COMPARATIVE PHYSIOLOGICAL AND BEHAVIORAL RESPONSES TO PSP TOXINS IN TWO BIVALVE MOLLUSCS, THE SOFTSHELL CLAM, *Mya arenaria,* AND SURFCLAM, *Spisula solidissima.*

V. Monica Bricelj*, Allan D. Cembella**, David Laby*, Sandra E. Shumway***and Terry L. Cucci****

* Marine Sciences Research Center, State University of New York, Stony Brook, N.Y. 11794-5000, U.S.A.;
** Institute for Marine Biosciences, National Research Council, 1411 Oxford Street, Halifax, N.S. B3H 3Z1 Canada;
*** Southampton Campus, Long Island University, Southampton, Long Island, N.Y. 11968, U.S.A.;
**** Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine 04575, U.S.A.

ABSTRACT: *Mya arenaria* (F. Myacidae) and *Spisula solidissima* (F. Mactridae) with no prior history of exposure to PSP toxins, were fed unialgal cultures of 3 isolates of *Alexandrium* spp. of varying toxicity, and a non-toxic diatom, *Thalassiosira weissflogii.* Clearance rate of *Spisula* was not affected by dinoflagellate toxicity, but was significantly reduced (by 17-fold) in *Mya* offered monospecific suspensions of isolate PR18b [toxicity = 74 pg saxitoxin equivalents (STXeq) cell^{-1}]. The ability of clams to reburrow in sediment following exposure to toxic *Alexandrium* cells provided a useful index of *in vivo* sensitivity to PSP toxins. Longer-term (11 days) exposure to PR18b did not affect burrowing in *Spisula*, but *Mya* were impaired (unable to burrow) within $\leq$ 7h of exposure, and showed marked individual variability in susceptibility to PSP toxins, as measured by this index. Under identical experimental conditions, the two species also exhibited remarkable differences in maximum toxin body burden (30.4 x 10^3 vs 1.3 x 10^3 µg STXeq 100 g^{-1} in *Spisula* and *Mya* respectively), and in the composition of individual PSP toxins in tissues. In contrast to *Mya*, *Spisula* were extremely effective in converting the weakly toxic sulfocarbamoyl (C1+2) toxins present in ingested dinoflagellates and produced high levels of decarbamoyl gonyautoxins through biotransformation. These differences in sensitivity and capacity for toxin bioconversion serve to explain observed differences in toxin kinetics of field populations of the two species.

INTRODUCTION

On the Atlantic coast of North America, paralytic shellfish poisoning (PSP), caused by blooms of toxic dinoflagellates (*Alexandrium* spp.) has historically resulted in closure of commercially harvested beds of softshell clams, *Mya arenaria*, and surfclams, *Spisula solidissima. Spisula* can accumulate high toxin levels in the field [1] and in the laboratory, even when fed a low-toxicity dinoflagellate strain [2], whereas *Mya* typically attain 2-8x lower toxicities than the mussel, *Mytilus edulis* [3]. Differences among bivalve species in the ability to accumulate PSP toxins have been correlated with their *in vitro* nerve sensitivity to saxitoxin (STX) and ability to maintain active feeding during toxic blooms [4]. However, discrepancies have been noted between the ranking of bivalves based on *in vitro* vs. behavioral or physiological responses (e.g. shell valve closure or feeding inhibition) [5]. Toxin uptake rates are also dependent on the toxicity and density of toxic dinoflagellates cells present in the water column, especially in so-called "sensitive" species, and on the relative cell density of non-toxic phytoplankton, which may stimulate feeding activity on toxic cells [5].

Softshell clams and surfclams also differ in that *Mya* detoxifies fairly rapidly (1 to 4 wks [3]), whereas *Spisula* shows prolonged retention of toxins (2+ years [1]). Detoxification rate may be affected by a species' ability to convert ingested toxins from less potent to more potent derivatives in tissues. *S. solidissima* is one of few bivalve species identified as capable of *de novo* production of decarbamoyl toxins (dcSTX in field-collected clams [6], and dcGTX2+3 in a controlled toxification study [2]). Yet little is known about the pathways and rates of toxin conversion occurring in this species, especially in relation to the source of toxin.

The main objectives of this study were to compare *M. arenaria* and *S. solidissima* in terms of: 1) the effect of algal cell toxicity on feeding rates, 2) the effect of PSP toxins on burrowing activity, and 3) their capacity for accumulation and *in vivo* transformation of PSP toxins. Feeding and burrowing activities are used as indicators of the relative sensitivity of these two bivalve species to PSP toxins.

MATERIALS AND METHODS

Feeding rates in relation to cell toxicity. Weight-specific clearance rate (CR/DW) was determined for individual, juvenile clams from populations with no history of exposure to PSP: *M. arenaria* (mean shell length, L = 33.1 mm; mean wet weight of soft tissues, WW = 1.28g) were collected from Lawrencetown, southern Nova Scotia, Canada, and *S. solidissima* (mean L = 27.5 mm; mean WW = 0.82g) were obtained from a Maine, USA, hatchery. Clams were acclimated to the experimental temperature and salinity (16°C; 30ppt) for 2-3 wk prior to experiments under flow-through conditions, and to the experimental diet for 2-3 h prior to CR measurements. These were determined as: CR = $(\ln C_f - \ln C_0) \times V/t$, where C_f and C_0 = final and initial cell densities, V = volume of suspension (69 to 95 ml), and t = time interval, which ranged from 1 to 5 min for all treatments, except that of the pure isolate, PR18b, for which t = 20-23 min. An airstone was used to maintain algae in suspension.

Clams were offered unialgal suspensions of the non-toxic diatom *Thalassiosira weissflogii* (11 µm equivalent spherical diameter), and the following dinoflagellate isolates: *Alexandrium excavatum*, PR18b (length, L = 36.8 µm), a highly toxic isolate from the lower St. Lawrence estuary,

Quebec, *A. tamarense*, Gt429 (L = 29.4 µm), a strain of intermediate toxicity from Ipswich Bay, Massachusetts, and *A. tamarense*, PLY173 (= NEPCC183; L = 34.6 µm), a presumed non-toxic isolate from Plymouth, UK. Algal concentrations were equalized in terms of cell volume to compensate for differences in cell size. PR18b was also offered in a mixture of 30%PR18b:70% *T. weissflogii* by volume. Dinoflagellate cell densities were determined microscopically and densities of *T. weissflogii* cells were obtained with a Coulter Epics V flow-cytometer. Only the toxin content of the viscera (excluding the foot) was determined for *Mya*, and both viscera and other tissues were analyzed for *Spisula*.

Burrowing response during long-term toxification. Juvenile *Spisula* (mean L = 33.4mm; mean WW = 1.51g) were obtained from a Maine hatchery and *Mya* (mean L = 34.4mm; mean WW = 2.49g) from a population on the north shore of Long Island, New York, USA, with no history of exposure to PSP. *Mya* and *Spisula* were held together in each of 2 recirculating tanks (temperature = 15°C, salinity = 28ppt), filled with ca. 65 l of 0.45 µm filtered seawater and a 10 cm-deep layer of sand. One tank was fed *T. weissflogii* (mean density = 2236 cells ml^{-1}),and the other was fed strain PR18b (104 cells ml^{-1}). Algae were delivered continuously via a peristaltic pump from concentrated stocks. Each tank contained 54 *Mya* and 54 *Spisula*: 30 clams of each species were separated in one half of the tank by a plastic mesh divider, and removed periodically for repeated measures of burrowing response. The remaining clams were subsampled for toxin analysis (3 replicate samples, each with pooled tissues from 2 clams). The proportion of clams exposed on the sediment surface that burrowed by the end of 2 h, was determined in two 30 l tanks, one for control clams containing *T. weissflogii*, and one for toxified clams, containing isolate PR18b, throughout the course of toxification (11 d). Clams were then transferred into another tank and fed *T. weissflogii*. Burrowing response was determined over 4 d of depuration to determine the potential for recovery of impaired individuals.

Toxin analysis. Toxin extracts of dinoflagellates and lyophilized clam tissues for HPLC analysis were prepared in 0.03N and 0.1N acetic acid respectively, following previously described methods [5], and analyzed by the method of Oshima et al. [7]. Toxicity was calculated using toxin-specific conversion factors [in Mouse Units (MU) µmol^{-1} of toxin] [11], assuming 0.23 µg STXeq MU^{-1}.

RESULTS

Feeding response. Surfclams showed no significant differences in CR in response to algal toxicities of up to 64 pg STXeq $cell^{-1}$ (Fig. 1). In contrast, CR of *Mya* were significantly inhibited ($p<0.001$), by 95% relative to the non-toxic control, when fed a monospecific suspension of PR18b. However, CR was comparable on *T. weissflogii*, and the dioflagellate isolates Gt429 and PLY173, offered at approximately equal biovolumes (ca. 2000 diatom cells ml^{-1} and 94 to 135 dinoflagellate cells ml^{-1}). It is noteworthy that PLY173, originally selected as a non-toxic dinoflagellate control diet, exhibited detectable levels of toxin (0.9 pg STXeq $cell^{-1}$) in our cultures, as was also independently reported by Teegarden and Cembella [this volume]. Clearance rates of *Mya* were not suppressed when PR18b was fed (at 50 cells ml^{-1} = 30% of total algal biomass) in a mixed suspension with *T. weissflogii*. The relative depletion of *T. weissflogii* and PR18b cells (CR_{Tw}/CR_{PR18b}) provides an index of feeding selectivity. However, this ratio (mean = 0.9) did not differ significantly from 1, indicating that *Mya* fed non-selectively on the two algal species. No pseudofeces

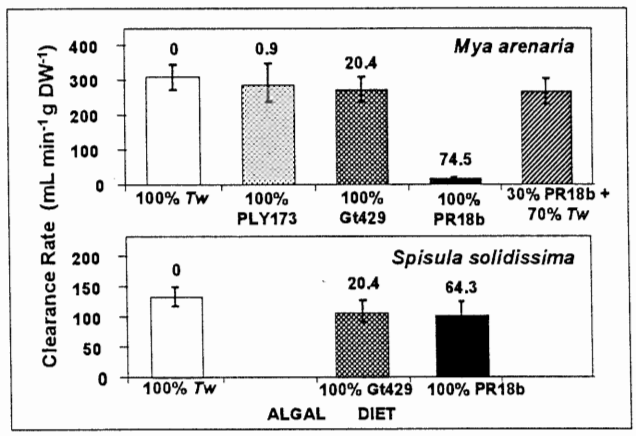

Fig. 1. Clearance rates (CR) (mean ± SE, in ml min^{-1} g^{-1} dry tissue weight) of *Mya* and *Spisula* fed *Thalassiosira weissflogii* (*Tw*) and 3 *Alexandrium* strains of varying toxicity (indicated above each bar in pg STXeq $cell^{-1}$).

Table 1. Rate of PSP toxin accumulation in viscera [in µg STXeq h^{-1} g wet weight $(WW)^{-1}$], and rate of toxin ingestion [in µg STXeq h^{-1} g dry weight $(DW)^{-1}$ of whole soft tissues] in *M. arenaria* and *S. solidissima* fed various experimental diets for 3-6 h.

Species / Diet	Toxin uptake (in µg STXeq h^{-1} g^{-1})		Toxin ingestion (in µg STXeq h^{-1} g^{-1})
M. arenaria	mean	(SE)	mean
100% Gt429	4.24	(0.53)	31.37
100% PR18b	7.76	(0.26)	8.59
30% PR18b + *Tw*	18.63	(3.47)	59.76
S. solidissima			
100% Gt429	2.15	(0.50)	20.12
100% PR18b	35.92	(3.94)	44.94

were produced by either clam species; therefore the product of CR x cell density x cell toxicity was used to calculate an instantaneous rate of toxin ingestion (Table 1). The rate of toxin accumulation in clam tissues (toxicity of

viscera/duration of exposure) provides a time-integrated, relative measure of toxification under the various feeding regimes used (Table 1), which is affected by biotransformation of individual toxins of variable potency within tissues as well as toxin ingestion. *Mya* became considerably more toxic when PR18b was fed in a mixed suspension (at a lower toxin concentration = ca. 3700 pg STXeq ml^{-1}) than when offered alone (at 8800 pg STXeq ml^{-1}).

The dinoflagellate isolates Gt429 and PR18b differ in relative toxin composition as well as specific-toxicity: the low potency, N-sulfocarbamoyl toxins, C1+2, are the dominant component (62% of total toxin on a molar basis) in PR18b, whereas gonyautoxins, GTX1-4, contribute most (59%) of the toxin in Gt429 (Fig. 2). Overall. the toxin composition of ingested dinoflagellates experienced relatively small changes in *M. arenaria* tissues, but exhibited major transformations in *Spisula* within only a few hours of toxification. C-toxins were reduced to negligible levels, and this loss was accompanied by a reciprocal increase in the more potent decarbamoyl gonyautoxins (dcGTX), mainly dcGTX3 and to a lesser extent its epimer dcGTX2. Their contribution to the toxin pool in clam tissues thus varied in direct proportion to the contribution of C-toxins in the dinoflagellate isolate used as diet (Fig. 2). C2 made up 100 and 97% of the C-toxins in Gt429 and PR18b respectively, and 80 to 87% of total C-toxins in *Mya*.

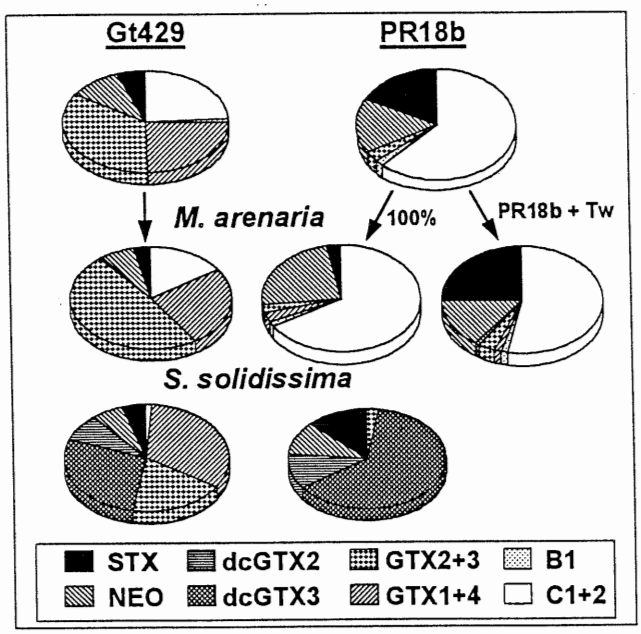

Fig. 2. Relative toxin composition (% molar) of *M. arenaria* and *S. solidissima* and of *Alexandrium* strains offered during 3-6 h feeding experiments. Viscera were analyzed in all cases except *Spisula* fed clone Gt429, for which whole tissues were analyzed. In both species the viscera typically contains >82-89% of the toxin body burden during toxification (Bricelj et al., unpubl. data). Toxins found in trace amounts (<0.5% of total toxin) are not shown.

Burrowing response. The ability to burrow in sediment was markedly impaired in *Mya* exposed to the high-toxicity isolate PR18b, but remained unaffected in *Spisula* (Fig. 3). Burrowing was severely inhibited in *Mya* within <7 h of toxin exposure, when clams averaged 128 µg STXeq 100 g^{-1} WW of whole tissues. No mortalities were recorded, and toxic softshell clams fully recovered their burrowing ability within 4 d of depuration, even though they still averaged high toxin levels (887 µg STXeq 100 g^{-1}). Significantly, during toxification, 7 to 31 % of the test population was insensitive to the effects of toxin loading. Under identical experimental conditions, and *ad libitum* supply of toxic algae, surfclams accumulated PSP toxins at levels an order of magnitude higher than those of softshell clams (Fig. 3). Toxicities peaked at 30429 and 1341 µg STXeq 100 g^{-1} in *Spisula* and *Mya* respectively after 11 d-exposure to toxic cells.

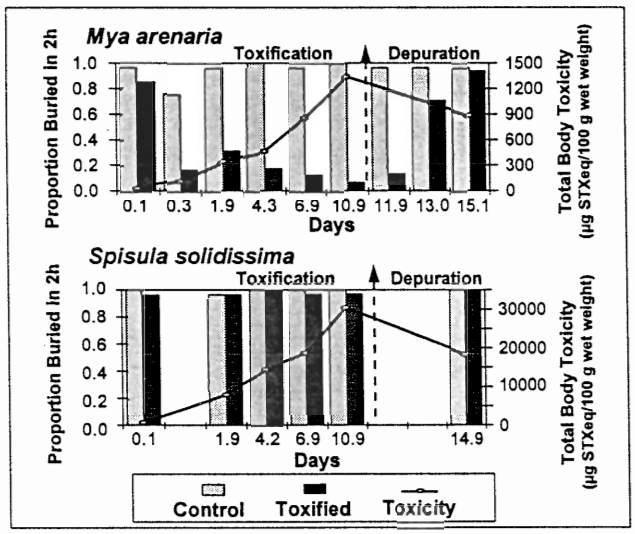

Fig. 3. Effect of toxification with *Alexandrium excavatum* (clone PR18b) on the burrowing response of *M. arenaria* and *S. solidissima* (controls fed *Thalassiosira weissflogii*).

DISCUSSION

Based on both feeding and burrowing responses *M. arenaria* was highly susceptible to the effects of toxigenic *Alexandrium*, whereas *S. solidissima* was insensitive. This correlates well with our finding that *Spisula* accumulates PSP toxins at much higher rates than *Mya* (2759 vs. 122 µg STXeq 100 g^{-1} day^{-1}, respectively, over 11 d [Fig. 3]), and confirms the ranking of *Mya* in terms of *in vitro* nerve sensitivity to STX [4]. In short-term feeding experiments only the high-toxicity isolate PR18b caused significant feeding inhibition in *Mya*, thus suggesting the existence of a toxicity threshold. Feeding depression was clearly unaffected by concentration-dependent effects, since equal biovolumes were used in all treatments. Shumway and Cucci [8] found that CR of *M. arenaria* was significantly depressed when a suspension of non-toxic phytoplankton was spiked with 200 cells ml^{-1} of strain Gt429. However, algal toxicity, known to vary greatly depending on growth and culture conditions, was

not measured in their study. In the present study, CR of *Mya* was not depressed when PR18b was present in a mixed suspension with *T. weissflogii*, suggesting that non-toxic phytoplankton may act as a phagostimulant or mask the inhibitory effect of toxic cells. This finding has important implications for toxin accumulation of softshell clams in the field, where *Alexandrium* co-occurs with other phytoplankton species, even during blooms. Curiously, feeding inhibition on PR18b alone did not appear to be related to toxin body burden, since *Mya* became 2.4x more toxic when fed Pr18b in a mixed rather than unialgal suspension (Table 1).

The toxin composition of ingested dinoflagellates was profoundly altered in *Spisula* but not in *Mya*. In the former species C-toxin concentrations dropped sharply within a few hours of toxin exposure and this was accompanied by *de novo* production of dcGTX2+3, which are characterized by ca. 4-7x higher specific toxicities than N-sulfocarbamoyl toxins. This conversion tends to magnify the differences in net toxicity between the two species resulting from differential feeding, and may also explain the large difference in toxin accumulation between *Spisula* fed Gt429 and PR18b (Table 1), which cannot be fully accounted for by differences in toxin ingestion rates. Biotransformation yielding decarbamoyl toxins is rare among bivalves but has been previously reported in two species of clams from Japan, *Mactra chinensis* and *Peronidia venulosa* [9], in the littleneck clam, *Protothaca staminea* [10], and in field populations of *S. solidissima* from the Gulf of Maine [6]. Efficient *in vitro* production of dcGTX from C1+2 and GTX via the activity of a carbamoylase enzyme which favors the hydrolysis of N-sulfocarbamoyl toxins, has been demonstrated in these species [9, 11]. In the present study the relative dominance of dcGTX2+3 tracked that of C-toxins in the dinoflagellates ingested, which argues strongly for direct metabolic decarbamoylation of C-toxins in *Spisula*. Our results corroborate the finding of Cembella et al. [1] that these toxins occur only briefly in association with toxic blooms in surfclam populations from the Gulf of Maine, although the presence of C-toxins was even more transient in our controlled experiments than in the field. Curiously, dcSTX, rather than dcGTX, was the dominant carbamoyl derivative in field-collected *Spisula* [6], whereas dcSTX occcurred only in trace amounts (0.2% molar) in surfclams fed PR18b for a few hours (Fig. 2), and did not make a substantial contribution in tissues until after longer-term toxification with this isolate.

In summary, information on feeding physiology and toxin compositional changes in clam tissues explain to a large degree the differences in toxin kinetics previously described in field populations of the two study species. Percent burial provided a rapid, readily determined behavioral index of relative susceptibility to PSP toxins, which may have useful application for other infaunal species. Juvenile bivalves are generally found at shallower depths than adults and are thus more likely to be exposed by tidal scour. *Mya* below legal harvestable size are also often discarded at the surface by intense clam digging activities in the intertidal. Therefore, toxin-induced loss of the ability to reburrow in sediments may be important in contributing to mortality (via increased desiccation and predation) of natural clam populations.

ACKNOWLEDGEMENTS

We thank N. Lewis and J. Uher (NRC, Halifax) for HPLC analysis and technical support, S. Bauer (SUNY) for maintaining algal cultures, and Y. Oshima (Tohoku University, Sendai, Japan), M.V. Laycock (NRC) and the MACS Program (NRC) for providing toxin calibration standards. This research was sponsored by the New York Sea Grant Institute, U.S. Dept. of Commerce. This is MSRC contribution #1011, and NRC #38102.

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