Behavioral and physiological responses to PSP toxins in *Mya arenaria* populations in relation to previous exposure to red tides

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ABSTRACT: Paralytic shellfish poisoning (PSP) poses a severe human health risk worldwide and can also adversely affect bivalve populations. This study investigates the intraspecific variation in sensitivity to paralytic shellfish toxins (PSTs) and in toxin accumulation capacity between 2 populations with contrasting histories of PSP in the softshell clam *Mya arenaria*, a species widely distributed in Atlantic North America. We determine the magnitude and potential ecological consequences of intrinsic variation in toxin susceptibility in *M. arenaria*, known to have a genetic basis, and the implications for prediction and management of PSTs in regions affected or threatened by PSP expansion. Burrowing, feeding, oxygen consumption (VO_2), toxin uptake and survival of 2 test populations were compared during 2 to 3 wk of laboratory exposure to a high-toxicity *Alexandrium tamarense* strain. Most clams from Lepreau Basin, Bay of Fundy (BF), an area with a long-term history of annual PSP events, exhibited high resistance measured by these parameters, relative to naïve clams from the Lawrencetown Estuary (LE). These were highly sensitive to PSTs, as reflected in significantly reduced clearance and VO_2 rates; they also failed to acclimate to the presence of toxins. BF clams attained significantly higher (up to 10-fold) tissue toxicities than LE clams. Toxicity of individual clams from the 2 populations varied up to 40-fold under the same experimental conditions. Toxin-induced mortalities were consistently higher among LE clams (up to 30%) compared to BF clams (2 to 8%). Our findings support the hypothesis that red tides result in natural selection for resistance to PSTs in natural populations.

KEY WORDS: Paralytic shellfish toxins · Softshell clams · *Mya arenaria* · Burrowing · Ecophysiology · *Alexandrium tamarense*

INTRODUCTION

Paralytic shellfish poisoning (PSP) poses a severe human health risk worldwide and toxic events have been increasing globally in frequency, intensity and geographic spread in past decades (Hallegraeff 2003). Severe economic impacts are associated with PSP events ranging from decreased shellfish consumption to indefinite bans on shellfish harvest, as is the case with the butter clam *Saxidomus giganteus* fishery in Alaska where clams remain toxic year-round. An extensive bloom along the New England coast in 2005 led to shellfish harvest closures from central Maine to Martha's Vineyard, Massachusetts, including 40 000 km² of offshore federal waters, prompting the governors of both Maine and Massachusetts to declare a state of emergency (Anderson et al. 2005). The economic losses from this PSP event are reported to exceed $15 million in Massachusetts alone (Anderson et al. 2005). Outbreaks of PSP in North America are caused by toxigenic, unicellular or chain-forming dinoflagellates 20 to 50 µm in size from the *Alexandrium* species complex. The species responsible for PSP on the Atlantic coast of North America are *A. tamarense* and *A. fundyense*, which range in distribution from the Gulf of St. Lawrence to Long Island, New

York (Maranda et al. 1985), whereas *A. catenella* is the source of paralytic shellfish toxins (PSTs) on the Pacific coast of North America, occurring from Alaska to southern California (Taylor 1984).

Bivalves exhibit marked interspecific variation in response to PSTs as measured by various physiological indices, such as *in vitro* nerve sensitivity, feeding rates and toxin kinetics (toxin uptake and detoxification rates), and thus toxicities attained (reviewed by Bricelj & Shumway 1998). The capacity for toxin accumulation is species-specific and is not related to taxonomic status. For example, it can vary considerably among oyster species (Ostreidae) (Shumway et al. 1990). Twarog et al. (1972) ranked PSP toxin sensitivity in several bivalve species from Atlantic North America by subjecting isolated nerves to increasing concentrations of purified saxitoxin (STX) and measuring the effects on the action potential. They found a 1000-fold difference in the STX concentration necessary to block the action potential among the 7 species tested. The blue mussel *Mytilus edulis* was the most resistant to STX, showing no signs of nerve block at the highest concentration tested (10^{-4} g ml^{-1}, 334 µM), whereas the eastern oyster *Crassostrea virginica* was extremely sensitive to STX, with nerve blocking at 10^{-7} g STX ml^{-1} (0.334 µM). The softshell clam *Mya arenaria* (collected from Woods Hole, Massachusetts) was ranked as having intermediate sensitivity, with full nerve block occurring at 10^{-5} g STX ml^{-1} (33.4 µM). It is important to note that the present study was conducted prior to the first documented occurrence of PSP in southern New England in 1972 (B. Twarog, University of Maine, pers. comm.), which resulted from southward transport of dinoflagellate cysts by a hurricane. Kvitek (1993) confirmed the intermediate sensitivity (nerve block at 5×10^{-6} g STX ml^{-1}) from a west coast (Carr Inlet, Washington), non-native population of *M. arenaria*. However, both studies used very small sample sizes (n = 3) for their nerve assays and were thus unable to detect individual variation within the test population.

Variation among individuals in response to PSP toxin exposure within a population of *Mya arenaria* was demonstrated by Bricelj et al. (1996) and Laby (1997). During toxification experiments these authors found that 7 to 31% of clams from a population from Mount Sinai Harbor, Long Island Sound, New York, were insensitive to the effects of toxins as determined by a burrowing index, which measures the ability of *M. arenaria* to reburrow in sediments following short-term (~6 to 24 h) exposure to toxic *Alexandrium* cells. The burrowing index allows rapid and non-destructive characterization of PST sensitivity of individuals in large samples. Burrowing incapacitation is reversible following removal of clams from the toxin source. Bricelj et al. (2004) used the burrowing index to char-

acterize the toxin sensitivity of *M. arenaria* populations along the east coast of North America and generally found a good correlation between resistance and the history of toxin exposure. Thus resistant clams were dominant in populations with a history of PSP outbreaks, whereas sensitive clams were prevalent in areas unaffected by PSP. They also found good agreement between resistance measured by the *in vitro* nerve assay and the burrowing index for 4 Atlantic *M. arenaria* populations. These results supported Twarog's (1974) previously untested hypothesis that bivalve populations recurrently affected by PSP outbreaks might undergo genetic adaptation to toxins through reduced fitness and natural selection against more sensitive individuals.

Our previous work described marked differences in the STX concentration necessary to elicit a nerve block response between representative *Mya arenaria* from 2 Atlantic populations with contrasting histories of PSP and attributed these differences to a point mutation in the sodium (Na$^+$) channel gene sequence of resistant clams that greatly reduced binding affinity to STX (Bricelj et al. 2005). The present study was designed to determine the lethal and sublethal effects of ~2 wk laboratory exposure of *M. arenaria* from these 2 populations. Key questions addressed by the present research include: (1) Does long-term history of exposure of a population to PSTs affect the clams' sensitivity to toxins, as measured by clearance rate (CR) on toxic cells, oxygen consumption (VO$_2$) as a measure of metabolic cost and toxin accumulation rates? (2) Do sensitive individuals acclimate feeding and burrowing responses to toxins after several weeks of laboratory toxification? Hence, can physiological acclimation contribute to toxin resistance? (3) Are PSTs lethal to sensitive clams, and if so, what is the timing of toxin-induced mortalities? As a result of this work, a molecular basis for resistance to PSTs in *M. arenaria* was demonstrated by the identification of a naturally occurring point mutation in Domain II of the Na$^+$ channel gene responsible for a 1000-fold increase in STX-binding affinity in resistant clams (Bricelj et al. 2005, Connell et al. 2007). From the results of the present study the potential fitness consequences of exposure of sensitive softshell clams to a highly toxic bloom of *Alexandrium tamarense* in the natural environment can be inferred.

MATERIALS AND METHODS

***Mya arenaria* collection and maintenance.** *M. arenaria* were collected from the mid-intertidal region to ensure similar immersion time among individuals sampled, using a traditional clam hack, from 2 sites: (1) Lepreau Basin, Bay of Fundy, New Brunswick (BF)

and (2) Lawrencetown River Estuary, Nova Scotia (LE). These sites were chosen because they differ in their long-term toxin exposure histories. Records of annual, recurrent PST levels in *M. arenaria* and *Mytilus edulis* are available for Lepreau Basin since 1943, with a historical maximum of 9000 µg STXeq 100 g^{-1} reported in 1976 (Martin & Richard 1996). The Lawrencetown River Estuary was chosen as representative of sites where shellfish have not been previously exposed to PSP. There is no record of harvest closures due to PSP in the area, and samples from a nearby site (Chezzetcook, Nova Scotia) have never exceeded the regulatory limit of 80 µg STXeq 100 g^{-1} tissues (B. Moore pers. comm.). Only juvenile clams 33 to 47 mm in shell length (SL) were collected for the present study and transported in coolers to the National Research Council's (NRC) Marine Research Station (MRS), Ketch Harbour, Nova Scotia. Juveniles, which are more active, faster burrowers (Emerson et al. 1990), were used in all experiments to minimize the confounding effects of reproductive state as well as reduce the amount of cultured toxic algae necessary for experiments. Clams were collected in early spring prior to bloom initiation or in late fall after they had sufficient time to depurate toxins. The absence of detectable PSP levels in tissues was confirmed at each time.

Prior to experimentation, clams were held in flow-through raceways containing 10 cm of washed coarse sediment (sand) with a flow of ~10 l min^{-1} of ambient, unfiltered seawater (salinity ≈30‰). After several days under these conditions, the temperature was slowly increased (~1°C d^{-1}) to 16°C. Clams were acclimated at this temperature for a minimum of 3 wk (typically 3 to 12 wk). Ambient seawater was supplemented with the continuous addition of the diatom *Thalassiosira weissflogii* via a peristaltic pump.

Algal culture. The toxigenic dinoflagellate *Alexandrium tamarense* (strain PR18b isolated from the Estuary of the Gulf of St. Lawrence, Canada; 35 µm mean equivalent spherical diameter, ESD) was used in all experiments due to its high cellular toxicity. *A. tamarense* was cultured using a modified L1 medium formulation with the addition of NH$_4$Cl at a final concentration of 5×10^{-5} M (Guillard & Hargraves 1993) at 16°C with a 14 h:10 h light:dark cycle (146 µmol s^{-1} m^{-2} irradiance). Non-axenic, dinoflagellate cultures for Expt I were batch-cultured in 20 l plastic carboys filled with 0.22 µm cartridge-filtered, autoclaved seawater with gentle aeration, in a temperature-controlled environmental chamber. Those used in Expt II were cultured under semi-continuous conditions in a 200 l photobioreactor with pH and temperature control (664 µmol s^{-1} m^{-2} irradiance) (Bauder et al. 2001). All cultures used in experiments were harvested in late exponential growth phase.

Thalassiosira weissflogii (12 µm mean ESD) was used as a nontoxic control alga in Expt II and during acclimation. This diatom was cultured in a 200 l photobioreactor at 20°C with continuous light (664 µmol s^{-1} m^{-2} irradiance) using a commercially prepared (Fritz) F/2 medium formulation (Guillard & Ryther 1962).

Toxin analysis. Duplicate samples of experimental *Alexandrium tamarense* cultures were collected for toxin analysis to determine cell toxicity (pg STXeq cell^{-1}) and toxin composition. A predetermined culture volume was retained on a 20 µm sieve to yield 1×10^6 cells for toxin extraction in 0.03M acetic acid (HOAc) following methods described in Bricelj et al. (1990). *Mya arenaria* were individually sampled and dissected into 2 tissue pools for toxin analysis: (1) visceral mass (including the digestive gland, stomach complex and gonadal tissues) and (2) other (remaining) tissues including gills, mantle, foot, adductor muscles and siphons. Wet weight (WW) of tissue pools, SL, as well as foot and siphon response to stimuli, of each clam were recorded. Tissues were immediately frozen in liquid N$_2$ and stored at −80°C. Toxins were extracted from lyophilized tissues in 0.1M HOAc following previously described methods (Bricelj et al. 1990). Total body toxicity was calculated from the toxicity of individual tissue pools and their contribution to total body tissue weight, thus allowing comparisons between experimental and field toxicity data.

Toxin analysis of dinoflagellate and clam tissue extracts were performed by reverse-phase, ion-pair, high-performance liquid chromatography with post-column derivatization and fluorescence detection (HPLC-FD) according to the methods of Oshima (1995) with minor modifications. Three sample injections are required to quantify each of the 3 toxin groups: (1) STX, decarbamoyl saxitoxin (dcSTX) and neosaxitoxin (NEO); (2) N-sulfocarbamoyl C and B toxins and (3) gonyautoxins (GTXs) and decarbamoyl gonyautoxins (dcGTXs). Calibration toxin standards were obtained from IMB/NRC's Certified Reference Materials Program (CRMP), with the exception of dcGTX2&3 and C1&2. These toxins were quantified using toxin solutions provided by Maurice Laycock (IMB, NRC), which were checked at high concentration against the CRMP STX solution using capillary electrophoresis with UV detection. Toxicities were converted to saxitoxin equivalent units (STXeq) based on a conversion factor of 0.23 µg STXeq MU^{-1} (Cembella et al. 1993) using relative potency values of individual toxins (MU µmole^{-1}) derived from the mouse bioassay by Oshima (1995).

Experimental setup. All experimental systems were set up in a walk-in, temperature-controlled environmental chamber at 16°C. Clams were held in 160 l glass aquaria (80 cm L × 40 cm W × 33 cm H) with

10 cm of washed, coarse sediment and 140 l of 0.5 µm cartridge-filtered seawater (30‰), which was replaced every 2 to 3 d to minimize waste buildup. Each aquarium was fitted with 2 external, low-pressure pumps (Super King, Danner Mfg.) (placed at opposite ends) in order to keep the water oxygenated and algal cells in suspension.

The algal culture was continuously delivered via a multi-channel peristaltic pump (Masterflex console drive) from a stock reservoir at a rate necessary to balance consumption and maintain the desired treatment concentration. An algal concentration (Expts I & II) of 100 *Alexandrium tamarense* cells ml^{-1} was chosen to simulate bloom levels known to occur in the BF (Martin et al. 1998), whereas an approximately equivalent biovolume concentration (2000 cells ml^{-1}) of *Thalassiosira weissflogii* was used in the nontoxic control treatment (Expt II). Cell concentrations in the tank were determined in duplicate twice a day, morning and afternoon, using a Coulter Counter Multisizer particle counter (model IIe) fitted with a 140 µm aperture, and periodically confirmed by light microscopy (cell densities determined using the 2 methods showed good linear correlation [$r^2 = 0.98$], with an average difference of only 10%). Addition of algal culture was adjusted accordingly to maintain this concentration.

Burrowing response. The ability to re-burrow in sediment was used as a behavioral measure of the sensitivity of *Mya arenaria* to PSTs. Clams were removed from the sediment, redistributed on the sediment surface and allowed to burrow without interference for 2 h, a period of time determined to be adequate for burial for clams of that size based on previous studies (Bricelj et al. 1996, Emerson et al. 1990). At the end of 2 h, *M. arenaria* with their umbo below the sediment surface were counted as burrowed, and burrowing was expressed as a percentage of the total number of clams in the trial. The time required for burrowing of individual clams was also determined during Expt I. At the end of the trial, clams that failed to burrow during the trial were repositioned vertically in the sediment to maintain their normal feeding position.

Physiological rate measures. Custom-designed chambers were used to house individual *Mya arenaria*, allowing burrowing and acclimation in sediment prior to determination of physiological rates (CR and VO$_2$). Acrylic chambers were constructed based on an earlier design (Lewis 1996, Lewis & Cerrato 1997) comprised of a measurement chamber top and a sediment core bottom. The latter contained 15 cm of washed sediment and was held overnight in the treatment tank allowing clams to burrow and resume normal physiological functions. Prior to conducting physiological rate measurements, the chamber bottom was fitted to the chamber top (260 ml) with an air- and watertight O-

ring seal. The chamber top had inflow, outflow, sampling and oxygen probe ports (see MacQuarrie [2002] for a schematic). The chamber contents were mixed during trials using a 1 cm stir bar held in place at the top of the chamber by a large horseshoe magnet mounted on the outside of the chamber. The magnet was suspended from an 18 V electric hobby motor (Radio Shack) and controlled by a model train voltage regulator (PowerRail 1300). The treatment suspension was gravity fed from a head tank to the chamber assembly via a stopcock manifold. Outflow from the chamber was returned to the head tank with a peristaltic pump (Masterflex) to maintain constant head pressure and flow to the chambers. The head tank was mixed by aeration to ensure a homogeneous suspension. Clams were acclimated in this system and to the treatment suspension under flow-through conditions for 2 to 3 h prior to conducting physiological rate measurements. Only clams with siphons exposed at the sediment surface for the duration of the trial were included in these measurements.

Individual CR was determined using the closed system method described by Coughlan (1969), where: CR = [ln (C_i / C_f)] · V/t. Initial (C_i) and final (C_f) suspension samples were taken from each of the chambers immediately prior to turning off the flow from the head tank at the stopcock manifold and at the end of the incubation period respectively. Clams were allowed to feed during the incubation period (t) previously determined to ensure adequate removal (~20 to 30% depletion). Cell counts were determined in duplicate or triplicate with an electronic particle counter as previously described. The seawater volume (V) of each chamber was measured at the end of each trial.

VO$_2$ rates were determined using a Strathkelvin 6-channel respirometer (model 928) connected to a Toshiba Tectra 8000 portable computer with a microcathode oxygen electrode (model 1302, VO$_2$ ca. 0.5 to 3×10^{-10} mg O$_2$ min^{-1}) placed in each of the chambers. Oxygen probes were calibrated for 0 and 100% saturation with a 2% sodium sulfite solution and with oxygen-saturated filtered seawater at 16°C, respectively. The incubation period allowed reduction of the dissolved oxygen concentration to ≥70% saturation. Oxygen depletion curves were fitted with linear regression equations to determine the VO$_2$. Values were corrected for VO$_2$ due to sediment microfauna, bacteria and/or algae determined for a control chamber with no clam present. VO$_2$ rates were expressed on a weight-specific basis as the range of clam sizes was relatively small (1.24 to 3.15 g soft tissue WW).

Experimental design. *Expt I — burrowing, feeding and toxin uptake rates: Mya arenaria* from the 2 source populations were exposed to toxic *Alexandrium tamarense* (100 cells ml^{-1}) for 15 d in two 160 l aquaria, one

(Tank 1) used for repeated burrowing trials, the other (Tank 2) for acclimation of clams used in feeding trials and sampling for determination of toxin accumulation rates. The burrowing trial tank contained numbered *M. arenaria* to allow tracking of individual response over time (n = 50 for each of the BF and LE populations) yielding a tank stocking density of 312 clams m^{-2}. Burrowing trials (% burrowed after 2 h) were conducted in the same tank (Tank 2) in which the clams were held after 24 h, 3 d, 9 d and 13 d of exposure using the same numbered clams for each trial. Clams for CR measurements were held in the feeding chamber bases and transferred to the physiological rate measurement system to determine feeding rates. CR measurements (n = 5 clams) were carried out after 24 h, 7 d and 14 d of toxin exposure. Clams (n = 40 of each population, BF and LE) were held separately in a section of the tank separated by a plastic mesh divider and sampled for individual toxicity (n = 5 per population) every 2 d in order to determine toxin accumulation rates for each population. At the end of the experiment, *M. arenaria* used in feeding trials were also sampled for toxicity to correlate individual CR with tissue toxicity. The tank was monitored visually every 2 d for mortality, and any dead clams were removed. Tank replication was not possible due to the large amount of toxic algae required over 2 wk, but clams from the 2 source populations were exposed to a common, well-mixed suspension as verified in the experimental setup by preliminary trials. Toxicity patterns for both test populations were again obtained in a sequential experiment (Expt II below), which used duplicate tanks.

Expt II—Oxygen consumption and survival rates: Clams from the 2 test populations were exposed to 2 algal treatments (toxic and nontoxic or control) using 2 replicate tanks per treatment for 18 d. Clams in Treatment 1 were exposed to bloom levels of *Alexandrium tamarense* (100 cells ml^{-1}), and those in Treatment 2 were fed the nontoxic diatom *Thalassiosira weissflogii* (2000 cells ml^{-1}). Clams from both populations were color coded and randomly mixed prior to introduction in each tank. *Mya arenaria* (n = 50 from each population) were placed in one-half of each of the 4 tanks (Tanks 1 & 2: toxified tanks; Tanks 3 & 4: control tanks) (stocking density = 625 clams m^{-2}). Characterization of *M. arenaria* toxin sensitivity after 24 h exposure was conducted in the toxic tanks using the burrowing index (Bricelj et al. 2004), and each clam was identified as either sensitive or resistant with a mark on the shell. Mortalities were determined by removing all clams from the sediment at 2 d intervals, at which point dead clams were removed and their SL recorded; live clams were reintroduced to the sediment in an upright, normal feeding position.

Clams (n = 9 per tank for the toxic treatment to compensate for any losses due to mortality, n = 6 for the nontoxic treatment) used for aerobic metabolic rate measurements were individually numbered and segregated at opposite ends of the tanks. Measurements of VO$_2$ for the treatment group were conducted at 2, 9 and 18 d of exposure; at each time clams from both populations were removed from the tanks and placed in the chamber assembly for overnight acclimation in the physiological rate measurement system under flow-through conditions. VO$_2$ trials for the control group fed *Thalassiosira weissflogii* were conducted the day following the toxified trials due to the time constraints involved in obtaining these measurements simultaneously and were thus always offset by 1 d. At the end of the trial, clams were returned to their respective tanks until the next trial. Clams were removed from the toxified tanks for individual toxin analysis after 2, 5, 8, 12 and 18 d of exposure as previously described. Clams used for VO$_2$ measurements were also sampled for toxin analysis at the end of the experiment.

Statistical analysis. All statistical analysis was performed using the SYSTAT 8.0 (SPSS) software. A 2-way ANOVA repeated measures design allowed comparisons of feeding rates for each population over the duration of the experiment. The 2 populations were compared for each trial using planned comparisons. A 2-way ANOVA was used to compare toxicities for the 2 populations over time. Comparisons of VO$_2$ between populations over exposure time were made with a repeated measures 2-way ANOVA. All statistical tests were performed as 2-tailed tests.

RESULTS

Algal toxicity and toxin composition

Algal concentrations were very similar between treatment tanks and constant throughout the toxification period in Expt I (Tank 1, mean = 103 cells ml^{-1}, SE = 3.4; Tank 2 = 101 cells ml^{-1}, SE = 3.3). Cultures were harvested in late exponential growth phase 10 to 14 d from inoculation at a cell density of ca. 9000 to 13 000 cells ml^{-1}. Algal delivery rate by peristaltic pump averaged 70 000 and 47 000 cells min^{-1} for Tanks 1 and 2, respectively, in order to maintain the desired treatment concentration. Cell toxicity of *Alexandrium tamarense* (strain PR18b) cultures also remained relatively constant over time, averaging 60.7 pg STXeq cell^{-1} (SE = 2.6). Percent molar toxin composition of *A. tamarense* (strain PR18b) remained relatively constant over the experimental period. The N-sulfocarbamoyl toxin, C2, was the highest contribu-

Table 1. Percent *Mya arenaria* molar toxin composition (%M; mean ± SE) of *Alexandrium tamarense* cells and clam tissues from the 2 test populations (Expt I) averaged over the 15 d exposure period (only toxins present at levels >1% are included). –: non-detectable

	C1+2	GTX$_3$	GTX$_2$	NEO	STX
A. tamarense (PR18b)	66.9 ± 0.8	2.6 ± 0.1	–	22.6 ± 0.5	7.6 ± 0.6
Lepreau Basin (BF)					
Viscera	68.3 ± 0.9	2.6 ± 0.3	–	18.9 ± 1.1	8.7 ± 0.4
Other tissues	62.9 ± 0.4	3.3 ± 0.4	1.5 ± 0.3	22.5 ± 0.4	9.2 ± 0.4
Lawrencetown Estuary (LE)					
Viscera	67.4 ± 0.6	2.9 ± 0.2	–	16.5 ± 1.1	12.1 ± 1.1
Other tissues	56.5 ± 1.9	3.6 ± 0.5	1.6 ± 0.3	25.9 ± 1.4	12.1 ± 1.2

tor (66 to 69%) to total toxin concentration, followed by GTX3, NEO and STX; trace amounts of GTX4 and dcGTX3 were also present (Table 1). However because of the low potency of C2, the bulk of the toxicity (63 to 77%) was due to the highly potent NEO and STX derivatives.

In Expt II, *Alexandrium tamarense* (strain PR18b) cultured in the photobioreactor attained a 67% higher mean toxicity (98.1 pg STXeq cell^{-1}) per cell compared to carboy cultures used in Expt I (60.7 pg STXeq cell^{-1}). Cellular toxicity of cultures remained relatively constant over the 3 wk experiment except on Day 13 when carboy cultures were used due to limited availability of culture from the photobioreactor. Algal cell density in toxified treatments was comparable between the 2 replicate tanks, averaging 98 cells ml^{-1} (SE = 3.4) for Tank 1 and 102 cells ml^{-1} (SE = 3.7) for Tank 2. The control tanks contained an approximately equivalent biovolume concentration of nontoxic *Thalassiosira weissflogii* cells averaging 1807 cells ml^{-1} (SE = 85.7)
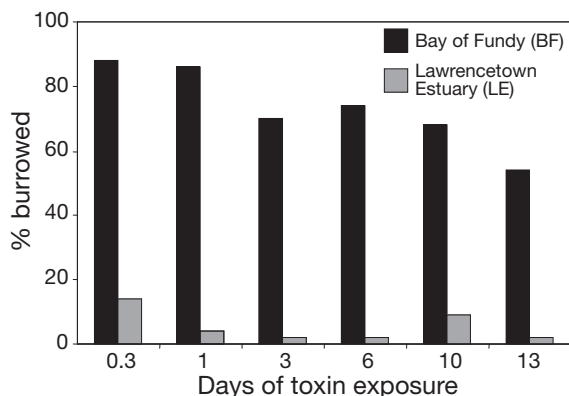

Fig. 1. *Mya arenaria*. Repeated burrowing response (percent of clams of each source population which burrowed after 2 h following exposure at the sediment surface) of clams exposed to the toxigenic dinoflagellate *Alexandrium tamarense* (strain PR18b) in Expt I

for Tank 3 and 1748 cells ml^{-1} (SE = 69.2) for Tank 4. Toxin composition of *A. tamarense* (strain PR18b) was similar to that of cultures used in Expt I except for the presence of a small amount of GTX4 (data not shown).

Burrowing response

Mya arenaria from the LE population were adversely affected by PSTs after only 7 h of toxin exposure, as evidenced by limp, non-retractable siphons (upon aerial exposure or tactile stimulation) and inability to close valves. After 7 h of exposure the ability of most individuals from the LE population to re-burrow in the sediment was significantly reduced by the presence of toxic *Alexandrium* cells, compared to clams from BF (p < 0.001, *F* = 96.4, df = 1) (Fig. 1). Incapacitated LE clams remained in a horizontal position on the sediment surface with the foot partially to fully extended (no probing of substrate) and non-responsive to physical stimuli. Less than 10% of the population was able to burrow throughout the experimental period. Percent burrowing of BF clams was 88% after 7 h of toxin exposure, but was significantly reduced over time (p = 0.005, *F* = 30.7) to a low of 54% after 13 d of exposure. A small proportion of individual LE clams (2 to 14%) were able to burrow throughout the experiment despite the presence of toxins. Conversely, a percentage of BF clams (12 to 46%) were unable to burrow during toxin exposure, thus indicating the presence of 2 distinct phenotypes within each of the 2 populations. There was no significant difference in time required to burrow between BF clams (mean = 44.5 min, range = 14.7 to 114.0 min, N = 49) and LE clams (mean = 50.1 min, range = 26.1 to 98.5 min, N = 12) (p = 0.430, *F* = 0.63, df = 1).

Feeding rates

Observations conducted during feeding trials showed flared, distended siphons with extended guard tentacles in BF clams, whereas most LE clams exhibited a reduced siphon diameter or crimped siphons (both inhalant and exhalant). CR of BF clams was significantly higher than that of LE clams for all 3 exposure times, Day 1 (p < 0.001), Day 7 (p < 0.001) and Day 14 (p < 0.001) (Fig. 2). Feeding rates of LE clams did not change significantly during the 14 d of exposure, remaining below 5 ml min^{-1} clam^{-1}. After 7 d of exposure feeding rates for BF clams were significantly
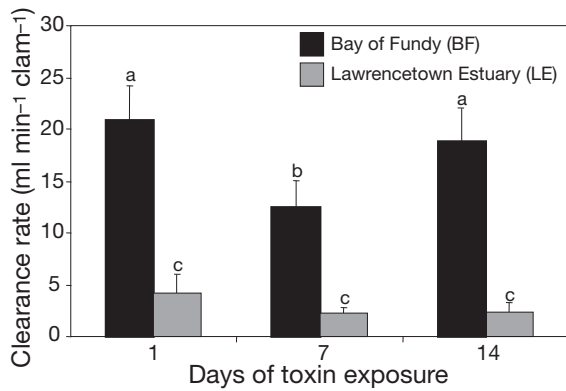
Fig. 2. *Mya arenaria.* Repeated clearance rate (mean ± SE) of clams from 2 source populations exposed to *Alexandrium tamarense* (strain PR18b) in Expt I. Different letters indicate statistically significant differences as determined by 2-way ANOVA (α = 0.05). Tissue wet weight (mean ± SE): LE = 1.87 g ± 0.06; BF = 2.14 g ± 0.10
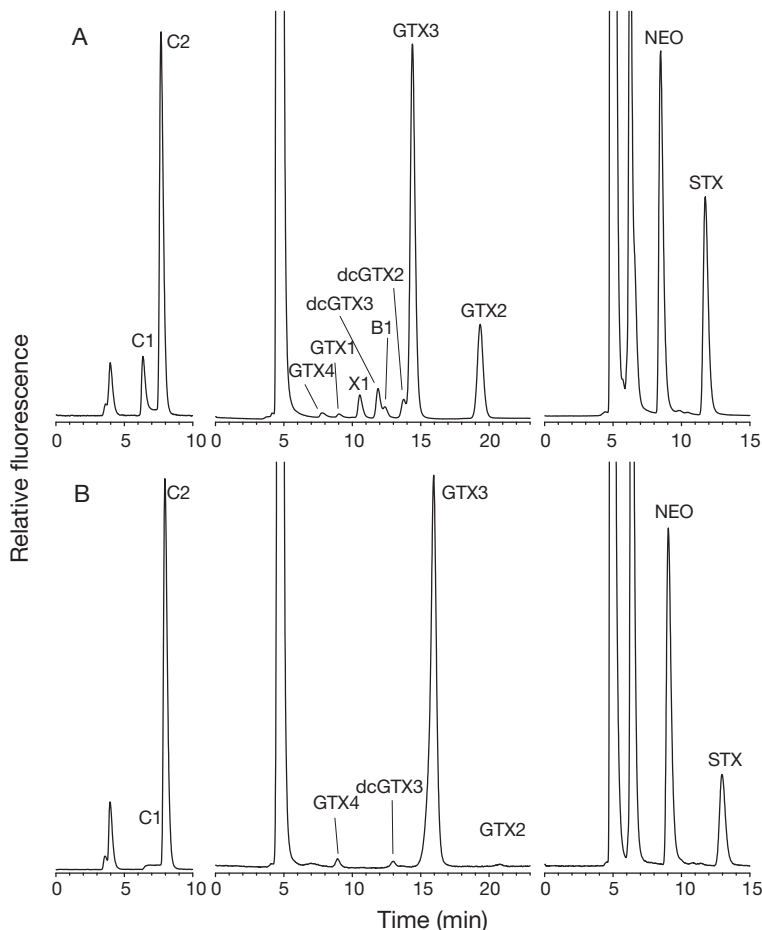

Fig. 3. *Mya arenaria.* Representative chromatograms from high-performance liquid chromatography (HPLC) toxin analysis. (A) *M. arenaria* visceral extract; (B) *Alexandrium tamarense* (PR18b) extract. Note the unknown toxin (X1) present in the clam tissues but not in the phytoplankton extract (identified by Quilliam et al. [2001] from samples provided from the present study)

lower (mean = 12.5 ml min^{-1} clam^{-1}) than those measured at 24 h and 14 d of exposure (means of 18.1 and 18.8 ml min^{-1} clam^{-1}, respectively) (p = 0.045, F = 4.2, df = 2).

Toxin accumulation

Toxin analysis revealed a previously unknown PST derivative in clam tissue extracts that was absent in the *Alexandrium tamarense* cell extracts. This toxin was not included in calculations of total toxicity as its relative potency remains unknown. Fig. 3 shows representative chromatograms of clam visceral extract compared to that of isolate PR18b.

Mya arenaria from the BF population accumulated significantly higher total body toxin levels over the exposure period compared to the LE population (p < 0.001, 2-way ANOVA, Fig. 4A). Removal of rare phenotypes from each population (based on individual burrowing response at 24 h exposure) resulted in a maximum 9-fold difference in mean toxicity at 7 d of exposure between the 2 populations (Fig. 4B). Although based on a few individuals on each sampling date, the toxicity of rare phenotypes within each test population was typically markedly different from that of common phenotypes, with burrowers (resistant clams) having a higher toxicity than non-burrowers (sensitive clams), supporting the theory that 2 distinct phenotypes exist within each population.

Clams from both populations exceeded the regulatory limit of 80 µg STXeq 100 g^{-1} after 24 h of toxin exposure, on average by 27-fold for BF clams and 15-fold for LE clams. Assuming a linear toxin accumulation rate over the first 24 h, BF and LE clams would have reached the regulatory limit in <1 h and <2 h, respectively. Toxicities of LE clams did not increase substantially after 24 h of toxin exposure, reaching only 1.5 times this level by the end of the experiment. Toxin levels in the viscera of the 2 populations (calculated only for the common or dominant phenotype) differed significantly (data not shown). Toxicity of BF clams peaked at 77 000 µg STXeq 100 g^{-1} (4037 nmol g^{-1}) after 15 d, whereas LE clams attained an order of magnitude lower toxicity at 8200 µg STXeq 100 g^{-1} (431 nmol g^{-1}). Peak toxicity in the other tissues among the common phenotypes
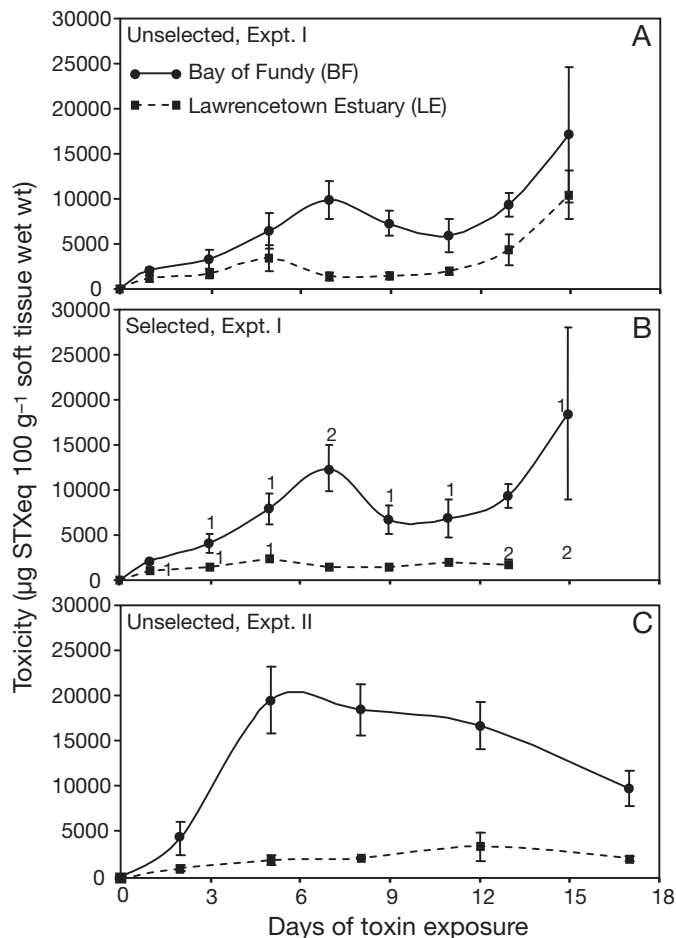
Fig. 4. *Mya arenaria.* Total body toxicity (µg STXeq $100 g^{-1}$) of clams from 2 populations exposed to *Alexandrium tamarense* (mean ± SE, n = 5 clams). (A) Expt I, all data included; (B) Expt I, only dominant phenotypes included, i.e. resistant BF clams and sensitive LE clams as determined from the burrowing response at 24 h. Numbers in bold indicate no. of clams with rare phenotypes removed; (C) Expt II, all data included. Curves drawn by smoothed line interpolation between data points

(data not shown) was twice as high in the BF population (1600 µg STXeq $100 g^{-1}$, 78 nmol g^{-1}) as in the LE population (800 µg STXeq $100 g^{-1}$, 17 nmol g^{-1}). Total body toxicity was 3 times higher in the BF clams used to determine feeding rates (9723 µg STXeq $100 g^{-1}$, SE = 1270) than in the LE clams (2916 µg STXeq $100 g^{-1}$, SE = 645) after 14 d of exposure (p = 0.002).

In Expt II, total body toxicity in BF clams reached a maximum of ~20 000 µg STXeq $100 g^{-1}$ (1053 nmol g^{-1}) after only 5 d of exposure (Fig. 4C), a level reached in Expt I only after 15 d of exposure. However, marked differences in toxicities attained by the 2 test populations were again evident. LE clams accumulated significantly less toxin (p < 0.001) reaching a maximum of only 3300 µg STXeq $100 g^{-1}$ (174 nmol g^{-1}) after 12 d of

exposure, a value representing only 16 % of the maximum of the BF population. Toxicity in BF clams exhibited a significant downward trend after reaching the maximum, attaining 50 % of peak toxicity at the end of 17 d, whereas no significant decline in toxicity was evident in the LE population. As in Expt I, the toxicity pattern of BF clams did not allow description of toxin kinetics by fitting simple, standard equations. Toxin accumulation in the viscera contributed ca. 80 % of the total body toxin burden and exhibited similar patterns as total body toxin accumulation. BF clams reached 68 000 µg STXeq $100 g^{-1}$ in the viscera at 8 d, whereas LE clams accumulated significantly less toxin (12 000 µg STXeq $100 g^{-1}$, p < 0.001) in the viscera after 12 d of exposure. Relative toxicity patterns in other tissues were similar to those described for the viscera with peak toxicities of 3200 µg STXeq $100 g^{-1}$ in BF clams and only 750 µg STXeq $100 g^{-1}$ in LE clams after 12 d. These differences were highly significant (p < 0.001). The 2 populations did not differ in gross anatomical compartmentalization of toxins. Consistent results were obtained in both experiments, indicating that although viscera only made up 15 to 20 % of total tissue weight, this tissue contributed 80 to 85 % of total toxins in both clam populations.

Percent molar toxin composition of the viscera of both populations did not differ significantly from that of *Alexandrium tamarense* cells (BF: p = 0.372, LE: p = 0.205) (Table 1). However, when comparing the toxin composition of the other tissues pool with *A. tamarense* cells, there was a significant increase in the more potent carbamate toxins (NEO + STX) (BF: p = 0.003, LE: p < 0.001) as well as a significant reduction in the less potent N-sulfocarbamoyl toxins (C1+2) (p < 0.001) in both populations. A comparison of the 2 major toxin groups between the 2 clam populations showed that there was no significant difference in either the relative amount (% molar) of N-sulfocarbamoyl (BF = 68.3 %, LE = 67.4 %, p = 0.392, F = 0.781) or carbamate toxins (BF = 27.6 %, LE = 28.6 %, p = 0.368, F = 0.865) in the viscera. The LE population did, however, have a significantly higher relative content of carbamate toxins (BF = 31.7 %, LE = 38.1 %, p = 0.014, F = 7.929) and a significantly lower one of N-sulfocarbamoyl toxins (BF = 62.9 %, LE = 56.5 %, p = 0.006, F = 10.648) in the other tissues compared to the BF population. This pattern of carbamate enrichment in the other tissues was also evident in the LE population in Expt II (data not shown).

Oxygen consumption rates

VO_2 rates for *Mya arenaria* fed *Alexandrium tamarense* were significantly reduced, by 80 %, in LE clams at 2 d (p = 0.002) and by 60 % at 18 d (p = 0.038)
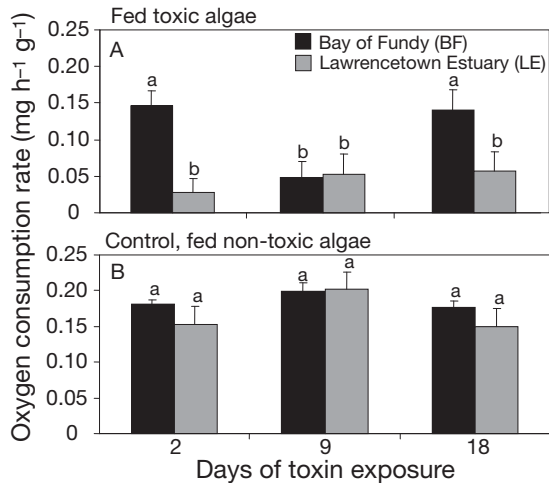
Fig. 5. *Mya arenaria.* Weight-specific oxygen consumption rates (mean ± SE, based on soft tissue wet weight) of clams from the 2 source populations over the 18 d of toxin exposure in Expt II. (A) Toxified treatment, (B) controls exposed to nontoxic algae (*Thalassiosira weissflogii*). Different letters indicate statistically significant differences as determined by 2-way ANOVA (α = 0.05)

relative to those of BF clams. However, VO$_2$ of clams from both populations was significantly reduced (and comparable) at 9 d of exposure relative to nontoxic controls (p < 0.001) (Fig. 5A). This reduction in VO$_2$ for the BF population at the midpoint of the toxin exposure period coincides with the reduction in feeding rates after 7 d in Expt I. However, there was no difference in VO$_2$ between BF clams fed toxic *A. tamarense* on Days 2 and 18. These overall differences in VO$_2$ between *M. arenaria* populations are consistent with the results of a preliminary VO$_2$ trial conducted in Expt I, which showed a 40% reduction in the sensitive (LE) relative to the resistant (BF) population fed *A. tamarense* after 8 d of exposure (data not shown). Measurements of VO$_2$ for clams from both populations fed the nontoxic *Thalassiosira weissflogii* control diet showed no significant difference between populations or over time (Fig. 5B).

Mortality rates

In Expt I LE clams exhibited marked cumulative mortalities which started after 7 d of toxin exposure and reached 42% among clams that remained undisturbed in the sediment and 26% for clams repeatedly removed from sediment after 16 d of exposure. In contrast, BF clams experienced <2% mortality by the end of toxification (Fig. 6). Although cumulative mortality among LE clams that were left undisturbed was higher than that for clams that were regularly removed from the sediment during sampling (handled), differential

mortality of LE relative to BF clams was evident in both groups. Clams from both populations from the same collection date as the experimental clams, held in parallel in the raceway system and fed nontoxic algae, suffered no mortality over the course of the experiment. The exact timing of mortality among sensitive clams was difficult to determine as live but incapacitated (paralyzed) clams are difficult to distinguish from recently dead clams. Only clams exhibiting obvious signs of morbidity (blackened mantle and siphon, strong odor, decomposing soft tissues) were removed and included in mortality counts. Thus PSTs were lethal to sensitive clams and differentially affected survival in the 2 test populations.

In Expt II, the dominantly sensitive population (LE) suffered significantly higher mortalities than the dominantly resistant population (BF) (p < 0.001) (Fig. 7): mortalities of LE clams began within ca. 1 wk as in Expt I, and reached 16% at 23 d of toxin exposure, whereas they began at 10 d of exposure and reached 8% in BF clams. During the same exposure period, no mortality occurred in the 2 nontoxic, control tanks for either population. Phenotype-specific mortalities were calculated, although this experiment was not designed for this purpose; their determination is the subject of a follow-up study using larger sample sizes of rare phenotypes within each population. Based on burrowing response at 24 h of toxin exposure, sensitive individuals (non-burrowers) showed higher mortalities compared to resistant individuals (burrowers) within the same population (Fig. 7 inset). However, caution should be exercised in interpreting these preliminary results due to the small sample sizes (n = 6 to 14) of the rare phenotypes within each population.
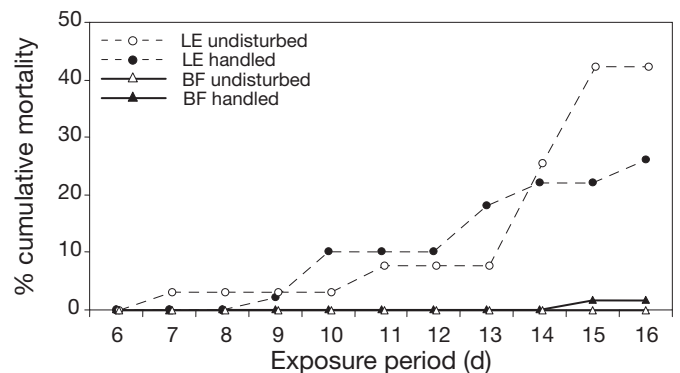

Fig. 6. *Mya arenaria.* Cumulative mortality of clams from 2 source populations (Expt I) exposed to *Alexandrium tamarense* (strain PR18b). Solid line = Bay of Fundy (BF); broken line = Lawrencetown Estuary (LE); solid symbols = handled (Tank 1); open symbols = undisturbed clams (Tank 2; see text)
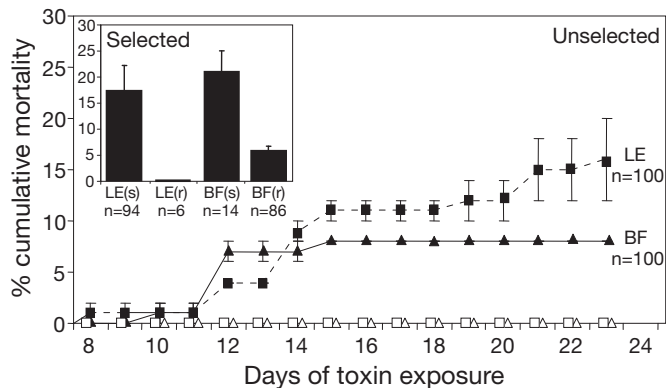
Fig. 7. *Mya arenaria.* Cumulative mortality (mean of 2 tanks ± SE) for the 2 test populations exposed to *Alexandrium tamarense* (strain PR18b) (Expt II). Toxified clams including both resistant and sensitive phenotypes as determined by burrowing at 24 h (solid symbols), and non-toxic controls (open symbols); solid line = Bay of Fundy (BF); broken line = Lawrencetown Estuary (LE). Inset compares cumulative mortalities of clam phenotypes at the end of the experiment (Day 23); s = sensitive, r = resistant

DISCUSSION

Overall, this study demonstrates that there are marked differences in burrowing behavior, feeding, metabolic rates, toxin accumulation and survival between sensitive and resistant populations of *Mya arenaria* and that these differences are maintained over 2 wk of exposure to toxic dinoflagellate cells. These findings support and expand on previous research which showed that LE and BF populations differ greatly in prevalence of resistant and sensitive clams, as determined by the *in vitro* nerve effects of STX, and in their genotype composition determined from the Domain II Na$^+$ channel gene sequence (Bricelj et al. 2005, Connell et al. 2007). The combined results of these studies strongly support Twarog's (1974) hypothesis that exposure to PSTs can result in selection for toxin resistance in natural shellfish populations.

Behavioral responses

Mya arenaria populations with contrasting histories of PST exposure exhibited marked variation in their behavioral responses (burrowing and siphon retraction) during laboratory exposure to toxins. Paralytic shellfish toxins block Na$^+$ channels in nerves, thus preventing the conductance of an action potential along nerve fibers (Narahashi et al. 1967). Burrowing in *M. arenaria* is a complex, hydraulic process, involving several muscular systems that work simultaneously (Trueman 1966, Checa & Cadée 1997). Each of these is under nervous control and thus can be affected by

PSTs. The foot is innervated by the pedal nerve under the control of the pedal ganglia, whereas siphon retraction is controlled by the visceral ganglia via the pallial nerve (Stefano et al. 1990). It is difficult to discern which of these neuromuscular systems is most affected by PSTs, but visual observations of foot extension, valve gape and lack of siphon retraction among sensitive individuals suggest that burrowing inhibition is the result of loss of nervous control of all or a combination of these systems.

The percentage of sensitive (non-burrowers) to resistant (burrowers) individuals varied among the 2 *Mya arenaria* populations in relation to their previous PSP histories: the LE population (no history of toxin exposure) was predominantly comprised of sensitive individuals, whereas the BF population (annual, recurrent PSP outbreaks) was dominated by resistant individuals. The percent of resistant clams (4 to 8%) in the LE population was consistent between Expts I and II. Bricelj et al. (1996) found similar effects on burrowing when a *M. arenaria* population from Long Island, New York, (no history of PSP) was exposed to a toxic *Alexandrium tamarense* (strain PR18b): only 7 to 31% of clams were able to burrow in the presence of toxins over the 11 d exposure period.

Impaired burrowing and siphon retraction in infaunal bivalves can have important ecological consequences in the natural environment. In addition to the toxin-induced mortalities demonstrated in the laboratory, clams that are sensitive to PSTs are likely to be exposed to increased predation and desiccation in the intertidal during toxin events. Impaired clams are likely to be exposed at the sediment surface due to tidally induced scour in a high-energy environment such as the BF and are thus expected to be more vulnerable to predation. Increased risk of predation by demersal fish and crabs during high tide and predation by birds, gulls in particular, during low tides was suggested by Emerson et al. (1990) when sublegal-sized clams were discarded during intertidal harvesting. The lack of siphon response could lead to increased vulnerability to siphon-cropping fish, known to adversely affect growth of infaunal bivalves (Peterson & Skilleter 1994). Fitness differences between sensitive and resistant phenotypes due to direct or indirect effects of PSTs during red tides could thus lead to selection of the more resistant clams during toxin exposure in nature.

Feeding and VO$_2$ rates

Previous studies have shown interspecific differences in the feeding response of bivalves to PST-producing dinoflagellates. *Mytilus edulis*, a species with nerves resistant to STX, feeds readily on toxic

Alexandrium cells (Bricelj et al. 1990), whereas oysters *Crassostrea virginica* (Shumway & Cucci, 1987) and *C. gigas* (Bardouil et al. 1993, 1996) exhibit severe feeding inhibition. In the present study, we first demonstrate significant differences in feeding rate on toxic cells among bivalve populations of the same species related to their prior history of toxin exposure and genotypic composition (as reported in Bricelj et al. 2005). Thus, exposure to a unialgal suspension of highly toxic *A. tamarense* cells caused severe feeding inhibition (up to 80 %) among *Mya arenaria* from a population with no prior exposure to PSP, whereas clams from a historically exposed population maintained CRs on toxic cells significantly higher than those of sensitive clams. The mean CR of clams from the 2 populations also correlated with their nerve sensitivity to PSTs (Bricelj et al. 2005) and their differential toxin accumulation capacity, as demonstrated in the present study.

Weight-specific CRs of resistant clams (mean = 50 ml min^{-1} g^{-1} dry wt) were comparable to those obtained for similar-sized *Mya arenaria* fed nontoxic diets under similar conditions in other studies (e.g. CR: 49 ml min^{-1} g^{-1} dry wt in Riisgård & Seerup [2003]). Bivalves can reduce the ingestion of undesirable or toxic particles via reduction of pumping rate and/or selective rejection in pseudofeces. Shumway & Cucci (1987) exposed *M. arenaria* from a population in Maine, USA, to a nontoxic suspension spiked with toxic *Alexandrium tamarense* cells (strain GT429, at 500 cells ml^{-1}, cell toxicity not quantified). Clams removed toxic cells from this suspension at a rate 28 % below that of the nontoxic control, but rejected cells via copious pseudofeces production. In the present study, at a bloom density of 100 *A. tamarense* cells ml^{-1}, no production of pseudofeces was observed. Thus, the observed differences in feeding rates between sensitive and resistant clams can be attributed to differences in CR (or pumping rate, as *A. tamarense* exceeds the size threshold for 100 % retention efficiency by the gill [Møhlenberg & Riisgård 1978]). Bricelj et al. (1996) compared CR of *M. arenaria* (from the LE population) fed 3 strains of *A. tamarense* ranging from nontoxic to highly toxic and a nontoxic control of *Thalassiosira weissflogii*. They found no reduction in CR in clams offered the moderate (20 pg STXeq $cell^{-1}$) or nontoxic strain of *A. tamarense* compared to the control following short-term (2 to 3 h) exposure, but CR on the highly toxic PR18b strain was significantly reduced (by 90 %). CR for clams offered the highly toxic strain in a mixture with nontoxic *T. weissflogii* (30 % toxic *A. tamarense* cells based on volume) did not differ significantly from that of the control. The present study used unialgal suspensions of toxic *Alexandrium* to simulate red tides, which are largely monospecific at the peak of the

bloom. Future studies should determine whether feeding rate differences between resistant and sensitive clams are also observed in mixed assemblages of toxic and nontoxic phytoplankton, as often observed during bloom initiation and termination phases.

The high individual and temporal variability in CR shown in the present study within a given *Mya arenaria* population suggests that CR inhibition is less useful than burrowing incapacitation for characterization of individual toxin sensitivity in this species. Feeding rate measurements, however, may be very useful to determine toxin sensitivity of epifaunal, non-burrowing species.

In bivalves, food particles are drawn into the mantle cavity by gill ciliary feeding currents, captured on the gill and transported to the mouth in ciliated food tracts. The amount of current generated by the gills is a function of the ciliary beat frequency (CBF) of the lateral cilia which can be altered as a means of varying the amount of particles brought in contact with the gills. Although ciliary beating is autonomous, CBF is under nervous control by the visceral ganglion via the branchial nerve (Aiello & Guideri 1965). Aiello (1960) discovered that by cutting the branchial nerve in *Mytilus edulis* the cilia continued to beat, but at a highly reduced rate which could persist for several days. Video-endoscopy observations of *Mya arenaria* during feeding on *Alexandrium tamarense* (strain PR18b) showed muscular convulsions, contraction and collapse of the gill, typically within 10 min of exposure to toxic cells (Bricelj et al. 1998). In an individual clam exhibiting no response to tactile stimuli (after 10 h of exposure), the gill appeared fully relaxed and no intra-pallial feeding currents were detected. Thus, the loss of nervous system control of the gill caused by toxin-induced block of the Na^+ channel in sensitive individuals could explain the feeding inhibition observed among sensitive clams. The mechanism involved appears to be post-ingestion incapacitation rather than chemosensory detection of PSTs. This hypothesis is supported by our visual observations indicating that sensitive clams can feed readily upon initial exposure to toxic cells, but subsequently experience feeding inhibition, including loss of ability to extend, contract or dilate the siphon for feeding.

Mya arenaria from the sensitive LE population also showed significant reduction (60 to 80 %) in VO_2 in the presence of toxic *Alexandrium tamarense* compared to clams from the resistant BF population and to those fed a nontoxic, control alga. VO_2 of BF clams did not differ significantly from that of the control group with the exception of measurements conducted midway through the experiment (9 d exposure), when VO_2 was significantly reduced (75 %) in both test populations. A reduction in CR was also observed for BF clams in

Expt I after a similar exposure period (7 to 9 d), suggesting that even resistant clams are eventually affected by sufficiently high PST levels in the tissues. Physiological functions such as feeding and metabolic rate may thus be negatively affected once a threshold toxicity level is reached. In contrast to these results, Shumway et al. (1985) found that 5 d of exposure of *M. arenarium* to toxic *Alexandrium* cells caused an increase in VO_2 with a subsequent decrease to normal values after 1 wk of recovery. The collection site was not specified, so clams are of unknown PST sensitivity. However, their results for the sea scallop *Placopecten magellanicus* and surf clams *Spisula solidissima* are consistent with results from the present study that show a reduction in VO_2 during toxin exposure. Gainey & Shumway (1988) showed no significant effect of toxic cells on cardiac activity in *M. arenaria* collected from Long Cove, Searsport, Maine, USA.

The reduction in mantle cavity irrigation rate caused by PST-induced loss of nervous control of the gill could result in an oxygen deficit and an increase in anaerobic metabolism. *Mya arenaria* experience daily aerial exposure in the intertidal. However, extended toxin-induced paralysis (on the order of days to weeks) could result in reduced intrapallial flushing and lead to hypoxic or anoxic conditions and accumulation of anaerobic end products. Future studies should investigate the contribution of anaerobic metabolism to total metabolic rate in relation to toxin resistance.

It is difficult to predict the overall impacts of differential toxin sensitivity on scope for growth of *Mya arenaria* populations, as other parameters such as absorption efficiency of *Alexandrium* cells and total metabolic rate (including anaerobic metabolism) would need to be determined. Scope for growth of sensitive individuals may not necessarily be reduced relative to resistant clams given that, in the former, both VO_2 and ingestion rate decrease during toxin exposure.

Toxin accumulation

High variability in toxin accumulation in individuals within a bivalve species in relation to environmental parameters such as position in the intertidal zone and microhabitat differences has been described previously (Quayle 1969, Shumway et al. 1994, Curtis et al. 2000). However, this study in conjunction with that of Bricelj et al. (2005) presents the first detailed investigation of intraspecific behavioral and physiological differences associated with history of toxin exposure and its relationship with genotypic differences. Toxin uptake in tissues is expected to be correlated with ingestion rates of toxic cells in suspension-feeding bivalves, and this is demonstrated for *Mya arenaria* in

the present study. Most of the toxin is generally retained in the visceral mass (~80%) during toxification (Bricelj & Shumway 1998), as confirmed in the present study for *M. arenaria* from both sensitive and resistant populations. Sensitive clams readily fed upon toxic cells during initial exposure, until a threshold level in total body toxicity (2000 to 3000 µg STXeq $100\,g^{-1}$) was reached, which in turn was followed by a reduction in feeding rates. This pattern of ingestion for sensitive clams is evident in the toxin accumulation patterns where a maximum toxin level is reached early during toxification and remains relatively unchanged with continued toxin exposure. Total toxin body burden in sensitive clams ranged from 1700 µg STXeq $100\,g^{-1}$ (89 nmol g^{-1}) in Expt I to 3300 µg STXeq $100\,g^{-1}$ (174 nmol g^{-1}) tissue WW in Expt II. In contrast, resistant *M. arenaria,* which were relatively unaffected by toxins, continued to ingest toxin cells and incorporate toxins in their tissues, reaching an extremely high total toxin body burden (18 000 and 20 000 µg STXeq $100\,g^{-1}$ [948 and 1053 nmol g^{-1}] in Expts I and II, respectively) during extended periods (2 wk) of exposure.

The pattern and magnitude of toxin accumulation in Expt I was influenced by the differential survival rates of sensitive and resistant clams within the 2 test populations. Selective mortality of more sensitive LE clams resulted in an increased proportion of resistant clams with a higher toxin accumulation capacity by the end of the exposure period (Fig. 3A,B). Non-asymptotic toxin accumulation was observed in Expt II in which maximum total body toxicity (19 500 µg STXeq $100\,g^{-1}$) was reached after 5 d of exposure, followed by a decrease in toxicity for the remainder of the 17 d experiment. Maximum toxicities attained by BF clams were comparable between the 2 experiments, ranging from 12 500 to 19 500 µg STXeq $100\,g^{-1}$. However, the toxicity maximum was reached earlier, after only 5 d of exposure in Expt II compared to 15 d in Expt I. This may be the result of the higher toxicity of *Alexandrium tamarense*, 98 pg STXeq cell^{-1} in this experiment, versus 61 pg STXeq cell^{-1} in Expt I. Fluctuations in feeding and toxin uptake in BF clams further support the hypothesis of intermediate toxin resistance in *Mya arenaria*, compared to highly resistant species such as *Mytilus edulis* which experience a monotonic increase in toxicity over time until an asymptote is reached (Bricelj et al. 1990). Prediction of toxin uptake kinetics in *M. arenaria* is thus expected to be a function of the composition of resistant and sensitive individuals in a given population.

There are few studies of laboratory PSP toxification of *Mya arenaria* with which to compare results from the present study. Bricelj et al. (1996) exposed *M. arenaria* (from Long Island, New York, an area with no history of PSP) and *Spisula solidissima* to *Alexandrium*

tamarense (strain PR18b, 74.5 pg STXeq cell^{-1}) under comparable conditions. *M. arenaria* reached 1340 µg STXeq 100g^{-1}, whereas *S. solidissima* reached a mean total body toxicity of ~30 000 µg STXeq 100g^{-1} after 11 d of toxin exposure. The toxicities for *M. arenaria* closely match those obtained for the LE sensitive population in the present study.

Toxicities for *Mya arenaria*, derived from the PSP monitoring program in Lepreau Basin, indicate that clams can reach field toxicities comparable to those obtained in the present laboratory study; a maximum total body toxicity of 9100 µg STXeq 100g^{-1} was recorded in 1976 (Martin & Richard 1996). These authors reported that *Mytilus edulis* from a BF population could reach toxicities >28 000 µg STXeq 100g^{-1}, whereas co-occurring *M. arenaria* only attained toxicities 30% of that value.

Findings of this study have important implications for management of shellfish PSTs and toxin monitoring of *Mya arenaria* in Atlantic North America. Regardless of site or individual toxin sensitivity, *M. arenaria* readily ingested toxic cells upon initial exposure to bloom conditions, exceeding the 80 µg STXeq 100g^{-1} regulatory limit within 4 h (all experiments) and in some cases within 1 h (assuming a linear toxin uptake during the first 24 to 48 h). Individual variation could account for up to a 10-fold difference in total body toxicity among clams within a population and up to a 52-fold difference among individuals from different populations toxified under identical conditions. Monitoring of PSP in North America relies on pooling of several individuals (to attain 100 g total soft tissue WW) for determination of toxicity by the mouse bioassay and as a result will mask this inter-individual variation and could thus potentially underestimate public health risk.

Toxin composition in both the viscera and other tissues from both resistant and sensitive populations generally varied little from that of the toxin composition of ingested dinoflagellates. This agrees with prior studies which show that *Mya arenaria* has a limited capacity for biotransformation of PSTs (Martin et al. 1990, Laby 1997, Fast et al. 2006). Nevertheless, some significant changes in toxin composition were documented in *M. arenaria* in the present study. A shift from the less potent N-sulfocarbamoyl toxins (C1&2) to the more potent carbamate toxins (NEO + STX) evident in the other tissues of the sensitive LE population is likely the result of non-enzymatic conversion. This is known to readily occur at low pH (~3) (Hall et al. 1990) and could result from acidic conditions present in the gut. This observed compositional shift might be explained by selective retention of individual toxins. Sensitive individuals reduce their intake of toxic cells after a short exposure period, typically <24 h, presumably due to

toxin incapacitation, while toxin transfer from the viscera to the other tissues continues despite the reduced influx of new toxins via ingestion. If carbamate toxins are selectively retained over N-sulfocarbamoyl toxins or both groups of toxins have differential transfer efficiencies, the effects would be more pronounced in sensitive individuals as a result of the fixed toxin pool in the viscera available for transfer to other tissues. However, the small relative difference in toxin composition between LE and BF clams is insufficient to explain the marked differences in toxin uptake documented, as these are even more pronounced when expressed as molar concentrations.

Toxin-induced mortality

Two sequential experiments showed differential mortalities between the 2 clam populations in relation to their history of exposure to red tides in nature. Mortalities of sensitive LE clams typically started after ~1 wk of toxin exposure. Although both experiments yielded higher mortalities for sensitive than resistant clams, cumulative mortalities after 2 wk exposure were highly variable between experiments, ranging from 16 to 42%. These differences cannot be explained by differences in algal cell toxicity, as this was higher in Expt II, or by the duration of toxin exposure, 23 d in Expt II compared to only 16 d in Expt I. Clam stocking density can also be ruled out as the cause of increased mortality, as this was higher in Expt II (625 clams m^{-2}) than Expt I (312 clams m^{-2}). An explanation for the variability in mortalities between experiments may be found in more subtle differences in experimental design, and in particular, the methods used to determine mortality rates. In Expt I, tanks were inspected daily for signs of moribund clams, but dead clams were not removed from the sediment. Clams showing signs of morbidity were often surrounded by a layer of black, anoxic sediment. In Expt II mortality estimates were based on removal of all clams from the sediment at 2 d intervals (and subsequent reintroduction of live clams to the sediment) to ensure that all clams were closely inspected and that the timing of mortality could be more accurately estimated. Periodic removal of the clams from the sediment in Expt II allowed flushing and re-oxygenation of sediments, thus likely preventing the establishment of anoxic conditions. This was also the only experiment in which BF clams suffered detectable (8%) mortality, and this may be associated with their proximity to the moribund LE clams within the tank. Both populations were kept mixed together in this experiment, whereas in Expt I populations were segregated at either ends of the tank.

Several potential causes of mortality of sensitive *Mya arenaria* exposed to PSTs can be excluded. Starvation resulting from suppressed feeding in toxified, sensitive clams cannot be attributed a role in clam mortalities. MacQuarrie (2002) showed that when LE and BF clams at the sizes used in the present study were starved in the absence of sediment for 3 wk, negligible mortalities occurred. Landsberg (1996) suggested a link between PST exposure and the incidence of neoplasia in bivalves, including *M. arenaria*. However, Morrison et al. (1993) showed low levels of neoplasia occurrence in *M. arenaria* from 5 of 22 sites surveyed in the BF. Furthermore, histological analysis of both control and toxified clams (BF and LE) in Expt II showed no increase in the prevalence of neoplasia among the toxified group, discounting it as a possible cause for the increased mortality rates (V. M. Bricelj et al. unpubl. data). Neoplasia (based on analysis of n = 20 clams per population), was detected in ≤10% of experimental clams and was thus typical of background levels for these populations.

Infaunal organisms, such as the lugworm *Arenicola marina* are known to enhance oxygen availability in the sediments through bioturbation and sediment reworking (Andersen & Kristensen 1991, Retraubun et al. 1996). Infaunal suspension-feeding bivalves also have the ability, although to a lesser degree, to rework sediments during burrowing (Checa & Cadée 1997) and oxygenate the surrounding sediments via circulation of water through the mantle cavity (Hansen et al. 1996). Sensitive clams are incapacitated by exposure to PSTs and are thus limited in their ability to generate feeding currents and reject or flush material from the pallial cavity, activities which allow replenishment of the food and oxygen supply in the body cavity and cleansing of incoming sediment. The observed reduction in VO_2 may not be a result of a decrease in O_2 demand but rather of a decrease in the exchange of gases at the gill and mantle surfaces due to reduced ventilation rates. This may lead to hypoxic and eventually anoxic conditions in the mantle cavity and the surrounding sediments and may be aggravated by bacterial decomposition of dead clams. This is supported by qualitative observations made during dissections, in which pockets of anoxic sediment were found within the mantle cavity of sensitive clams. *Mya arenaria* inhabits the intertidal zone and therefore experiences daily periods of anaerobiosis during low tides and has thus evolved mechanisms to tolerate periods of low oxygen levels. However, muscular paralysis and prolonged exposure to anaerobic conditions (>1 wk) may indirectly cause the observed mortality rates during prolonged toxin exposure. Our observations to date suggest that mortalities during exposure to toxic *Alexandrium* cells are not a direct effect of PSTs, but rather an indirect effect of toxin-induced paralysis and reduced irrigation of the mantle cavity. The association between anoxia/hypoxia within the pallial cavity and surrounding sediments and toxin-induced mortalities will be tested in future studies.

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