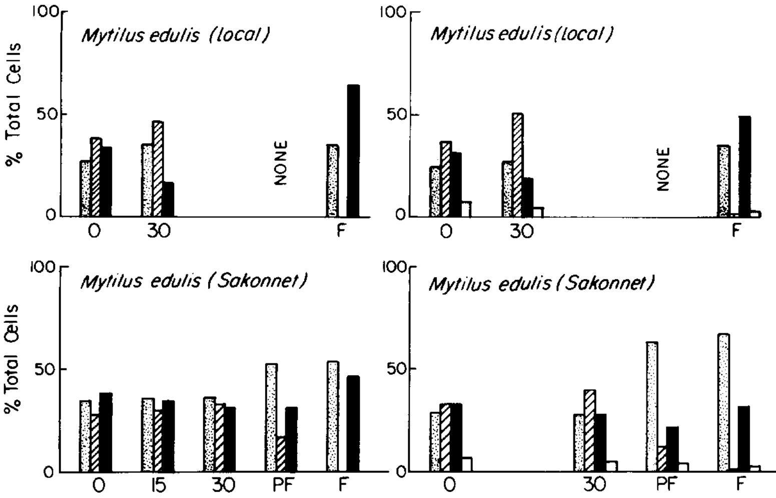

Fig. 4. Summary of the percent of the total cell count within an algal mixture of the clones 3H (stippled bars), 3C (striped bars), Exuv (solid bars), and GT429 (open bars) at time 0 and 30 min and within the pseudofeces (PF) and feces (F) during the grazing experiments.

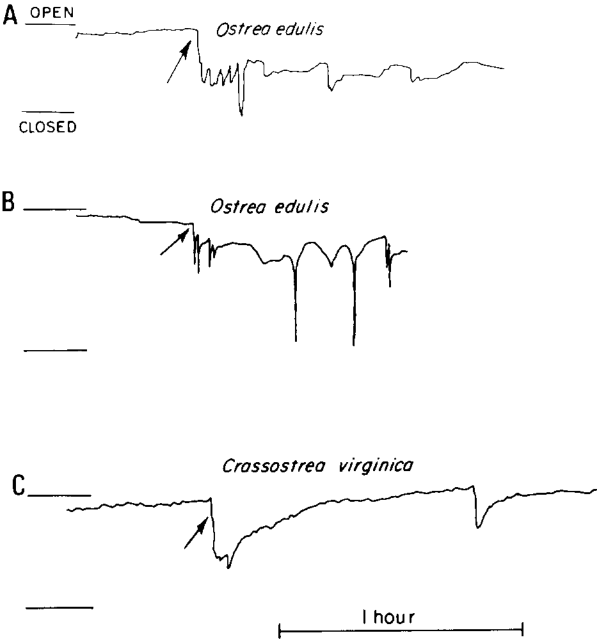

Fig. 5. Actual recordings of shell valve activity of oysters after exposure to the toxic dinoflagellate *P. tamarensis*.

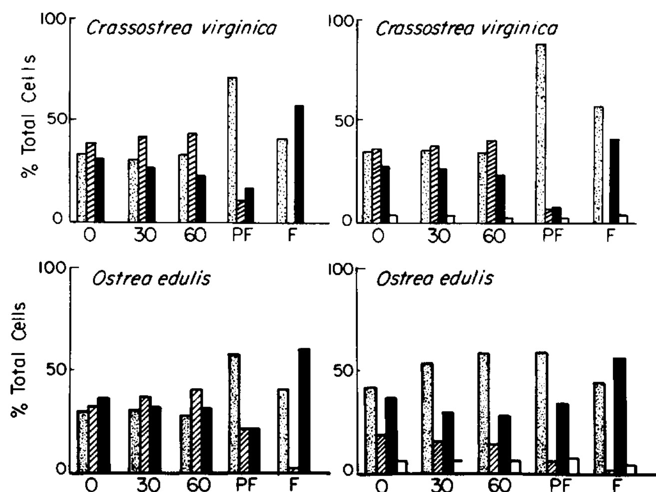

Fig. 6. Summary of the percent of the total cell count within an algal mixture of the clones 3H (stippled bars), 3C (striped bars), Exuv (solid bars), and after 60 min and within the pseudofeces (PF) and feces (F) during the grazing experiments.

Of the mixed diet that was ingested, both species showed a preferential absorption of the cryptomonad flagellate 3C. A similar preference for 3C has been demonstrated for several other bivalve species (Shumway et al., 1985a).

Actual traces of molluscan shell-valve activity patterns are shown in Figs. 3, 5, and 9. These results are discussed below in conjunction with the feeding patterns observed for the individual species.

M. edulis

Specimens of *M. edulis* from local waters showed no evidence of shell-valve closure when exposed to GT429 (Fig. 3a), nor was there any indication of selection for or against the toxic dinoflagellates in the feeding experiments (Fig. 4, Table III).

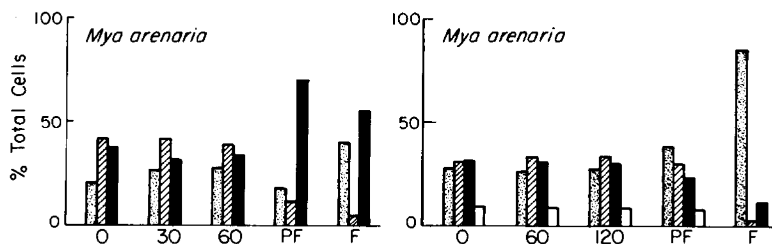

Fig. 7. Summary of the percent of the total cell count within an algal mixture of the clones 3H (stippled bars), 3C (striped bars), Exuv (solid bars), and GT429 (open bars) at time 0 and after 60 and 120 min and within the pseudofeces (PF) and feces (F) during the grazing experiments.

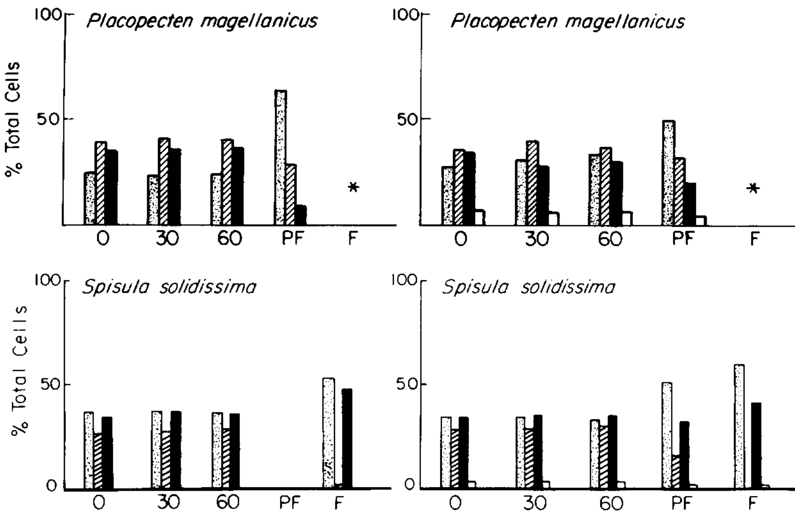

Fig. 8. Summary of the percent of the total cell count within an algal mixture of the clones 3H (stippled bars), 3C (striped bars), Exuv (solid bars), and GT429 (open bars) at time 0 and after 30 and 60 min and in the pseudofeces (PF). No whole cells could be detected in the feces (F) of *P. magellanicus*.

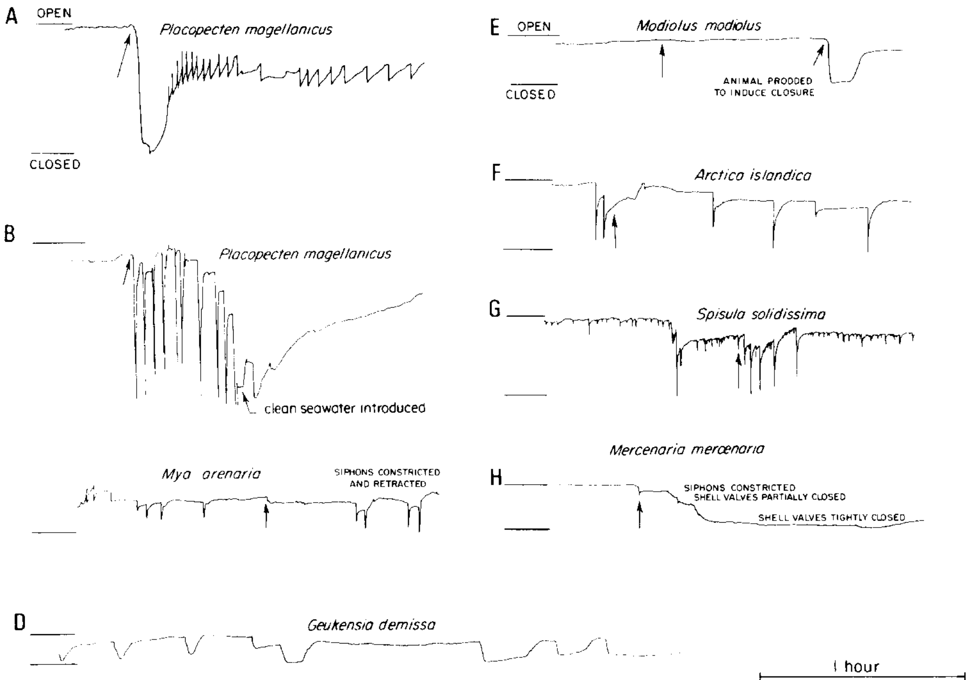

Fig. 9. Actual recordings of shell-valve activity patterns of various bivalve molluscs after exposure to the toxic dinoflagellate *P. tamarensis*. Arrows indicate addition of GT429.

GT429 was readily filtered and appeared in both the pseudofeces and feces of *M. edulis* from both localities. Specimens from other areas, however, exhibited a variety of responses. While local *M. edulis* showed no response to the addition of GT429, Sakonnet *M. edulis* showed varying degrees of shell-valve closure (Fig. 3b, c) when presented with GT429. While a few animals (three) were shown to continue normal activity patterns (Fig. 3b), the majority (15) exhibited at least partial shell-valve closure. Siphons (exhalent) were seen to be closed and the mantle edges in otherwise 'open' animals were retracted. Similar patterns of shell-valve activity were noted in Spanish *M. edulis* in the presence of GT429 (Fig. 3d, e). The majority of animals tested (eight of 12) showed initial, erratic shell-valve closure followed by complete closure. In addition, copious amounts of a white, mucus-like material were produced by *M. edulis* from both Rhode Island and Spain in the presence of GT429. A total of six animals from the Rhode Island waters died after exposure to GT429 and 10 of 12 Spanish *M. edulis* used in the experiments also died after exposure to GT429.

While there are no other data available for the responses of *M. edulis* to *P. tamarensis*, several authors have reported on the effects of other dinoflagellate species on various species of mussels. Buley (1936) demonstrated that *M. californianus* was capable of particle selection. He showed that, while the composition of the water column was 2.4% dinoflagellates and 97.5% diatoms, the dinoflagellates made up 97.4% of the stomach contents of *M. edulis*. We did not see any such selection in our studies, but the possible role of GT429 as a food source for *M. edulis* must not be ruled out. 'Local' *M. edulis* used in our studies actually showed an increase in both clearance and irrigation rates in the presence of GT429. Fox and Coe (1943) and Dupuy and Sparks (1968) also demonstrated that *M. californianus* fed readily in dinoflagellates (*Prorocentrum micans, Dinophysis* sp., *Dinodinium* sp., *Protogonyaulax* sp., and *Protogonyaulax catenella*, but also noted that many cells pass through the digestive tract intact. Sievers (1969) reported that *Brachidontes recurvus* failed to open in the presence of *Gonyaulax monilata*. More drastic effects have been reported in South Africa where *P. catenella* and *P. grindleyi* caused mass mortalities of *Choromytilus meridionalis* and *Donax serra* (Horstman, 1981). An outbreak of *P. tamarensis* was deemed responsible for *M. edulis* deaths in Massachusetts (Ceurvels and Der Hovanesian, 1972); however, *M. edulis* deaths caused by red-tide outbreaks are unknown in Maine (J.W. Hurst, pers. comm.).

Twarog and Yamaguchi (1975) suggested that the relative toxicities of various filter-feeding molluscan species, i.e., the ability of various species to accumulate toxin, might be the result of differing sensitivities of the nervous system of each species to saxatoxin. They further suggested that some species which are periodically exposed to dinoflagellate blooms have evolved mechanisms permitting them to exploit the toxic organisms as food. The possibility emerges from this study that *M. edulis* from Maine waters which are exposed to *P. tamarensis* blooms seasonally and throughout their lifetimes are in some way adapted to the toxins present. The possible role of the toxic dinoflagellates as food organisms is still under investigation.

Oysters C. virginica and O. edulis

Both *C. virginica* and *O. edulis* remained open and continued to filter in the presence of GT429 (Figs. 5, 6). *O. edulis* normally had the shell valves open and the mantle edges were visible. Addition of GT429 caused partial adduction of the shell valves in only four of the 14 animals studied (Fig. 5a) although pumping/filtering continued in all animals. Fig. 5b shows the typical response of *O. edulis* to the introduction of GT429 to the food source. There was an initial, partial adduction of the shell valves followed by periodic 'snaps'. This activity pattern continued until clean sea water was introduced.

C. virginica exhibited an initial shell-valve closure when GT429 was presented. This closure was followed by a gradual reopening (Fig. 5c) and the pattern repeated, although complete closure never occurred. This activity pattern is reflected in the slight decrease in clearance rate (8%) and 25% decrease in irrigation rate (Table III).

In both species, there was a preferential rejection of 3H in the pseudofeces and in the feces and a postingestion selection for the cryptomonad flagellate 3C. While *O. edulis* shows evidence of preferential clearance of the dinoflagellate Exuv, there is no evidence of any such preference for GT429. GT429 is present in both the feces and pseudofeces of both *C. virginica* and *O. edulis*, indicating that it has been ingested; however, quantitative estimates of digestion/absorption are still lacking.

In the presence of GT429, clearance rate and irrigation rate are both increased in *O. edulis*. These rate increases (similar to those seen in *M. edulis*) explain, at least partially, the fact that in Maine waters *O. edulis* have been found to become toxic several days prior to any other species, including *M. edulis* (J.W. Hurst, pers. comm.).

Previous studies of the effects of toxic dinoflagellates on oysters are scant and do not include *P. tamarensis*. Ray and Aldrich (1976) reported that *C. virginica* rarely opened and did not filter when exposed to *Gonyaulax monilata* (= *Gessnerium monilatum*, Loeblich), but opened frequently in the presence of *Gymnodinium breve* (= *Ptychodiscus brevis*, Steidinger). High mortality rates in the presence of *G. monilata* (10^6 cells $\cdot l^{-1}$) were reported by Sievers (1969). In contrast, Connell and Cross (1950) reported that oysters (presumably *C. virginica*, since it is the predominant species in Texas waters) could concentrate toxic dinoflagellates (*P. catenella*) with no obvious ill effects. *Crassostrea gigas* exhibited a reduction in volume of water filtered, vigorous clapping of shell valves or complete cessation of pumping when exposed to *P. catenella* (Dupuy and Sparks, 1968). Animals that did continue pumping, showed a 30% reduction in rate and also produced a large amount of pseudofeces. During the 1972 outbreak of PSP in Massachusetts, *C. virginica* did not become toxic although exposed to the bloom and presumably isolated themselves by shell-valve closure.

Twarog (1974) indicated that *C. virginica* nerves were the most sensitive to saxitoxin of any species studied. During the summer of 1985, preliminary experiments with the same species collected from the same area indicated a sensitivity two orders

of magnitude less than when tested 10 years previously. This data, coupled with the fact that Maine *C. virginica* which are regularly exposed to the toxic dinoflagellates readily accumulate toxin, whereas animals from Massachusetts did not accumulate toxin during what was presumably their first encounter with toxic dinoflagellates, again raises the possibility that animals frequently/routinely exposed to the toxins may, in some way, become immune to their effects.

M. arenaria

Initial feeding studies with *M. arenaria* confirmed our original findings and the preferential extrusion of the dinoflagellate Exuv in the pseudofeces and in the feces (Fig. 7) coupled with postingestive selection against Exuv and 3H. There was no GT429 present in the feces, probably indicative of the fact that they were not taking this species into the gut. Our previous studies with this species indicate that clams exposed to bloom conditions of GT429 in the laboratory remain nontoxic for up to 10 days, i.e., no uptake of the toxic dinoflagellates. Most activity in *Mya* is associated with the siphons rather than the shell valves. On addition of GT429 to the medium, most specimens showed withdrawn or partially withdrawn siphons which remained constricted and/or retracted for extended periods. A major decrease in clearance rate (28%) and irrigation rate (71%) were recorded and copious amounts of pseudofeces were produced in the presence of GT429.

In a preliminary experiment, both *Mytilus* (local) and *Mya* were exposed to bloom conditions (10^5 cells $\cdot$ l^{-1}) of GT429 for a period of five days. The animals were then sacrificed and toxicity levels determined using the standard mouse bioassay (AOAC, 1975). *Mytilus* showed toxicity levels ranging from 490 to 860 $\mu g \cdot 100\ g^{-1}$, whereas the *Mya* were essentially nontoxic ($< 50\ \mu g \cdot 100\ g^{-1}$). It was noted during these experiments that *M. edulis* began filtering immediately upon being placed in the tanks, whereas the clams tended to retract the siphons and remain relatively inactive. These results corroborate field observations made by personnel at the Maine Department of Marine Resources (J.W. Hurst, pers. comm.) who have noted that *Mytilus* invariably become toxic up to 10 days prior to *Mya* taken from the same locality.

The behavioral responses and concomitant decreases in clearance and irrigation rates reported here for *Mya* explain previous reports of nontoxic clams in the presence of other toxic bivalve species. During an outbreak of shellfish poisoning in northeast England, it was shown that soft-shell clams (*M. arenaria*) contained less than one-thirtieth of the concentration of the toxin in *M. edulis* from the same site (Ingham et al., 1968). J.W. Hurst (pers. comm.) has reported that during a rise in PSP levels, *M. edulis* are always more toxic than clams by a factor of about 10. Gilfillan and Hanson (1975) reported *M. edulis* sampled during an outbreak of PSP in Ogunquit, Maine, to be six times more toxic than *Mya* from the same locality. In addition, Twarog et al. (1972) found that *Mya* nerves were completely blocked by a concentration of saxitoxin 2 orders of magnitude lower than that which had

no effect of *M. edulis*, i.e., clams are more sensitive to the presence of the toxins than *M. edulis*.

Other species

Activity patterns were not interrupted and clearance rates and irrigation rates remained unchanged when *S. solidissima* were exposed to GT429 (Table III, Figs. 8, 9g). Under control conditions (no GT429), no pseudofeces were produced and there was a postingestive selection for 3C and against both 3H and Exuv. In the presence of GT429, large amounts of pseudofeces were produced and GT429 was present in both the feces and pseudofeces. *Spisula* is unique in that, although toxin accumulation is slow, once the animals become toxic, they may remain toxic for up to two years (J.W. Hurst, pers. comm.).

Although *P. magellanicus* continued to feed at normal rates after the introduction of GT429, the animals showed the most striking behavioral responses of any species studied. While two animals showed no response, the majority (14) exhibited an immediate closure of the shell valves followed by either violent swimming activity, partial, sustained shell-valve closure, or a combination of the two. Swimming/clapping activity patterns were never observed to last for more than 30 min to 1 h. On addition of clean sea water, the activity ceased (Fig. 9b) and the animals remained open with the mantle edges and tentacles freely exposed. Thompson et al. (1980) demonstrated a similar reaction in *P. magellanicus* when confronted with starfish extract. There was an initial series of rapid adductions followed by fatigue. While such activity may provide the animals with a temporary escape from predators, it does not provide refuge from phenomena such as toxic dinoflagellate blooms. Under such conditions, the activity patterns demonstrated here may actually prove detrimental to *P. magellanicus*. In their study, Thompson et al. (1980) showed that the rapid adductions of the shell valves resulted in an accelerated heart rate, increased stroke volume and an enhanced cardiac output. Anaerobic pathways were invoked and there was an increase in oxygen uptake after the animals reopened. They estimated that the restoration of physiological functions to normal values takes several hours.

P. magellanicus exposed to bloom conditions of GT429 produced copious amounts of a white mucus-like substance similar to that seen in *M. edulis* from Rhode Island and Spanish waters. 3H was preferentially removed in the pseudofeces and all algal species presented were extruded in the pseudofeces in the experiments with GT429. Feces production was low in these animals and no whole cells could be recognized.

Modiolus modiolus showed no response to the addition of GT429 and readily filtered the dinoflagellate from the medium. *Mercenaria mercenaria* showed an initial retraction of the siphons followed by complete closure of the shell valves. The animals did not re-open until after the addition of clean sea water and consequently, feeding studies were impossible. Twarog et al. (1972) reported that *M. mercenaria*

nerves were highly resistant to saxatoxin, yet these animals did not accumulate nearly as much toxin as did soft-shelled clams (*Mya*) during a bloom of *P. tamarensis*. It has also been noted that during the 1972 PSP outbreak in Massachusetts *M. mercenaria* did not accumulate toxin. This is probably a result of isolation from the external environment.

G. demissa, also from Rhode Island, showed an activity pattern under normal conditions indicating that the animals were 'open' most of the time, and closed for approximately 3 min in every 20 min. Fig. 9d shows animals which closed on addition of GT429 and then re-opened after approximately 1 h. The animal then closed and showed intermittent opening for the next 22 h. This response was observed in nine animals and in *M. edulis* that were open, mantle edges were retracted in the presence of GT429. No filtration was seen in the presence of GT429 and again, copious amounts of pseudofeces/mucus were produced.

The results reported here clearly demonstrate that the responses of bivalve molluscs to the presence of the toxic dinoflagellate *P. tamarensis* are species-specific and range from no response to complete isolation from the external medium. A better understanding of the species-specific responses to the presence of GT429 will make it possible to define more clearly areas for potential closures. More importantly, managers can utilize knowledge of species-specific responses to determine harvest areas which can be safely kept open and thus minimize losses to the fishing industry.

While we are by no means proposing the use of behavioral studies as a means of monitoring the occurrence of toxic dinoflagellate blooms, aquaculturists may benefit from our knowledge of species' responses to the presence of *P. tamarensis*. The two species most favored by Maine aquaculturists are *M. edulis* and *O. edulis*. Both of these species readily consume *P. tamarensis*, and although the potential for *P. tamarensis* as a food source is still not clear, its utilization could enhance growth. Since the periods during which the shellfish quality is best for marketing (November–April), and the threat of paralytic shellfish poisoning is most prevalent (May–October) do not coincide, the presence of *P. tamarensis* may prove to be a factor worthy of consideration when selecting favorable grow-out areas/potential locations for new aquaculture enterprises.

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