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Paralytic Shellfish Toxins in Bivalve Molluscs: Occurrence, Transfer Kinetics, and Biotransformation

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ABSTRACT: This is a critical review of the global distribution, sources of variation in toxicity, anatomical partitioning, metabolism, and detoxification kinetics of paralytic shellfish poisoning (PSP) toxins (carbamate, *N*-sulfocarbamoyl, and decarbamoyl saxitoxin derivatives) in bivalve molluscs. Marked interspecific differences in toxin accumulation are related to differences in toxin sensitivity, determined from neurological, physiological, and behavioral responses. Toxicity also varies considerably with body size, immersion time, off-bottom position, and over distances ≤ 1 km. Bivalve species can be broadly classified as rapid (e.g., *Mytilus edulis*) or slow detoxifiers (e.g., *Placopecten magellanicus*). The former takes weeks to detoxify to the regulatory level (up to 15% toxin loss day⁻¹); the latter takes months to years to detoxify ($\leq 3\%$ loss day⁻¹). Toxin biotransformation, which may lead to changes in net toxicity, varies greatly among species. A few clam species, such as *Protothaca staminea* and *Spisula solidissima*, exhibit rapid enzymatic decarbamoylation, whereas other bivalves (e.g., *Mya arenaria* and *M. edulis*) show limited toxin metabolism and thus are useful indicators of the toxigenic source. Pronounced changes in toxin composition occur when algae are rich in low-potency, *N*-sulfocarbamoyl toxins. Analysis of toxin composition and relative toxin levels of viscera and other tissues can be used to predict the timing of toxic blooms. This review highlights information required to select aquaculture species and effectively manage stocks in PSP-affected areas. Caveats in the interpretation of existing data and needs for future research are identified.

KEY WORDS: paralytic shellfish toxins, bivalves, dinoflagellates, detoxification, biotransformation.

I. INTRODUCTION

Suspension-feeding bivalve molluscs are the principal vectors for the transfer of several major groups of phycotoxins (toxins of algal origin) that pose a health hazard to humans. These include paralytic shellfish poisoning (PSP) toxins, the focus of this review, diarrhetic shellfish poisoning (DSP) toxins, and domoic acid, the causative agent of amnesic shellfish poisoning (ASP). Contamination of bivalves is facilitated by their trophic role as primary consumers, limited mobility, ability to concentrate phytoplankton by pumping large volumes of water per unit time, and the relative insensitivity of some species, compared with finfish, to PSP toxins.

Blooms of PSP toxin-producing dinoflagellates cause serious economic losses worldwide due to closure of shellfish harvesting grounds, the negative "halo" effects on seafood marketing generated by such events, and the need for costly monitoring programs to ensure product safety for human consumption. For example, the total economic loss to the oyster industry from a single PSP incident on the Pacific U.S. coast

in 1980 was estimated at U.S. \$10 million. In 1989, a ban on mussel harvesting in the Pacific Northwest resulted in an estimated loss of \$U.S. 2.2 million. In 1990, the annual cost of PSP toxin in the Pacific Northwest, Columbia, Canada, was valued at \$U.S. 10 million, representing about 4 to 5% of the total value of the shellfish harvest. Harmful algae may directly affect bivalve populations (Shumway et al., 1995). This review focuses primarily on the biotransformation and elimination of PSP toxins and the ecological effects of PSP toxin monitoring and reviews (e.g., Nishitani and Shumway et al., 1995), and the need for future research.

In contrast to the recent interest in PSP, PSP has been known in the Pacific Northwest for over a century, providing a rich historical record. In the last decade, however, advances in the increased availability of PSP toxins, a refined understanding of the toxicity of PSP toxins, ingestion of toxigenic algae, and manipulation of toxin exposure have combined monitoring of PSP in various regions, have also furthered our understanding. Finally, increased attention to the regional and global spread of PSP, especially in southern regions (Shumway, 1989; Hallegraeff, 1995), and the impact of aquaculture activities in coastal areas.

II. CAUSATIVE MICROALGAE AND DISTRIBUTION OF PSP

The microalgae responsible for PSP are dinoflagellates (Dinophyceae), including *Gymnodinium* spp. (formerly included in *Gyrodinium aureolum*) and *Pyrodinium bahamense*, cells armored with cellulose plates. These microalgae are responsible for PSP outbreaks in the Pacific Northwest, the Pacific U.S. coast, and other regions.

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in 1980 was estimated at U.S. \$ 0.6 million (Conte, 1984), and an 8-month ban on mussel harvesting in the Philippines in 1983, resulted in an estimated loss of \$U.S. 2.2 million (Estudillo and Gonzalez, 1984). The annual cost of PSP toxin monitoring in the Bay of Fundy and British Columbia, Canada, was valued at U.S. \$102 K and \$82 K, respectively, representing about 4 to 5% of the value of shellfish harvested in 1988 (Cembella and Todd 1993). Recently, it has become apparent that harmful algae may directly compromise survival and growth in some bivalve populations (Shumway, 1990); however, little is known about the ecological effects of PSP toxins on field populations. Therefore, this review focuses primarily on factors controlling the fate (uptake and elimination) of PSP toxins in various bivalve species. Detailed descriptions of PSP toxin monitoring programs were provided in previous reviews (e.g., Nishitani and Chew, 1988; Cembella and Todd, 1993; Shumway et al., 1995), and are not included in the present study.

In contrast to the recent documented occurrence of DSP and ASP, PSP has been known in North America since the late 1880s, thus providing a rich historical database for the present study. In the past decade, however, advances in the chemical analysis of PSP toxins and the increased availability of analytical standards have led to a more refined understanding of their metabolism in bivalve tissues following ingestion of toxigenic algae. Laboratory studies allowing controlled manipulation of toxin exposure conditions using cultured isolates, and combined monitoring of toxic phytoplankton and shellfish in some regions, have also furthered our knowledge of toxin transfer dynamics. Finally, increased attention has been drawn to this subject by the recent regional and global spread of PSP outbreaks to previously unaffected areas, especially in southern South America and Southeast Asia (Anderson, 1989; Hallegraeff, 1993), and the associated threat to expanding aquaculture activities in coastal waters worldwide.

II. CAUSATIVE MICROALGAE AND GLOBAL DISTRIBUTION OF PSP

The microalgae responsible for PSP in the marine environment are dinoflagellates (Dinophyceae, unicellular members of the phytoplankton), including *Gymnodinium catenatum* (unarmored cells), *Alexandrium* spp. (formerly included in the genus *Gonyaulax* or *Protogonyaulax*), and *Pyrodinium bahamense* var. *compressum*, both of which have cells armored with cellulose thecal plates. The latter species is largely responsible for PSP outbreaks in tropical waters in the Indo-West Pacific

(e.g., Borneo, Sabah, Brunei, Philippines, as far south as Papua-New Guinea), as well as the tropical east Pacific (off the coast of Guatemala and Mexico) (global distribution reviewed by Hallegraeff, 1993). *Gymnodinium catenatum*, a temperate, chain-forming species, is presently distributed in the Gulf of California, Gulf of Mexico, Argentina, Japan, the Philippines, Palau, Tasmania, the Mediterranean, and the Atlantic coast of Morocco, Portugal, and Spain, where it is the most important source of PSP in the galician rias (Hallegraeff, 1993).

A number of toxic *Alexandrium* spp., belonging to the "tamarensis/catenella" species complex, are the cause of PSP in temperate waters and are widely distributed in both Atlantic and Pacific oceans. They typically occur as unicellular or short-chain (< 4 cells) morphotypes (e.g., *A. tamarense*), or as longer chains (> 8 cells) (e.g., *A. catenella*), and range in size from ca. 20 to 50 μm in cell width. Along the Atlantic coastline of North America (from the Gulf of St. Lawrence to Long Island, New York), *A. tamarense* (also cf. *A. excavatum*) and *A. fundyense* are the species implicated in PSP outbreaks, whereas *A. catenella* is the primary source of PSP toxins on the Pacific coast, from Alaska to southern California (Taylor, 1984). *Alexandrium tamarense* is also important in British Columbia waters, where it is usually contiguously distributed (with some overlap) with *A. catenella*. Both *A. catenella* and *A. tamarense* co-exist, although temporally segregated, off the coast of Japan, another region severely affected by PSP. In the southern Atlantic, where the highest PSP toxicities in shellfish have been recorded (Figure 1), *A. tamarense* (cf. *excavatum*) occurs along the Argentine Sea (Carreto et al., 1986), and *A. catenella* is found in the Magallanes Strait (Benavides et al., 1995). Other PSP-causing *Alexandrium* species include *A. minutum* (from Europe, southern Australia, and New Zealand), *A. ostenfeldii* (distributed from Iceland to Spain, and recently found in British Columbia, the Gulf of St. Lawrence and Nova Scotia, Canada), *A. cohorticula* (Thailand); *A. acatenella* (Pacific North America); *A. fraterculus* (S. Japan); and *A. tamiyavanichi* from the Gulf of Thailand.

Maximum historical PSP toxicities achieved by bivalves worldwide, irrespective of the toxigenic dinoflagellate involved in each region, are shown in Figure 1, in order to identify "hot spots" of high toxicity and potential latitudinal patterns in toxicity. Only data for mussels (primarily *Mytilus* spp.) are used for comparison, because bivalves are known to vary greatly in their ability to accumulate PSP toxins. Mussels were selected because they are ubiquitous in coastal waters, and they are commonly used as the indicator organism in PSP toxin-monitoring programs worldwide. Caution must be exercised, however, in the interpretation of these global patterns, for a number of reasons. For example,

other mussel species (e.g., West Pacific region) are the fact that direct comparison among mussel species is available. Higher toxicities in intertidal populations due to feeding time on toxic algal cells (Hallegraeff et al., 1990; Hallegraeff et al., 1993). Although most PSP toxicity data are from the intertidal, only a few are available in a few regions. Confidence in the historical data is depending on the reliability of the sampling frequency. Longer-term data are available in Canada, where shellfish poisoning was first reported in the early 1940s, whereas in New Zealand by PSP outbreaks. For example, in New Zealand (Chang

Despite these caveats, a global analysis. Highest shellfish toxicity was recorded on the west coast (maximum of 127 $\mu\text{g STX/g}$), followed by the Atlantic coast (comparable maxima of 20 $\mu\text{g STX/g}$). Lower levels generally occur in Europe (maxima ranging from 1 to 10 $\mu\text{g STX/g}$ for one high record off the coast of Ireland). These geographic differences in column toxicity ($\mu\text{g STX/g}$) and cell toxicity of dinoflagellates (see section on latitudinal patterns).

It has been argued that the global distribution of PSP has increased (Hallegraeff, 1993). The causes for this increase in the frequency of PSP outbreaks, however, remain controversial. (1) increased primary production in macronutrient ratios favorable for PSP (Hallegraeff, 1990); (2) increased awareness and an increase in monitoring; (3) anthropogenic activities in previously toxin-free areas, such as

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other mussel species (e.g., the green mussel, *Perna viridis* in the Indo-West Pacific region) are used where data for *Mytilus* are lacking, despite the fact that direct comparisons of the potential for toxin accumulation among mussel species under identical exposure conditions are unavailable. Higher toxicities are generally obtained in subtidal rather than intertidal populations due to differences in immersion time and therefore feeding time on toxic algae and/or bloom patchiness (Desbiens et al., 1990; Hallegraeff et al., 1989, 1995; see section on toxin accumulation). Although most PSP toxin-monitoring programs rely on mussels collected from the intertidal, only data for mussels in suspended culture were available in a few regions (see caption for Figure 1). Furthermore, confidence in the historical maxima reported varies among regions, depending on the reliability of the mouse bioassay data and the length and sampling frequency of the monitoring records available. Thus, longer-term data are available for North America, especially eastern Canada, where shellfish monitoring for PSP toxins was implemented in the early 1940s, whereas some regions have been affected only recently by PSP outbreaks. For example, PSP was first reported as recently as 1993 in New Zealand (Chang et al., 1995).

Despite these caveats, some general patterns emerge from this analysis. Highest shellfish toxicities occur along the South American coast (maximum of $127 \times 10^3 \mu\text{g}$ saxitoxin equivalents [STXeq] 100 g^{-1}), followed by the Atlantic and Pacific coasts of North America (with comparable maxima of 28 and $30 \times 10^3 \mu\text{g}$ STXeq 100 g^{-1} respectively). Lower levels generally occur in the Indo-West Pacific region and in Europe (maxima ranging from 0.2 to $4.0 \times 10^3 \mu\text{g}$ STXeq 100 g^{-1} , except for one high record off the Norwegian coast [van Egmond et al., 1993]). These geographic differences presumably reflect differences in water column toxicity (μg STXeq L^{-1}), that is, the product of cell concentration and cell toxicity of dinoflagellate strains occurring in each region (see section on latitudinal patterns below).

It has been argued convincingly that a historical increase in the global distribution of PSP has occurred, especially since 1970 (Hallegraeff, 1993). The causes for this geographic spread, and for a suggested overall increase in the frequency and intensity of harmful algal blooms, however, remain controversial. These have been variously attributed to: (1) increased primary productivity caused by eutrophication and a shift in macronutrient ratios favoring harmful species in coastal waters (Smayda, 1990); (2) increased awareness resulting from improved detection methods and an increase in coastal mariculture activities (Anderson, 1989); (3) anthropogenic activities that provide seed populations to previously toxin-free areas, such as the release of ship ballast water and movement

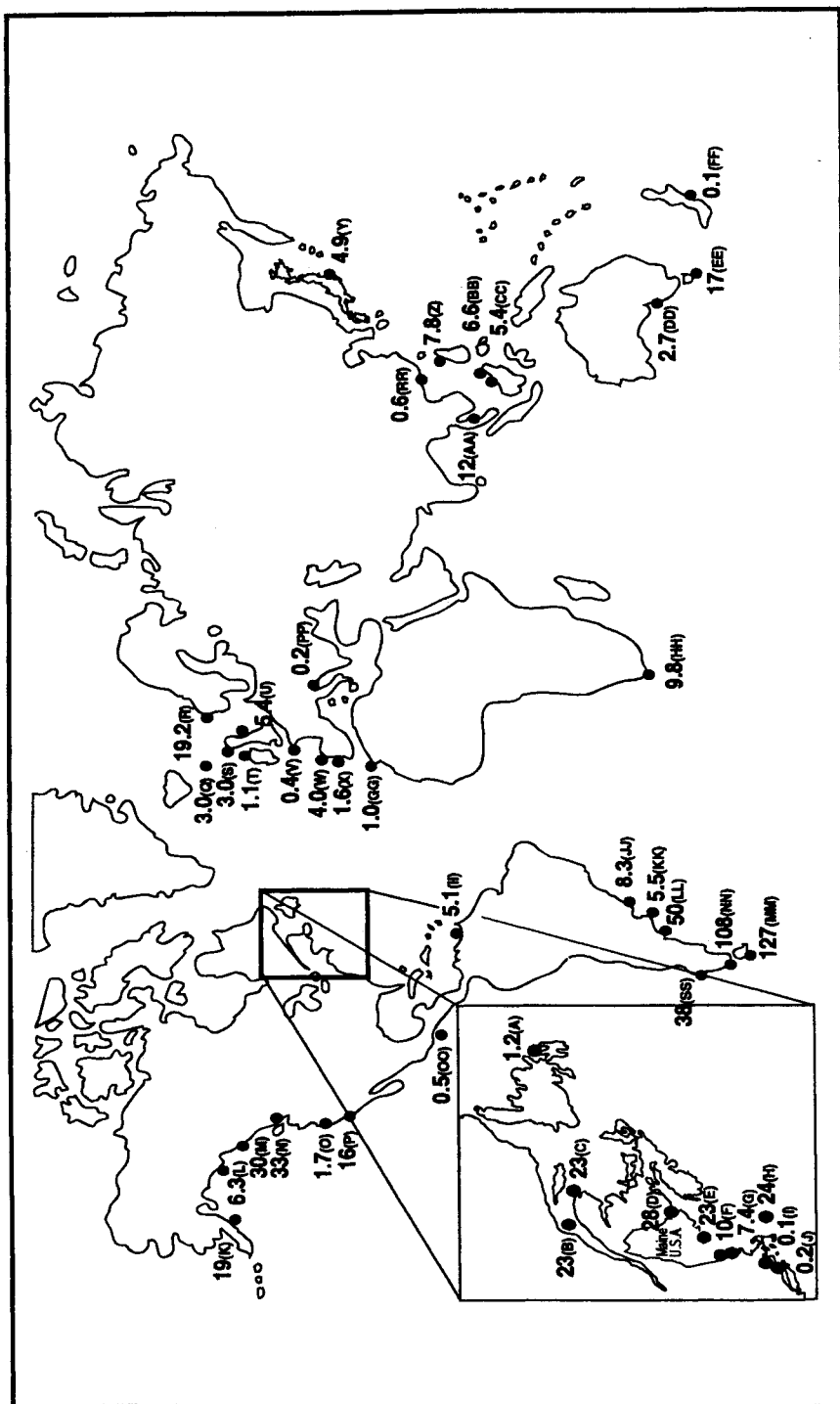


FIGURE 1. Maximum historical PSP toxicities determined by mouse bioassay (plotted values multiplied by 10^3 yield $\mu\text{g STXeq } 100 \text{ g}^{-1}$) throughout the world in mussels (*Mytilus* spp. [*californianus*, *edulis*, *galloprovincialis* or *chilensis*] unless otherwise indicated). A conversion factor of $0.2 \mu\text{g STXeq per mouse unit (MU)}$ used where values reported in MU. **References:** (A) White and White, 1985; (B) Prakash et al., 1971; (C) Desbiens and Cembella, 1993 (mussels in suspended culture); (D) Prakash et al., 1971; (E) Yentisch et al., 1975; (F and G) Twarog, 1974; (H) White et al., 1993a; (I and J) Nuzzi and Waters, 1993; (K) State of Alaska Dept. of Health and Social Services, Aug. 17, 1994 memorandum; (L) Shimizu et al., 1993a; (M) Chiang, 1988; (N) Lutz and Ince, 1979; (O and P) Nishitani and Chew, 1988; (Q) Gaard and Poulsen, 1988; (R) van Egmond et al., 1993; (S) Wyatt and Sabordo-Rey, 1993; (T) McCughey and Campbell, 1992; (U) Ingham et al., 1968; (V) Erard-Le Denn, 1991; (W) Anderson et al., 1989; (X) Franca and Almeida, 1989; (Y) for 1981 PSP outbreak, M. Yamasaki, Tohoku Nat. Fisheries Research Institute, Japan, pers. comm.; (Z) for 1993 PSP outbreak, F.F.A. Bajarias, Bureau of Fisheries and Aquatic Resources, Philippines, personal communication (*Perna viridis*); (AA) Sudara et al., 1984 (*P. viridis*); (BB) Ting and Wong, 1989 (*P. viridis*); (CC) Maclean, 1989 (*P. viridis*); (DD) Hallegraeff et al., 1988;

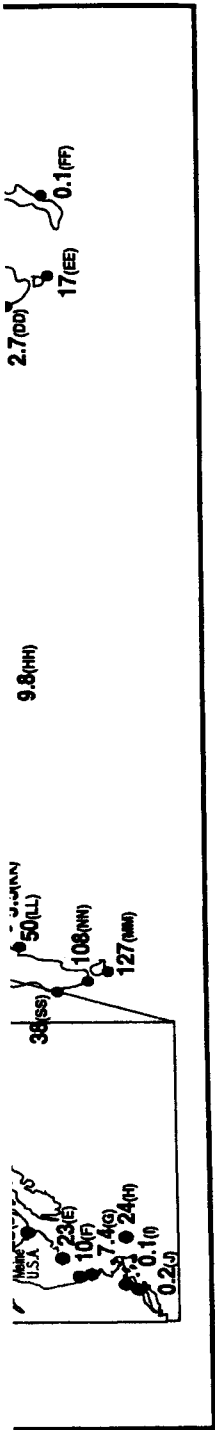


FIGURE 1. Maximum historical PSP toxicities determined by mouse bioassay (plotted values multiplied by 10^3 yield $\mu\text{g STXeq } 100 \text{ g}^{-1}$) throughout the world in mussels (*Mytilus* spp. [*californianus*, *edulis*, *galloprovincialis* or *chilensis*] unless otherwise indicated). A conversion factor of $0.2 \mu\text{g STXeq per mouse unit (MU)}$ used where values reported in MU. **References:** (A) White and White, 1985; (B) Prakash et al., 1971; (C) Desbiens and Cembella, 1993 (mussels in suspended culture); (D) Prakash et al., 1971; (E) Yentsch et al., 1975; (F and G) Twarog, 1974; (H) White et al., 1993a; (I and J) Nuzzi and Waters, 1993; (K) State of Alaska Dept. of Health and Social Services, Aug. 17, 1994 memorandum; (L) Shimizu et al., 1978; (M) Chiang, 1988; (N) Lutz and Incze, 1979; (O and P) Nishitani and Chew, 1988; (Q) Gaard and Poulsen, 1988; (R) van Egmond et al., 1993; (S) Wyatt and Saborido-Rey, 1993; (T) McCughey and Campbell, 1992; (U) Ingham et al., 1968; (V) Erard-Le Denn, 1991; (W) Anderson et al., 1989; (X) Franca and Almeida, 1989; (Y) for 1981 PSP outbreak, M. Yamasaki, Tohoku Nat. Fisheries Research Institute, Japan, pers. comm.; (Z) for 1993 PSP outbreak, F.F.A. Bajarías, Bureau of Fisheries and Aquatic Resources, Philippines, personal communication (*Perna viridis*); (AA) Sudara et al., 1984 (*P. viridis*); (BB) Ting and Wong, 1989 (*P. viridis*); (CC) Maclean, 1989 (*P. viridis*); (DD) Hallegraef et al., 1988; (EE) Hallegraef et al., 1995 (mussels in suspended culture); (FF) L. Mackenzie, personal communication, Cawthorn Institute, New Zealand (cultivated *P. canaliculus*); (GG) Baddys, 1992; (HH) Horstman 1981 (*Choromytilus meridionalis*); (II) La Barbera-Sanchez et al., 1993 (*Perna perna*); (JJ) Medina et al., 1993; (KK) Carreto et al., 1993; (LL) Carreto et al., 1996; (MM) Benavides et al., 1995; (NN) L. Vergara, personal communication, cited in Benavides et al., 1995; (OO) Cortés-Altamirano et al., 1993 (*Choromytilus palliopunctatus*); (PP) Honsell et al., 1996; (RR) Yantang et al., 1993 (*Perna viridis*); (SS) Andrinolo et al., 1995 (toxin analysis by HPLC).

of shellfish from toxic areas (Hallegraeff, 1993), and (4) physical transport mechanisms (Franks and Anderson, 1992). Progressive regional spreading of PSP via physical transport of vegetative cells and/or benthic dinoflagellate cysts has been documented in several instances. A southward spread of PSP has been described on the Atlantic coast of North America: PSP, well known in eastern Canada and Maine since the late 1800s (Prakash et al., 1971), was first documented in Massachusetts in 1972, where its presence was attributed to dispersion of toxic cells induced by Hurricane Carrie in that year, and toxic shellfish were first reported in southern Connecticut and Long Island waters in the 1980s. In South America, an initial PSP outbreak in Peninsula Valdés in 1980 subsequently spread along much of the Argentine coast (El Busto et al., 1993), and in Southeast Asia PSP outbreaks, first reported in the Papua New Guinea in 1972, have since spread widely throughout the Indo-West Pacific (Maclean, 1989).

Vegetative *Alexandrium* cells were shown to remain viable and resume normal growth following gut passage and egestion in bivalve feces (Figure 2; Bricelj et al., 1993). Therefore, inadvertent seeding of toxic dinoflagellates to new areas could potentially occur via transfer or relaying of live bivalves, a practice commonly used to depurate shellfish from uncertified waters contaminated by fecal coliform bacteria, or the movement of spat from high-recruitment areas to growout aquaculture sites (Scarratt et al., 1993). However, this mechanism of transport of toxic cells has not yet been implicated in the spread of PSP to new areas.

III. PSP TOXINS

Paralytic shellfish poisoning (PSP) toxins are potent, water-soluble neurotoxins (tricyclic tetrahydropurine derivatives), whose mode of action involves a reversible and highly specific block of ion transport by the sodium channel and thus of the action potential in excitable membranes (nerve and muscle fibers) (Narahashi, 1988). Human fatalities resulting from consumption of toxic shellfish are caused by respiratory paralysis. The most common symptoms associated with PSP in humans are paraesthesias and perioral numbness and tingling (Gessner and Middaugh, 1995). More than 20 structurally related PSP derivatives have so far been identified in toxigenic dinoflagellates and filter-feeding bivalves that consume them (Figure 3). These vary widely in their potency or biological activity (see insert in Figure 1): the carbamate toxins (saxitoxin, STX, neosaxitoxin, NEO, and gonyautoxins (GTX_{1,2,3,4}) are the most potent, the *N*-sulfocarbamoyl toxins (B and C toxins) are the least potent, and the decarbamoyl (dc) toxins exhibit intermediate specific toxicities.

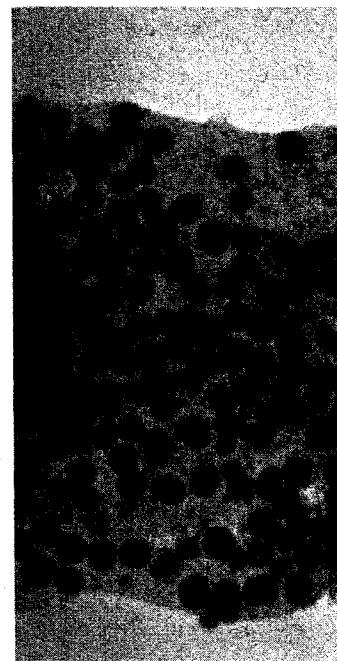


FIGURE 2. Fecal ribbon of juvenile bivalve with a mixed suspension of *Alexandrium* cells ml^{-1} ; toxicity = 96 pg STX (70:30 cell volume equivalent of *Alexandrium* cells following gut passage).

Net toxicity is measured by the Official Analytical Method (OAM) worldwide to monitor PSP. However, analytical methodology with fluorescence (Oshima, 1995a) are more accurate. The concentration of individual toxins ($\mu\text{g STXeq}$) is then calculated (μmol^{-1}) of individual toxins. Between shellfish toxicity (Oshima et al., 1983; Oshima et al., 1995b) conditions (heating in water, partial hydrolysis of the toxins) and extracts for HPLC analysis (0.03 to 0.1 N acetic acid) can cause a considerable discrepancy. This may occur, especially with

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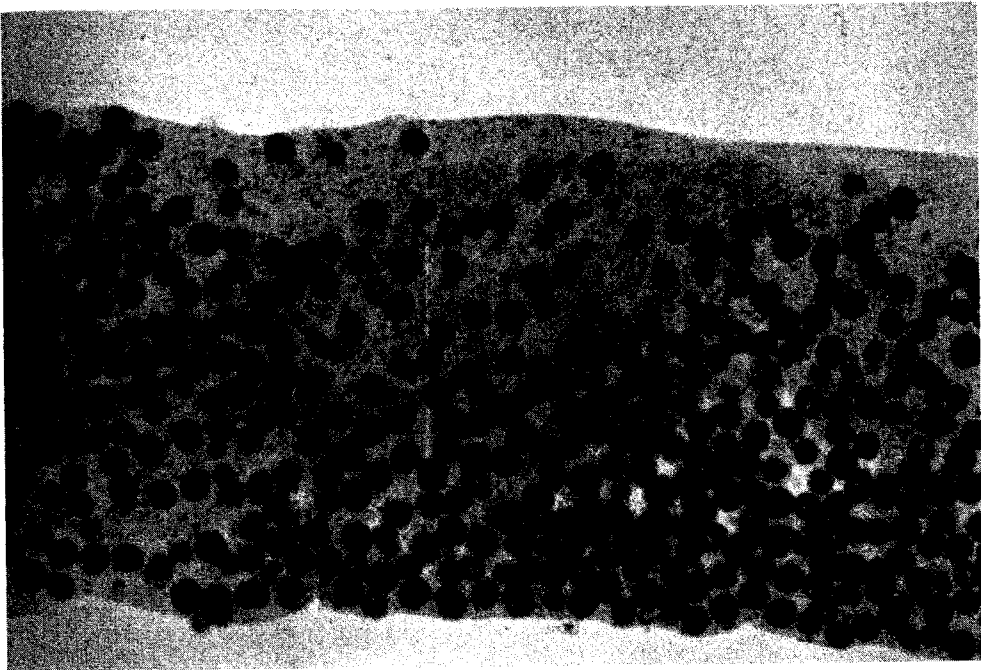


FIGURE 2. Fecal ribbon of juvenile quahogs, *Mercenaria mercenaria*, fed experimentally with a mixed suspension of the dinoflagellate *Alexandrium fundyense* (strain GtCA29 at 100 cells ml⁻¹; toxicity = 96 pg STXeq cell⁻¹) and the nontoxic diatom *Thalassiosira weissflogii* (70:30 cell volume equivalents). Note the presence of numerous intact, vegetative dinoflagellate cells following gut passage.

Net toxicity is measured by the standard mouse bioassay (Association of Official Analytical Chemists, AOAC, 1990), the method adopted worldwide to monitor the safety of shellfish for human consumption. However, analytical methods (e.g., high-performance liquid chromatography with fluorescence detection, HPLC-FD [Sullivan and Wekell, 1986, Oshima, 1995a] are more sensitive and can be used to determine the concentration of individual PSP toxins. Net toxicity (expressed in µg STXeq) is then calculated from the molar specific potencies (MU µmol⁻¹) of individual toxins. Generally, there is a good correlation between shellfish toxicities measured by the two methods (e.g., Sullivan et al., 1983; Oshima et al., 1988). However, because the harsh extraction conditions (heating in 0.1 N HCl) used by the AOAC method cause partial hydrolysis of the more labile *N*-sulfocarbamoyl toxins, when extracts for HPLC analysis are prepared under mild acidic conditions (0.03 to 0.1 N acetic acid) to maintain the integrity of these toxins, considerable discrepancies between results obtained by the two methods may occur, especially when samples are relatively rich in *N*-sulfocarbamoyl

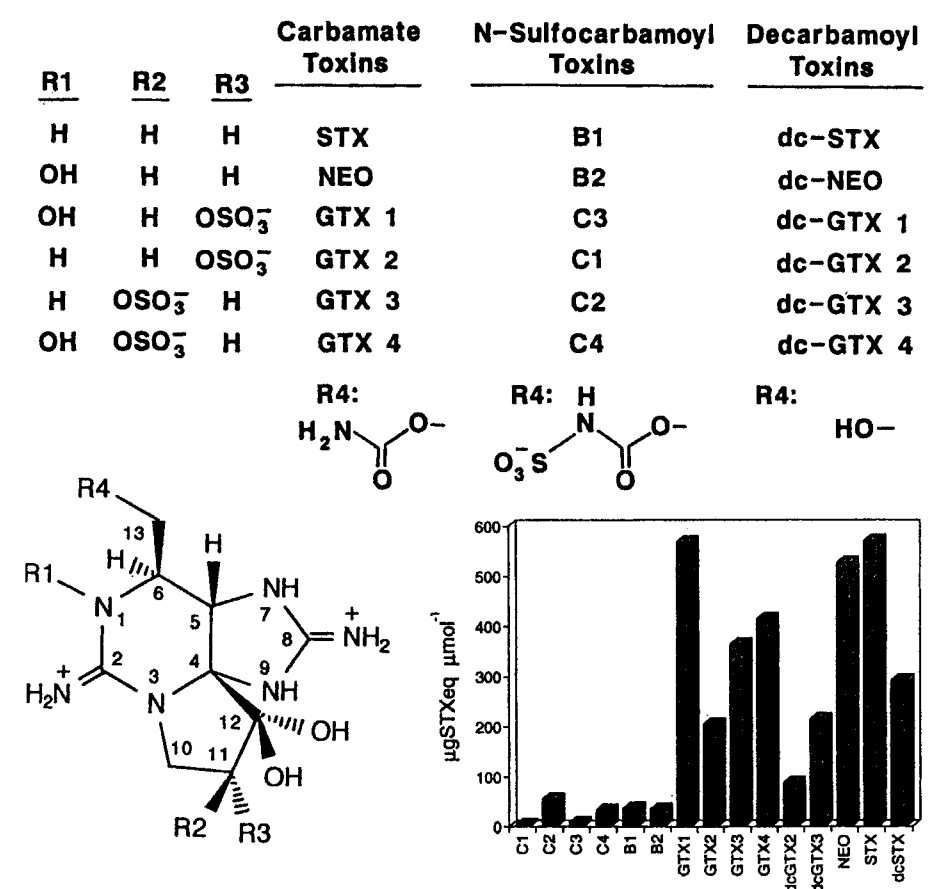


FIGURE 3. Structure of PSP toxins (carbamate: STX = saxitoxin, NEO = neosaxitoxin, GTX_{1,2,3,4} = gonyautoxins 1,2,3,4, N-sulfocarbamoyl and decarbamoyl derivatives). Only toxins whose potency has been determined are included. Insert shows the relative potency of individual PSP toxins, as measured by their specific toxicity (μg STXeq μmol⁻¹; based on a conversion factor of 0.23 μg STXeq MU⁻¹). (From Cembella et al., 1993.)

compounds (e.g., Cembella et al., 1993). A regulatory level (RL) of 80 μg STXeq 100 g wet weight of tissues⁻¹ (or 400 MU 100 g⁻¹) has been adopted by most countries for the safe human consumption of shellfish (detection limit of the mouse bioassay = 32 to 58 μg STXeq 100 g⁻¹). It must be noted, however, that the conversion factor from mouse units to μg STXeq varies somewhat with the sensitivity of the mouse strain used for the bioassay, typically ranging from 0.16 to 0.23 μg STXeq MU⁻¹, and that the AOAC standard protocol and calibration standards did not become available until 1965, thus affecting the reliability of earlier data.

Different dinoflagel (STXeq cell⁻¹), dependi the relative proportion vative property of a g growth phase (Cembell toxicity of *Alexandrium*. was first described by from 0.9 pg STXeq cell the St. Lawrence estu: subsequently confirme existence of at least tw their toxin profiles (det concentrations. High-t generally characterized GTX_{2,3}, whereas southe levels of less potent to toxins are also characte the Gulf of St. Lawrenc the relationship betwe toxins may not be gen latitudinal pattern in to erally reflected in the along nearshore Atlanti estingly, our compilati ure 1) suggests that a si decreasing latitude may (from Ushuaia to Urug however, to confirm th latitudinal differences i

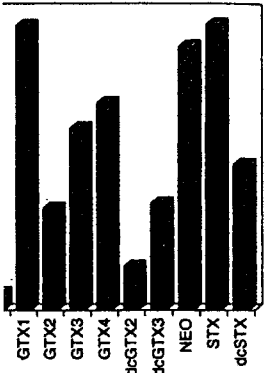
IV. TEMPORAL PA

Monitoring bivalve toxi integrated historical re blooms. Few long-term same bivalve species s: the hypothesis that P frequency and magnit relation to astronomical spanning several decac the Bay of Fundy, Cana

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oxin, NEO = neosaxitoxin, rbamoyl derivatives). Only shows the relative potency $\mu\text{g STXeq } \mu\text{mol}^{-1}$; based on et al., 1993.)

ory level (RL) of 80 $\mu\text{g U } 100 \text{ g}^{-1}$) has been assumption of shellfish $\mu\text{g STXeq } 100 \text{ g}^{-1}$). It r from mouse units to the mouse strain used $\mu\text{g STXeq MU}^{-1}$, and n standards did not ability of earlier data.

Different dinoflagellate strains vary greatly in their specific toxicity (STXeq cell^{-1}), depending on environmental and growth conditions, but the relative proportion of various PSP derivatives is a relatively conservative property of a given isolate for unstressed cells in exponential growth phase (Cembella et al., 1987). A latitudinal gradient in the specific toxicity of *Alexandrium* isolates along the Atlantic coast of North America was first described by Maranda et al. (1985), with toxicities increasing from 0.9 $\mu\text{g STXeq cell}^{-1}$ in Long Island waters to 130 $\mu\text{g STXeq cell}^{-1}$ in the St. Lawrence estuary (Cembella et al., 1988). This gradient was subsequently confirmed by Anderson et al. (1994) and attributed to the existence of at least two clusters of dinoflagellate isolates that differ in their toxin profiles (determined by HPLC), rather than total molar toxin concentrations. High-toxicity isolates from the northeastern U.S. are generally characterized by the predominance of more potent toxins, $\text{GTX}_{2,3}$, whereas southern U.S. low-toxicity isolates have relatively higher levels of less potent toxins $\text{C}_{1,2}$. However, high relative amounts of $\text{C}_{1,2}$ toxins are also characteristic of high-toxicity *Alexandrium* isolates from the Gulf of St. Lawrence region in northeastern Canada, indicating that the relationship between low toxicity and paucity of *N*-sulfocarbamoyl toxins may not be generally valid (Cembella and Destombe, 1996). The latitudinal pattern in total toxicity of dinoflagellate isolates is also generally reflected in the distribution of mussel, *Mytilus edulis*, toxicities along nearshore Atlantic North American waters (insert, Figure 1). Interestingly, our compilation of maximum toxicity levels in mussels (Figure 1) suggests that a similar pattern of decreasing shellfish toxicities with decreasing latitude may occur along the Atlantic coast of South America (from Ushuaia to Uruguay). Insufficient data are available at present, however, to confirm this gradient and determine whether it is due to latitudinal differences in dinoflagellate cell toxicity.

IV. TEMPORAL PATTERNS

Monitoring bivalve toxicity levels provides a valuable, time- and space-integrated historical record of the occurrence and intensity of toxic blooms. Few long-term, continuous time series of PSP toxicity for the same bivalve species sampled at the same location are available to test the hypothesis that PSP outbreaks have indeed intensified in their frequency and magnitude over time, or establish their periodicity in relation to astronomical, meteorological, or hydrographic events. Records spanning several decades exist for *Mytilus edulis* and *Mya arenaria* in the Bay of Fundy, Canada and the coast of Maine, U.S.A (Figures 4 and 5,

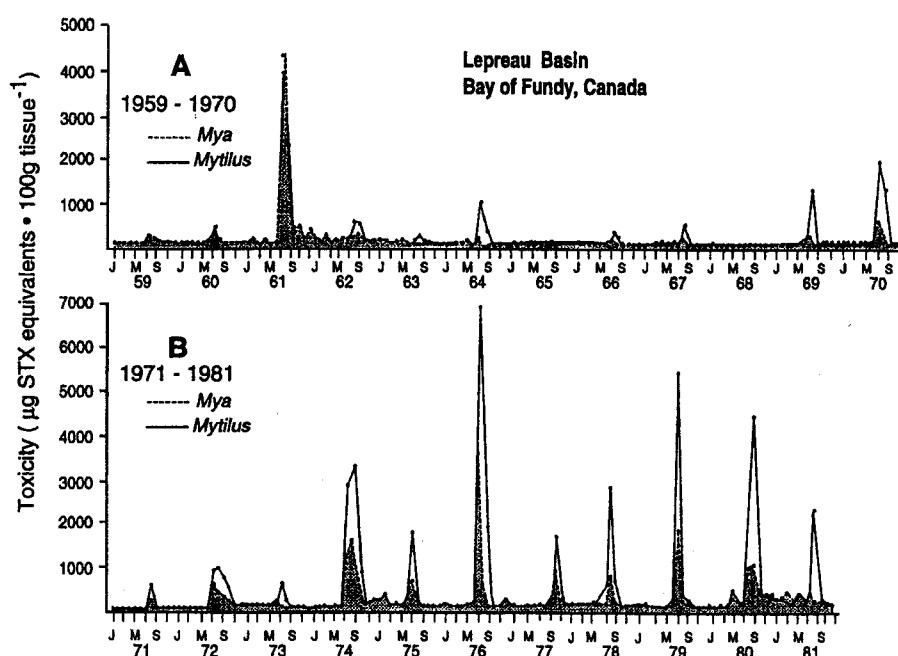
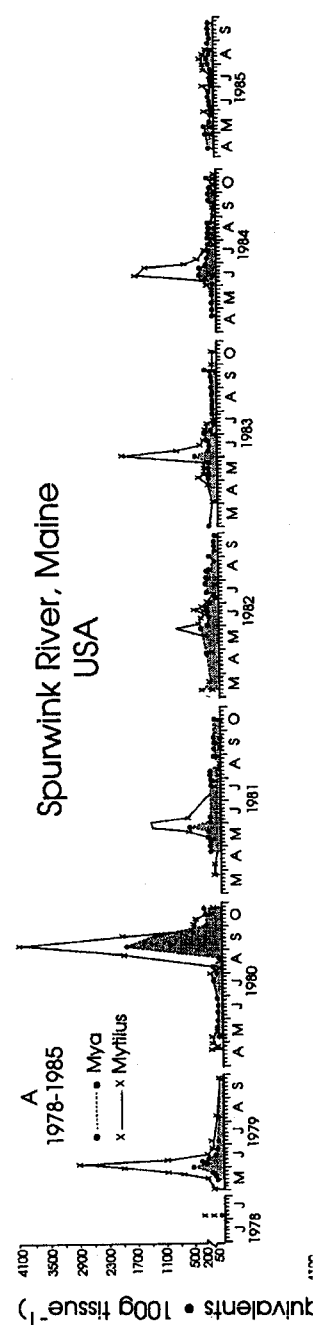
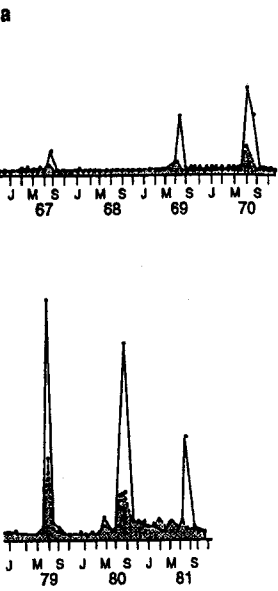


FIGURE 4. Temporal patterns in PSP shellfish toxicity, 1959–1981: mean monthly toxicity (in $\mu\text{g STXeq } 100 \text{ g}^{-1}$ wet tissue weight) in blue mussels, *Mytilus edulis*, and softshell clams, *Mya arenaria*, at Lepreau Basin, southwestern Bay of Fundy, New Brunswick, Canada (redrawn from White, 1982). Extended records from Martin and Richard (1996) indicate that peak bivalve toxicity peaks also occurred in the mid-1940s and mid-1990s.

respectively). An approx. 18.6-year cycle in the toxicities of Bay of Fundy bivalves was correlated with the lunar cycle and thus tidally induced exposure to toxic cells (White, 1982; Martin and Richard, 1996). Caution must be exercised, however, in the interpretation of data prior to 1980, because interrupted sampling may at times have led to misrepresentation of annual maxima (Martin and Richard, 1996). White (1982) also noted an overall increase in peak toxicity levels in the mid-1970s and observed that in the late 1970s and early 1980s toxicities above the regulatory level extended beyond the typical summer period and persisted into the winter at some stations, thus resulting in year-round closure of shellfish harvesting grounds. This intensification, however, appears to have discontinued in recent years (White, 1988). Messieh and El-Sabh (1990) described a 5- to 10-year interval between major PSP outbreaks in the Bay of Fundy, corresponding with a cycle in sea level changes and the incidence of sunspots. In Maine, PSP toxicities $> 10^3 \mu\text{g STXeq } 100 \text{ g}^{-1}$ appear to occur every 1 to 4 years, but intensification of PSP outbreaks





-1981: mean monthly toxicity is *edulis*, and softshell clams, ly, New Brunswick, Canada l Richard (1996) indicate that nd mid-1990s.

xicities of Bay of Fundy d thus tidally induced Richard, 1996). Caution 1 of data prior to 1980, ed to misrepresentation hite (1982) also noted id-1970s and observed ove the regulatory level and persisted into the nd closure of shellfish r, appears to have dis- eh and El-Sabh (1990) r PSP outbreaks in the . level changes and the > 10³ µg STXeq 100 g⁻¹ ation of PSP outbreaks

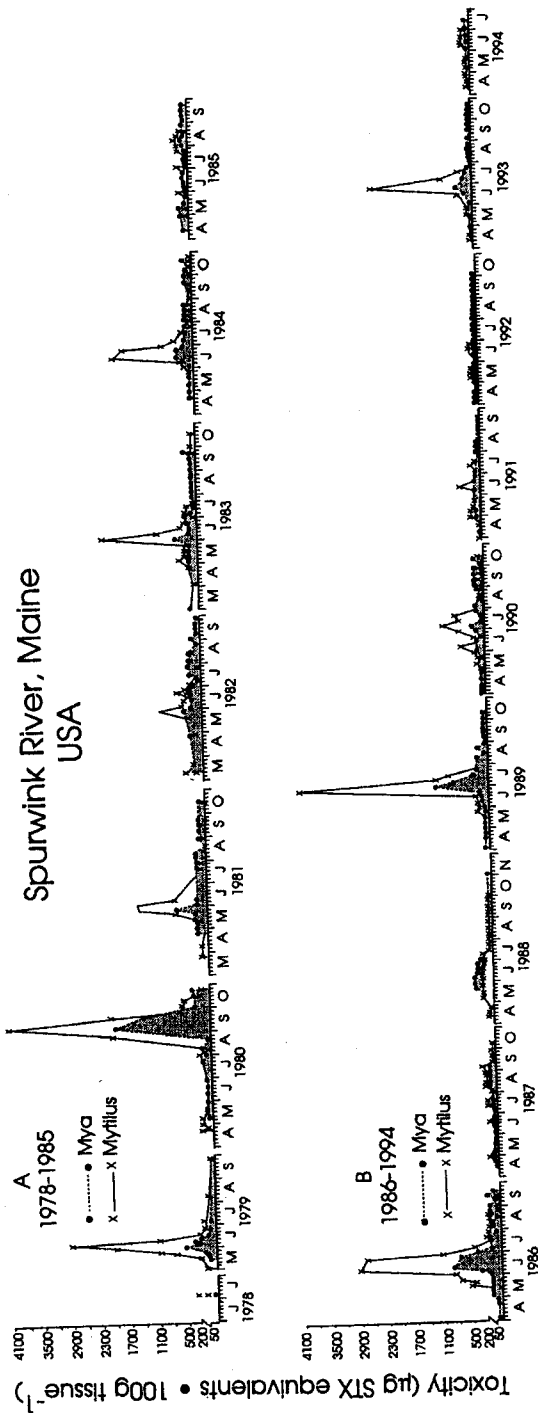


FIGURE 5. Temporal patterns in PSP shellfish toxicity from 1978 to 1994 (in µg STXeq 100 g⁻¹) in mussels, *Mytilus edulis*, and softshell clams, *Mya arenaria*, from the Spurwink River, Maine, U.S.A (State of Maine Dept. of Marine Resources PSP monitoring records, courtesy of J. Hurst).

over the years is not apparent (Figure 5). On the coast of British Columbia, Gaines and Taylor (1985) described a 7- to 8-year periodicity in PSP outbreaks rather than a pattern of progressive intensification. Attempts to identify long-term cycles of PSP outbreaks in the Gulf of St. Lawrence have met with little success (Therriault et al., 1985; Beaulieu and Ménard, 1985). In conclusion, while geographic expansion of PSP has been well documented, the evidence for long-term periodicity in PSP outbreaks or their intensification over time at a given location remains speculative.

In high latitudes, the annual timing of peak PSP toxicities in bivalves varies regionally, depending on the timing of toxic blooms: in North America maximum levels typically occur in the summer or early fall (e.g. Bay of Fundy and southern British Columbia [Gaines and Taylor 1985]), or in late spring, early fall (nearshore Maine waters). In the southern Gulf of Maine, Franks and Anderson (1992) found a strong association between the southward temporal progression of shellfish toxicity and the alongshore advection of toxic *Alexandrium* cells, providing further evidence that shellfish toxicity is a good indicator of the occurrence of toxic cells in the water column. Bivalve toxicities exceeding the regulatory level may in some cases persist or recur during the winter (Bourne, 1965; Cembella et al., 1993) and are attributed to a reduction in the rate of detoxification at low temperatures, or the ingestion of overwintering cysts (hypnozygotes) resuspended from sediments (Schwinghamer et al., 1994). Although unlikely, it is also possible that winter toxicities are caused by the presence of toxic, vegetative cells that remain undetected due to the reduced frequency of phytoplankton monitoring at this time of the year.

Alexandrium resting cysts are known to be as toxic (White, 1986) or even more toxic than vegetative cells (Oshima et al., 1992) and can occur at high densities in surface sediments in depositional environments (up to 800 to 8000 cysts ml⁻¹ of sediment [Anderson 1984]). Cysts have also been identified in the gut contents of sea scallops and surfclams from the Gulf of Maine (Shumway et al., 1987; Shumway et al., 1994). Furthermore, maximum PSP toxicity levels of *Mytilus edulis* at various sites along the Newfoundland coast were found to correlate positively with the abundance of cysts in sediments and with that in gut contents of mussels collected in the fall and winter (Schwinghamer et al., 1994). Although strongly suggestive, the evidence implicating cysts as the source of winter shellfish toxicities from such correlative field studies remains inconclusive, largely because assimilation and toxin accumulation from cysts, which have more resistant cell walls than vegetative cells, has not been demonstrated experimentally.

V. TOXIN ACCUM

A. *INTERSPECIFIC*

Bivalves show significant differences in their ability to accumulate STX. Table 1, which shows the results of various bivalve species selection of candidate activities. Species that accumulate STX include *Mytilus edulis* (Figure 1) and *Mytilus* sp. (Figure 2). *Mytilus* is known to provide early warning of toxins in coastal waters the toxin levels in the water column are 12 days on average (Gardner and Hargrave 1992). *Mytilus* *arenaria* (Figure 5) and *Mytilus* *edulis* (Figure 6) are equate protection for the bivalves. It is documented that *M. edulis* is more toxic than neighboring or co-occurring species (White, 1982; Larocque and Hargrave 1992). *Mytilus* typically becomes toxic in the water column (Sribhibhadh, 1963). The toxin levels in the water column often co-occurs with *M. edulis* (Figure 1), can also accumulate STX. STXeq 100 g⁻¹ in the water column is 5000 µg STXeq 100 g⁻¹ in the water column.

Caution must be exercised in comparing species reported in Table 1. In ecological maxima, as demonstrated by example, the maximum concentration of *solidissima* is $8 \times 10^3 \mu\text{g/g}$; whereas, the toxicified adults attained representative maximum concentrations of $10^3 \mu\text{g/g}$ which extensive data are available in comparing toxin levels in these species. These species occupy a different ecological niche (e.g., intertidal vs. subtidal) and specific comparisons are not warranted (1963; Cembella et al. 1973). The different bivalve species are known to be toxic to toxic cells (bloom data) and such studies are available for other bivalves are mentioned.

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V. TOXIN ACCUMULATION

A. INTERSPECIFIC DIFFERENCES

Bivalves show significant (up to 100-fold) interspecific differences in their ability to accumulate PSP toxins in their tissues (illustrated in Table 1, which shows maximal PSP toxicities achieved globally by various bivalve species). These differences can be exploited in the selection of candidate species for PSP monitoring or for aquaculture activities. Species that rapidly achieve elevated toxin levels, such as *Mytilus edulis* (Figure 1), are obviously ideally suited as sentinel organisms to provide early warning of a PSP outbreak. For example, in Maine coastal waters the toxicity of *M. edulis* is predictably detected about 12 days on average (range = 5 to 22 days) earlier than that of *Mya arenaria* (Figure 5 and Hurst and Gilfillan, 1977), thus providing adequate protection for harvesting of the latter species. It is also well documented that *M. edulis* generally becomes 2 to 4x times more toxic than neighboring or co-occurring *Mya arenaria* (Hurst and Gilfillan, 1977; White, 1982, Larocque and Cembella, 1991). Similarly, *M. californianus* typically becomes toxic 2 to 4 weeks before the oyster *Crassostrea gigas* (Sribhibhadh, 1963). The horsemussel *Modiolus modiolus*, a species that often co-occurs with *M. edulis* (and is not included in Figure 1 or Table 1), can also accumulate relatively high toxin levels: up to ca. 9000 µg STXeq 100 g⁻¹ in the Bay of Fundy (Jamieson and Chandler, 1983) and 5000 µg STXeq 100 g⁻¹ on Georges Bank (White et al., 1993a).

Caution must be exercised when comparing toxicity maxima among species reported in Table 1, as these may not necessarily reflect physiological maxima, as determined in laboratory feeding studies. For example, the maximum toxicity reported for field populations of *Spisula solidissima* is 8 × 10³ µg STXeq 100 g⁻¹ (Table 1), whereas experimentally toxified adults attained 19 × 10³ µg STXeq 100 g⁻¹ (Table 2). Furthermore, representative maxima are more likely to be obtained for species for which extensive data are available than for poorly sampled species. Bias in comparing toxin levels among species may also be introduced where these occupy a different habitat within a common body of water (e.g., intertidal vs. subtidal, or nearshore vs. offshore populations). Interspecific comparisons are best made from field (Figure 6; Sribhibhadh, 1963; Cembella et al., 1993) or laboratory studies (Table 2) in which different bivalve species experience comparable conditions of exposure to toxic cells (bloom duration, cell density, and specific toxicity), but few such studies are available. Direct comparisons between scallops and other bivalves are made difficult by the fact that toxicities are often

TABLE 1
Maximum PSP Toxicities (µg STXeq 100 g Wet Tissue Weight⁻¹) Recorded in Bivalve Molluscs (Excluding Mussels)
Toxified in the Natural Environment

Shellfish Species (common name)	Toxicity (µg STXeq 100 g ⁻¹)			Ref.
	Whole animal	Visceral mass	Location and year of sample collection	
CLAMS, COCKLES AND ARKS				
<i>Scapharca broughtonii</i> (blood ark shell)		40600*	Senzaki Bay, Japan (1986)	Ikeda et al. (1989)
<i>Saxidomus nuttalli</i> (Washington clam)	14000		Campbell Cove, California USA (1980)	Price et al. (1991)
<i>Saxidomus giganteus</i> (butter clam)	9600 8640 2299		Gilford Island, northern BC, Canada (1985) Theodosia Inlet, southern BC Canada (1965) Sequim Bay, Washington, USA (1990)	Chiang (1988) Quayle (1969) WA Dept. Health (L. Hanson, personal communication)
<i>Mya arenaria</i> [†] (softshell)	9600 9000 4900 ^b 2854 9100*		Merrimack estuary, Massachusetts USA (1972) Lepreau Basin, Bay of Fundy, Canada (1976) Crow Harbour, Bay of Fundy, Canada (1986) Wells, Maine, USA (1972) Tungkang, South Taiwan (1986)	Twarog (1974) Martin and Richard (1996) Martin et al. (1990) Yentisch et al. (1975) Hwang et al. (1987)
<i>Soletellina diphos</i> (purple clam)	7283		Campbell Cove, California, USA (1980)	Price et al. (1991)
<i>Schizothaerus (=Tresus) nuttalli</i> (Pacific gaper)	7640		Mazatlán Bay, Gulf of California, Mexico (1979)	Mee et al. (1986)
<i>Donax gracilis</i> (coquina)				
<i>Clinocardium nuttalli</i> (Nuttall cockle)	6700 1860		Charter Creek, BC, Canada (1992) San Juan Is., Washington, USA (1981)	DFO (1992) Erickson and Nishitani (1985)
<i>Spisula solidissima</i> (Atlantic surfclam)	6423 7934 5104 3740 2939	21000	Georges Bank, USA (1990) Head Beach, Maine, USA (1981) Sagadahoc Bay, Maine, USA (1980) Lepreau Basin, Bay of Fundy, Canada (1961) Hampton, New Hampshire, USA (1972)	Shumway et al. (1994) Shumway et al. (1994) Quayle and Bourne (1972) Sasner et al. (1975)
<i>Tapes (= Venerupis = Ruditapes)</i> <i>philippinarum (= japonica)</i> (Japanese littleneck)	6086 2000* 1710	29000*	Okeover Inlet, BC, Canada (1986) Senzaki Bay, Yamaguchi Prefecture, Japan (*1986) Ise Bay, Japan (1976) Portage Bay, Washington, USA (1992)	R. Chiang, DFO, (personal communication) Ikeda et al. (1989) Noguchi et al. (1978) WA Dept. Health (L. Hanson, personal communication)
<i>Amphichaena kindermanni</i> (Kindermann's false donax)	6000*		Champerico, Guatemala (1987)	Rosales-Loessener (1989)
<i>Cardium edule</i> (common European cockle)	5300*		Laguna d'Obidos, Portugal (1959)	DeSousa and Silva (1963)
<i>Protothaca staminea</i>	5053		Water Bay, BC, Canada (1986)	DFO (R. Chiang

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Table 1 (continued)
Maximum PSP Toxicities (µg STXeq 100 g Wet Tissue Weight⁻¹) Recorded in Bivalve Molluscs (Excluding Mussels)
Toxified in the Natural Environment

Shellfish Species (common name)	Toxicity (µg STXeq 100 g ⁻¹)			Ref.
	Whole animal	Visceral mass	Location and year of sample collection	
<i>Panopea generosa</i> (=abrupta) (Pacific geoduck)		2200	British Columbia, Canada (1989)	DFO (1989) in Bellier (1992)
<i>Paphies subtriangulata</i> (tuatua clam)	412		Bay of Plenty, New Zealand (1993)	Cawthron Institute (L. Mackenzie, personal communication) Jaafar and Subramaniam (1984)
<i>Anadara granosa</i> (granular ark; tembayan cockle)	188		Brunei Bay, Brunei (1980)	
OYSTERS				
<i>Spondylus butleri</i> (thorny oyster)	22000*	46000*	Arumizu Bay, Koror Island, Palau (1981)	Harada et al. (1982)
<i>Crassostrea gigas</i> = (iredalei) (Pacific oyster)	9929		Okeover Inlet, BC, Canada (1986)	DFO (R. Chiang, personal communication) Corite (1984)
	5500		Drakes Estero, California, USA (1980)	Onoue et al. (1980)
	2400*	22600*	Senzaki Bay, Yamaguchi Prefecture, Japan (1979)	Ikedo et al. (1989)
	2376		Senzaki Bay, Yamaguchi Prefecture, Japan (1986)	WA Dept. Health (L. Hanson, personal communication)
	236		Minter Bay, Washington, USA (1988)	Nezan and Piclet (1991)
	1500		Aber Wrach's estuary, Brittany, France (1988)	Ting and Wong (1989)
	2600*		Sabah, Malaysia (1987)	Harada et al. (1982)
			Arumizu Bay, Toror Island, Palau (1981)	
<i>Lopha cristagalli</i> (cock's comb oyster)	1720		Mazatlan Bay, Gulf of California, Mexico (1979)	Mee et al. (1986)
<i>Crassostrea iridescens</i> (rock oyster)	810		Bays of La Ventosa, Gulf of Tehuantepec, Mexico (1989)	Corrés-Alamirano et al. (1993)
<i>Saccostrea</i> (=Crassostrea) <i>mordax</i> (=scucculata) (hooded or pink oyster; tentip)	1336*		Kumbla Estuary, Mangalore, W. India (1983)	Karunasagar et al. (1984)
	487		Serasa, Brunei (1988)	Jaafar et al. (1989)
<i>Ostrea edulis</i> (edible oyster)	1300	4200*	Arumizu Bay, Koror Island, Palau (1981)	Harada et al. (1982)
<i>Crassostrea virginica</i> (eastern oyster)	214		Long Point Creek, Maine, USA (1986)	Shumway et al. (1990)
			Acadian peninsula, SE Gulf of St. Lawrence, Canada (1988)	Worms et al. (1993)
SCALLOPS				
<i>Patinopecten yessoensis</i> (Japanese scallop)	220000*		Japan (1982)	Noguchi et al. (1984)
	70000*		Ofunato Bay, Iwate Prefecture, Japan (1981)	Mariyama et al. (1983)
	15000*		Funka Bay, Hokkaido, Japan (1979)	Nishihama (1980)
<i>Placopecten magellanicus</i> (sea scallop)	150000		Digby, Bay of Fundy, NS, Canada (1978)	Jamieson and Chandler (1983)
	65000		Mascarene, Pasamaguddy Bay, Canada (1980)	Jamieson and Chandler (1983)
	10864		Georges Bank, USA (1990)	calc. from White et al. (1993)
	2200		Mascarene, Pasamaguddy Bay, Canada (1981)	Jamieson and Chandler (1983)
	1200*		Lepreau Basin, Bay of Fundy, Canada (1945)	Medcof et al. (1947)
			Damariscotta River estuary, Maine, USA (1988)	Cembella et al. (1993)
<i>Pecten albicans</i> (Japanese baking scallop)		30000*	Senzaki Bay, Yamaguchi Prefecture, Japan (1987)	Ikedo et al. (1989)
<i>Crassostrea nioensis</i>	13503	95000	Timber Cove, California, USA (1980)	Bellier (1980)

(cock's comb oyster)				
<i>Crassostrea iridescens</i>	1720			Mee et al. (1986)
(rock oyster)	810			Cortés-Altamirano et al. (1993)
<i>Saccostrea (=Crassostrea) mordax (=cucullata)</i>	1336*			Karunasagar et al. (1984)
(hooded or pink oyster; terlip)	487			Jaafar et al. (1989)
<i>Ostrea edulis</i>		4200*		Harada et al. (1982)
(edible oyster)	1300			Shumway et al. (1990)
<i>Crassostrea virginica</i>				
(eastern oyster)	214			Worms et al. (1993)

SCALLOPS				
<i>Patinopecten yessoensis</i>				Noguchi et al. (1984)
(Japanese scallop)		220000*		Maruyama et al. (1983)
		70000*		Nishihama (1980)
<i>Placopecten magellanicus</i>		15000*		Jamieson and Chandler (1983)
(sea scallop)		150000		Jamieson and Chandler (1983)
		65000		calc. from White et al. (1993)
	10864			Jamieson and Chandler (1983)
<i>Pecten albicans</i>		25000		Jamieson and Chandler (1983)
(Japanese baking scallop)	2200			Medcof et al. (1947)
<i>Crassadoma gigantea</i>	1200*			Cembella et al. (1993)
(=Hinnites multirugosus)		7200*		Ikedo et al. (1989)
(giant rock scallop)		4180		
<i>Chlamys hastata</i>		30000*		
(spiny scallop)				
<i>Chlamys rubida</i>				
(reddish scallop)				
<i>Chlamys tehuelicha</i>				
(Darwin's or Patagonian scallop)				
<i>Amusium pleuronectes</i>				
(Asian moon scallop)				
<i>Argopecten irradians</i>				
(bay scallop)				
<i>Pecten maximus</i>				
(great scallop)				
<i>Chlamys nipponensis akazara</i>				
(akazara scallop)				

Note: Calculated values have been rounded to the nearest hundred. Species are ranked in order of decreasing whole body toxicity within each group of bivalves (clams, cockles, and arks; scallops, oysters). Toxicities of whole tissues or visceral mass (also referred to as digestive gland, midgut gland, or hepatopancreas) determined by the AOAC mouse bioassay unless otherwise indicated. Only levels exceeding the regulatory level of 80 µg STX eq 100 g⁻¹ are given. Where available, common names for bivalve molluscs are according to Turgeon et al. (1988) and Abbott (1983), otherwise from personal communications.

* Used a conversion factor of 1 mouse unit (MU) = 0.2 µg STX.
b HPLC analysis of AOAC extracts.
c 1 MU = 0.16 µg STXeq.

TABLE 2
Maximum PSP Toxicities Attained by Bivalves Experimentally Fed Cultured *Alexandrium* spp. (temperature = 16–17°C; 10 to 19 day exposure), and weight-normalized, daily ingestion rate of toxic cells [for a standard animal 1 g in wet weight of soft tissues, $I_{(g)} = I_x \cdot (W_x^{-b})$, where b = exponent of the allometric relationship between I and W], unless specified. Mean dinoflagellate toxicity in pgSTXeq cell⁻¹; mean density in cells ml⁻¹; SL = mean initial shell length in mm/WW = wet weight of soft tissues in g. Dinoflagellates offered in a monospecific suspension unless indicated. Toxicities determined by HPLC analysis of acetic acid extracts unless otherwise specified

Bivalve sp. (SL/WW)	(μg STXeq 100 g ⁻¹ WW)			Ingestion rate, <i>I</i> (dinoflagellate cells day ⁻¹ g ⁻¹)	Source
	Toxicity of whole tissues	Toxicity of visceral mass	Dinoflagellate strain fed (toxicity; density) ^a		
<i>Mytilus edulis</i>					
Adults	2,371 ^b	—	MOG835 (7; —)	—	Lassus et al., 1993
35–45 mm	1,100 ^b	—	MOG835 (—; —)	6.67 × 10 ^{5a}	Lassus et al., 1989
42 mm/9.56 g	5,800	18,300	GtLI22 (7; 212)	20.27 × 10 ⁵	Lee, 1993
10 mm	10,900	—	GtCA29 (10; 173)	—	Bricelj et al., 1993
42 mm/5.38 g	17,970	49,970	GtCA29 (29; 205)	11.03 × 10 ⁵	Bricelj, Lee and Cembella, unpublished
44 mm/2.30 g	47,000	175,490	GtCA29 (66; 256)	9.82 × 10 ⁵	Bricelj et al., 1990
<i>Spisula solidissima</i>					
23 mm/0.60 g	16,810	56,660	AL1V (5; 190)	20.50 × 10 ⁵	Bricelj and Cembella, 1995
27 mm/0.60 g	32,755 ^a	105,130	GtCA29 (14; 196)	—	Bricelj, Laby and Cembella, unpublished
34 mm/1.36 g	30,429	154,024	PR18b (28; 104)	—	
61 mm/8.06 g	19,265 ^a	72,316	GtCA29 (14; 196)	—	
<i>Mercenaria mercenaria</i>					
23 mm/0.61 g	2,150	5,470	GtLI22 (6; 112)	11.06 × 10 ⁵	Bricelj et al., 1991
26 mm/0.78 g	10,543	29,180	GtCA29 (96; 159) ^c	3.18 × 10 ⁵	
<i>Mya arenaria</i>					
42 mm/2.63 g (NS, Canada)	10,175 ^d	30,650 ^d	PR18b (184; 150)	—	Cox, 1994
35 mm/2.23 g (NY, USA)	1,341	3,766	PR18b (28; 104)	—	Bricelj, Laby and Cembella, unpublished
<i>Pecten maximus</i>					
35–45 mm	2,700 ^b	—	MOG835 (7; —)	3.98 × 10 ^{5a}	Lassus et al., 1989
<i>Ruditapes philippinarum</i>					
35–45 mm	160 ^b	—		0.82 × 10 ^{5a}	
<i>Crassostrea gigas</i>					
35–45 mm	ca. 80 ^b	—		2.62 × 10 ^{5a}	

35–45 mm

^a GiC429 = A. fundyense. GiL22 and MOC835 = A. tamarensis; AL1V = A. minutum; PR18b = A. tamarensis cf. excavatum.

^b Toxicities determined by AOAC mouse bioassay.

^c Toxic cells offered in a mixed suspension with nontoxic diatoms (50%:50% by volume in the first week, followed by 70% Alexandrium:30% diatoms).

^d Does not include toxin contribution of foot and adductor muscles.

^e Weight-specific ingestion rates (IWR) estimated from Figure 2.

^f Toxicity reported in Lassus et al. (1994).

^g Does not include the toxin contribution of adductor muscles.

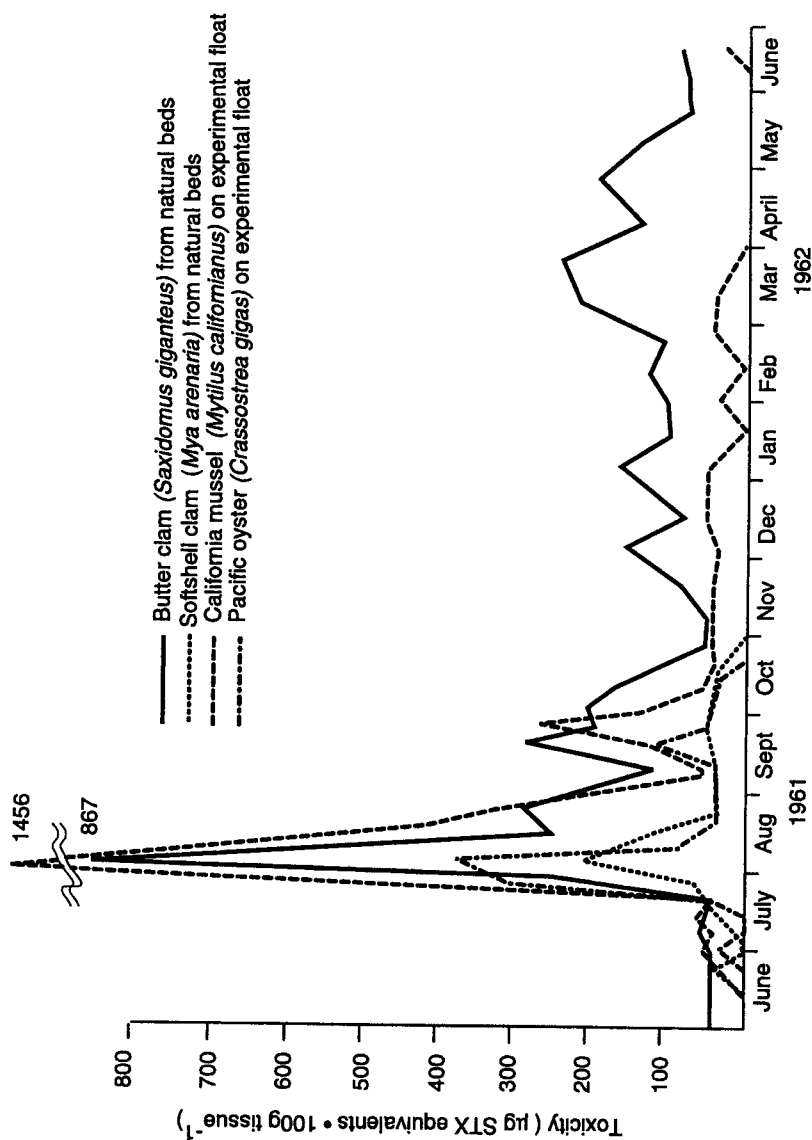


FIGURE 6. Levels of PSP toxins in four species of bivalves in experimental floats and natural beds in Sequim Bay, Washington, U.S.A. (After Sparks et al. [1962]).

measured for different and whole tissues for the toxicity of intertidal with that of scallops h. In Japan, where are available, scallops (become about 3 times

Differences in toxin correlated with variation by the *in vitro* response tetrodotoxin (TTX), a (Table 3). Interspecific development of a no action potential (Two species-specific binding the sodium channel, commented in other taxa (not appear to be clear Pacific clams *Protothaca* long to the family V sensitivity ranking (Tal et al. (1972) provides bivalves in terms of the toxin accumulation. However this method to adequately are discussed below.

In general, bivalves (e.g., *Mytilus edulis*, T 1990) and thereby attain relatively low to highly sensitive to PSP behavioral mechanism range from feeding rate scallops and complete. It is important to note observed between the ity to STX or TTX, and cells. For example, the insensitive to STX based to accumulate relative (Twarog et al., 1972) ; closure in the presence

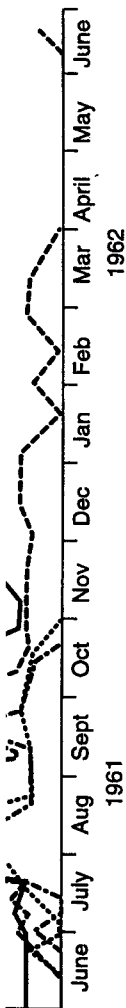


FIGURE 6. Levels of PSP toxins in four species of bivalves in experimental floats and natural beds in Sequim Bay, Washington, U.S.A. (After Sparks et al. [1962]).

measured for different tissues, namely, the digestive gland for scallops and whole tissues for clams, mussels and oysters (Table 1). Additionally, the toxicity of intertidal clams and mussels, cannot be readily compared with that of scallops held in suspended culture or from subtidal populations. In Japan, where direct comparisons of digestive gland toxicities are available, scallops (*Patinopecten yessoensis* and *Chlamys nipponensis*) become about 3 times as toxic as *M. edulis* (Oshima et al., 1982).

Differences in toxin accumulation among bivalve species have been correlated with variation in their sensitivity to PSP toxins, as determined by the *in vitro* response of isolated, unsheathed nerves to STX or tetrodotoxin (TTX), a toxin structurally and functionally similar to STX (Table 3). Interspecific variability in resistance does not depend on development of a non-sodium mechanism for the generation of the action potential (Twarog, 1974), but rather may be associated with species-specific binding characteristics of polypeptidic receptor sites at the sodium channel, or production of STX-binding proteins, as documented in other taxa (Daigo et al., 1988; Mahar et al., 1991). It also does not appear to be clearly related to taxonomic rank. For example, the Pacific clams *Protothaca staminea* and *Humiliaria kennerlyi* both belong to the family Veneridae, yet lie at opposite extremes of the sensitivity ranking (Table 3A). The nerve bioassay developed by Twarog et al. (1972) provides a useful, general classification scheme to rank bivalves in terms of their sensitivity to PSP toxins and thus potential for toxin accumulation. However, some notable exceptions in the ability of this method to adequately characterize a species' response to the toxins are discussed below.

In general, bivalve species with nerves insensitive to PSP toxins (e.g., *Mytilus edulis*, Table 3A) readily feed on toxic cells (Bricelj et al., 1990) and thereby accumulate high toxin levels. In contrast, species that attain relatively low toxicities (e.g., the oyster, *Crassostrea virginica*) are highly sensitive to PSP toxins (Table 3A) and exhibit physiological and behavioral mechanisms to avoid or reduce exposure to toxic cells. These range from feeding rate inhibition (Figures 7 and 8) to shell clapping in scallops and complete shell valve closure (Gainey and Shumway, 1988). It is important to note, however, that several discrepancies have been observed between the ranking of bivalves based on their nerve sensitivity to STX or TTX, and that based on whole-organism response to toxic cells. For example, the northern quahog, *Mercenaria mercenaria*, is insensitive to STX based on the *in vitro* assay (Table 3A), but is known to accumulate relatively low toxin levels during a major PSP outbreak (Twarog et al., 1972) and shows significant feeding inhibition and shell closure in the presence of a highly toxic *Alexandrium* isolate (Bricelj et

TABLE 3
Ranking of Marine Bivalve Species in Terms of Their *In Vitro* Nerve Sensitivity to (A) Saxitoxin, STX [*S. giganteus* Collected from Three Sites with Different Histories of PSP Contamination; a: Twarog et al. 1972 (Specimens from the Atlantic Coast of the U.S.A), b: Kvitek and Beitler, 1991 (Specimens from the Pacific U.S. Coast)]; (B) Tetrodotoxin, TTX (Twarog et al. 1972). Species Listed in Order of Increasing Sensitivity, as Determined by the Block of Action Potential by These Toxins. +: Block; (+): Partial Block; 0: No Effect; nd: Not Determined

(A) Species	Common name	Block of action potential by STX (g ml ⁻¹)					
		10 ⁻⁸	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻³
<i>Mytilus edulis</i> ^a	Blue mussel	0	0	0	0	0	nd
<i>Placopecten magellanicus</i> ^a	Sea scallop	0	0	0	0	0	nd
<i>Humilaria kennerleyi</i> ^b	Kennerly venus	0	0	0	0	0	
<i>Mercenaria mercenaria</i> ^a	Northern quahog	0	0	0	0		
<i>Saxidomus giganteus</i> ^b	Butter clam						
Sequim (always toxic)		0	0	0	0		
Mukilteo (occasionally toxic)		0	0	0	0		
Hood Canal (never toxic)		0	0	0	0		
<i>Saxidomus nuttalli</i> ^b	Washington clam	0	0	0	0		
<i>Modiolus demissus</i> ^a		0	0	0	0		
(= <i>Geukensia demissa</i>)	Ribbed Mussel	0	0	0			
<i>Mya truncata</i> ^b	Truncate softshell	0	0	0			
<i>Pecten irradians</i> ^a							
(= <i>Argopecten irradians</i>)	Bay scallop	0	0				
<i>Mya arenaria</i> ^{a,b}	Softshell	0	0				
<i>Tresus capax</i> ^b	Fat gaper	0					
<i>Protothaca staminea</i> ^b	Pacific littleneck	0					
<i>Crassostrea virginica</i> ^a	Eastern oyster	0					

TABLE 3 (continued)
Ranking of Marine Bivalve Spec [*S. giganteus* Collected from Th et al. 1972 (Specimens from the from the Pacific U.S. Coast)]; (E Increasing Sensitivity, as Deterr Partial Block; 0: No Effect; nd:

(B) Species
<i>Mytilus edulis</i> (Massachusetts)
<i>Mytilus californianus</i>
<i>Placopecten magellanicus</i>
<i>Saxidomus nuttalli</i>
<i>Mya arenaria</i>
<i>Modiolus modiolus</i>
<i>Modiolus</i> (=Geukensia) <i>demissus</i>
<i>Mercenaria mercenaria</i>
<i>Schizothaerus</i> (=Tresus) <i>nuttalli</i>
<i>Petricola pholadiformis</i>
<i>Ensis directus</i>
<i>Zirfaea pilsbryi</i>
<i>Hinnites multirugosus</i> (=Crassadoma
<i>Pecten</i> (=Argopecten) <i>irradians</i>
<i>Tagelus gibbus</i> (=divisus)
<i>Spisula solidissima</i>
<i>Crassostrea virginica</i>
<i>Protothaca staminea</i>

10^{-6}	10^{-5}	10^{-4}	10^{-3}
0	0	0	nd
0	0	0	nd
0	0	0	0.9
0	0		nd
0	0		1
0	0		
0	0		
0	0		
0	0		
0			
0			

TABLE 3 (continued)

Ranking of Marine Bivalve Species in Terms of Their *In Vitro* Nerve Sensitivity to (A) Saxitoxin, STX [*S. giganteus* Collected from Three Sites with Different Histories of PSP Contamination; a: Twarog et al. 1972 (Specimens from the Atlantic Coast of the U.S.A), b: Kvitek and Beitler, 1991 (Specimens from the Pacific U.S. Coast)]; (B) Tetrodotoxin, TTX (Twarog et al. 1972). Species Listed in Order of Increasing Sensitivity, as Determined by the Block of Action Potential by These Toxins. +: Block; (+): Partial Block; 0: No Effect; nd: Not Determined

Partial Block; 0: No Effect; nd: Not Determined		Block of action potential by TTX (g ml ⁻¹)			
(B) Species	Common name	10 ⁻⁸	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵
<i>Mytilus edulis</i> (Massachusetts)	Blue mussel	0	0	0	0
<i>Mytilus californianus</i>	California mussel	0	0	0	0
<i>Placopecten magellanicus</i>	Sea Scallop	0	0	0	0
<i>Saxidomus nuttalli</i>	Washington clam	0	0	0	0
<i>Mya arenaria</i>	Softshell	0	0	0	0
<i>Modiolus modiolus</i>	Northern horsemussel	0	0	0	+
<i>Modiolus (=Geukensia) demissus</i>	Ribbed mussel	0	0	0	+
<i>Mercenaria mercenaria</i>	Northern quahog	0	0	0	+
<i>Schizothaerus (=Tresus) nuttalli</i>	Pacific gaper	0	0	0	+
<i>Petricola pholadiformis</i>	False angelwing	0	0	0	+
<i>Ensis directus</i>	Atlantic jackknife	0	0	0	+
<i>Zirfaea pilsbryi</i>	Pilsbry piddock	0	0	0	+
<i>Hinnites multirugosus (=Crassadoma gigantea)</i>	Giant Rock Scallop	0	0	0	+
<i>Pecten (=Argopecten) irradians</i>	Bay scallop	0	0	0	+
<i>Tagelus gibbus (=divisus)</i>	Purplish tagelus	0	0	0	+
<i>Spisula solidissima</i>	Atlantic surfclam	0	0	0	+
<i>Crassostrea virginica</i>	Eastern oyster	0	0	0	+
<i>Protothaca staminea</i>	Pacific littleneck	0	0	0	+

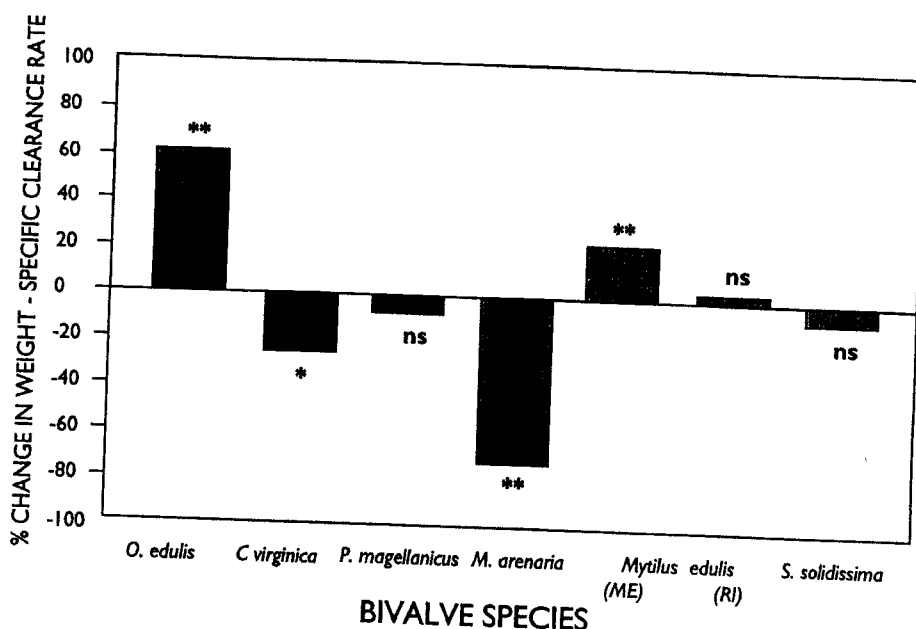
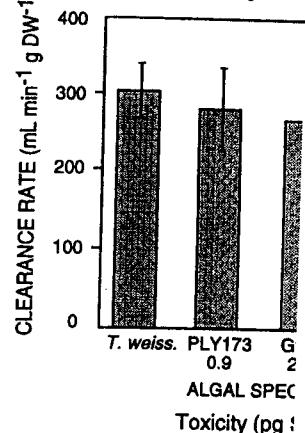
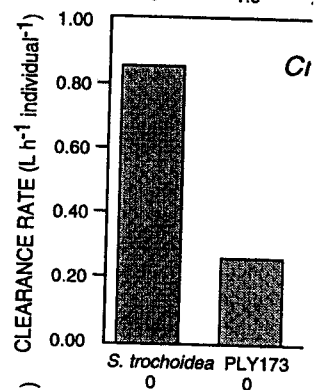
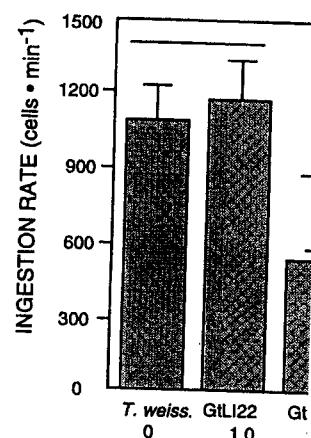
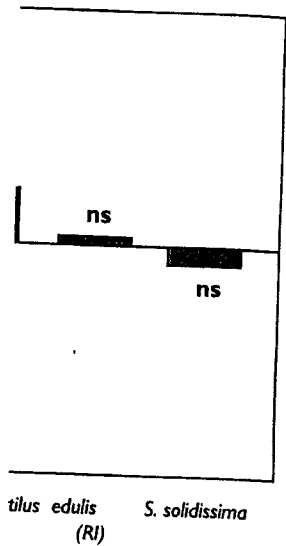


FIGURE 7. Percent change (increase or decrease) in weight-specific clearance rate of various bivalve species in response to addition of 500 cells ml^{-1} of toxic *Alexandrium tamarensis*, clone Gt429, to a nontoxic, control algal diet (mixed suspension of *Thalassiosira pseudonana*, *Chroomonas salina* and *Prorocentrum minimum* at 1.0×10^4 cells ml^{-1}) after 1 h of exposure (data plotted from Table 3 in Shumway and Cucci, 1987). Toxicity of *A. tamarensis* was not reported, but other studies (see Table 2) indicate that this strain is characterized by moderate to high toxicities, ranging from 10 to 96 pg STXeq cell^{-1} , depending on culture conditions. All bivalve species from Maine (ME) populations unless indicated: RI = Rhode Island. Results of statistical analysis: ns = nonsignificant; * and ** = significant at $p < 0.5$ and $p < 0.01$, respectively.

al., 1991). In contrast, the surfclam (or bar clam) *Spisula solidissima* is highly sensitive to TTX (Table 3B), but shows no feeding or burrowing inhibition in response to dinoflagellate toxicities as high as 74 pg STXeq cell^{-1} (Bricelj et al., 1996, Figure 8); this species also achieves extremely high PSP toxicities in both field and laboratory studies (reviewed in Tables 1 and 2, respectively). Thus, results of the nerve assay must be verified using alternate *in vivo* measures of sensitivity, such as physiological and behavioral responses. It is also important to note that although there is agreement between the sensitivity to STX and TTX for some species, especially those found at the extremes of the sensitivity range (*C. virginica* and the littleneck clam *P. staminea* >> sensitivity than *M. edulis* and the sea scallop *Placopecten magellanicus*), poor correlation is observed for others (e.g., *Saxidomus nuttalli* and *Mya arenaria*) (Table 3).



exposure to toxic dinoflagellates (no pseudofeces were produced by Clone Pr18b (*A. tamarensis*) of total volume) with *T. weissii*.



it-specific clearance rate of ml^{-1} of toxic *Alexandrium* suspension of *Thalassiosira* at 1.0×10^4 cells ml^{-1} after d Cucci, 1987). Toxicity of) indicate that this strain is 10 to 96 pg STXeq cell $^{-1}$, re (ME) populations unless = nonsignificant; * and ** =

Spisula solidissima is feeding or burrowing s high as 74 pg STXeq so achieves extremely studies (reviewed in : nerve assay must be itivity, such as physi- important to note that y to STX and TTX for mes of the sensitivity aminea >> sensitivity magellanicus), poor us nuttalli and Mya

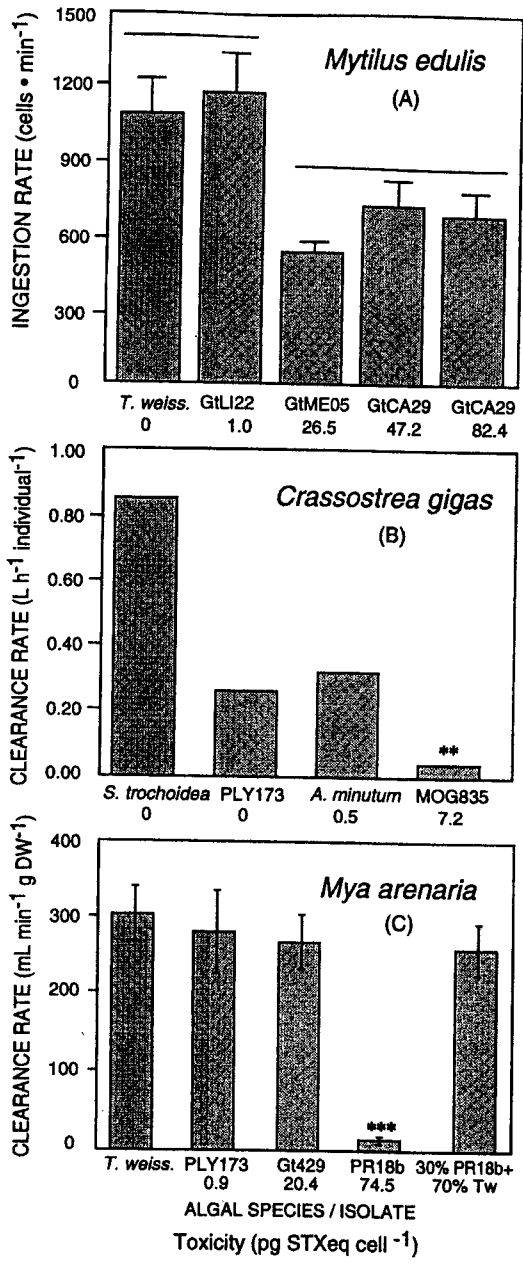


FIGURE 8. Effect of dinoflagellate PSP cell toxicity on bivalve feeding (algal species/clone, and toxicities, in pg STXeq cell $^{-1}$, indicated below each bar): **A.** Mean weight-normalized ingestion rate, in cells min^{-1} , for a standard animal 1 g in total body wet weight ($\pm$ standard error, SE), of juvenile (14 to 16 mm) mussels, *Mytilus edulis*, from Long Island, New York, after 4 to 5 h exposure to a monospecific diet of three toxic isolates of *Alexandrium* spp. (initial concentrations = 200 cells ml^{-1}) and the nontoxic diatom *Thalassiosira weissflogii* (volume-equivalent concentration of 3306 cells ml^{-1}) (from Lee, 1993). Note that mussels did not produce pseudofeces in any of the treatments. Horizontal bars indicate treatments that were not statistically significant from each other. GtLI22 = *A. tamarense*; GtCA29 and GtME05 = *A. fundyense*. **B.** Mean clearance rate, in L h $^{-1}$ individual $^{-1}$, of adult oysters, *Crassostrea gigas*, from Bourgneuf Bay, France, over 6-h exposure to various monospecific diets (*Scrippsiella trochoidea*, *A. minutum*, and *A. tamarense*, clones PLY173 and MOG835, respectively) (replotted from Bardouil et al., 1993). **: significantly different from the nontoxic PLY clone at $p < 0.01$. Oysters produced pseudofeces in all treatments (seston levels ranging from 7 to 11 mg L $^{-1}$). **C.** Mean weight-specific clearance rate, in mL min $^{-1}$ dry weight of soft tissues ($\pm$ SE) of juvenile (33 mm) softshell clams, *Mya arenaria* from southern Nova Scotia, Canada, after 2 to 3 h exposure to toxic dinoflagellates (94 to 125 cells ml^{-1}) or an equal volume of *T. weissflogii* (no pseudofeces were produced) (Bricelj et al., 1996). PLY173 and Gt429 = *A. tamarense*. Clone Pr18b (*A. tamarense* cf. *excavatum*) was also offered in a mixed suspension (30% of total volume) with *T. weissflogii*. ***: significantly different at $p < 0.001$.

The ability to burrow in the presence of toxic cells has been proposed recently as a rapid and easily determined index of sensitivity to PSP toxins for infaunal bivalves (Bricelj et al., 1996). Feeding response to toxic cells is also a good indicator of toxin sensitivity and the potential for toxin accumulation in bivalves. Significant inhibition of clearance rates by spiking of a nontoxic algal suspension with toxic *Alexandrium* cells was demonstrated in *C. virginica* and *Mya arenaria*, whereas *S. solidissima*, *P. magellanicus*, and *M. edulis*, three species ranked as highly resistant by the nerve assay, showed no feeding inhibition (Figure 7). Therefore, there is good agreement between sensitivity rankings based on the nerve assay and feeding response, except for *Mya arenaria*. The data shown in Figure 7 are useful for comparing the relative response among species. However, dinoflagellate cell toxicity was not measured in this study, and clearance rate inhibition may be partly attributable to concentration-dependent effects, because dinoflagellate cells were added at a relatively high cell density (500 cells ml⁻¹). Biomass-dependent effects may also confound results of feeding experiments in which toxic dinoflagellates and nontoxic algae differing greatly in cell size are offered in unialgal suspensions at comparable cell densities (e.g., Lesser and Shumway, 1993).

It is interesting to note that the two oyster species tested in Figure 7 showed opposite responses: clearance rate of *Crassostrea virginica* was significantly inhibited by addition of toxic cells, whereas that of *Ostrea edulis* was, in fact, stimulated. In support of these results, Table 1 shows that *O. edulis* from New England can achieve relatively high toxicities (maximum = 1300 µg STXeq 100 g⁻¹), whereas toxicities reported for *C. virginica* have never exceeded ca. 200 µg STXeq 100 g⁻¹, even during severe PSP outbreaks. Furthermore, field data from Maine (J. Hurst, unpublished) indicate that *O. edulis* became toxic prior to *M. edulis* from the same area. These differences between *O. edulis* and *C. virginica* are species- rather than genus-specific, because the Pacific oyster, *Crassostrea gigas*, can attain comparatively high toxicities (up to 9900 µg STXeq 100 g⁻¹) (Table 1).

B. FACTORS INFLUENCING TOXIN ACCUMULATION

1. Bloom Characteristics

The maximum toxin body burden accumulated by bivalves is dependent on dinoflagellate cell density and specific toxicity, as well as bloom duration. However, field studies in which seston toxin concentration (cell concentration x toxicity cell⁻¹) was measured concomitantly with

that in shellfish (e.g., relatively rare. Therefore, the relationship between peak toxin concentration and any given species. A maximum density of toxin in studies in which (Ogata et al., 1982; Ch duration of this lag pe

The maximum abundance of *Alexandrium* cells by up to 10⁵ to 10⁶ cells concentration range are *Alexandrium* cells by (Bricelj et al., 1990). A twofold variation in re STXeq cell⁻¹), ranging sized mussel (1 g in v *ad libitum* attained ma of exposure to simulat (Lassus et al., 1989; I nature, episodic bloom up to several weeks. species that are relativ time-saturating conditi in water column toxin global comparisons (E tively constant feeding tures (ca. 10 to 20°C) (species is likely to be l in temperature than influenced by seasona

Toxin accumulation have been reported f Gilfillan, 1977; Price e that *M. edulis* can exc to a highly toxic *Alexa have important impli routinely conducted incidence, and less fre uptake rates could p highly toxic blooms ; sumption, thus justifi STXeq 100 g⁻¹), well*

toxic cells has been used index of sensitivity (1996). Feeding response and the potential inhibition of clearance with toxic *Alexandrium* and *Mya arenaria*, whereas other species ranked as feeding inhibition (Figure 1). Sensitivity rankings except for *Mya arenaria*. Comparing the relative cell toxicity was not inhibition may be partly because dinoflagellate (10 cells ml⁻¹). Biomass-feeding experiments in differing greatly in cell comparable cell densities

Species tested in Figure 1: *Crassostrea virginica* cells, whereas that of *Mytilus edulis*. Of these results, Table 1 achieve relatively high (100 µg STXeq 100 g⁻¹), whereas toxicities (200 µg STXeq 100 g⁻¹, field data from Maine became toxic prior to between *O. edulis* and *C. virginica*, because the Pacific high toxicities (up to

CONCLUSION

Bivalves is dependent on feeding rate, as well as bloom and toxin concentration and concomitantly with

that in shellfish (e.g., Therriault et al., 1985; Chebib et al., 1993) are relatively rare. Therefore, it is difficult to describe a functional relationship between peak toxin concentration and maximum bivalve toxicity for any given species. A lag time is commonly observed between the maximum density of toxic phytoplankton and maximum shellfish toxicities in studies in which both parameters were measured concurrently (Ogata et al., 1982; Chebib et al., 1993; Martin and Richard, 1996). The duration of this lag period (days to weeks) is species-specific.

The maximum abundance of PSP-producing dinoflagellates can reach up to 10⁵ to 10⁶ cells L⁻¹. In a laboratory study, ingestion rate of toxic *Alexandrium* cells by *Mytilus edulis* varied by a factor of 2.4 over this concentration range and was maximized at densities of ca. 10⁵ cells L⁻¹ (Bricelj et al., 1990). At this concentration, ingestion rates also showed twofold variation in response to dinoflagellate cell toxicity (7 to 66 pg STXeq cell⁻¹), ranging from ca. 10 to 20 × 10⁵ cells day⁻¹ for a standard-sized mussel (1 g in wet weight of soft tissues) (Table 2). Mussels fed *ad libitum* attained maximal, saturating toxin levels within about 2 weeks of exposure to simulated bloom concentrations, regardless of cell toxicity (Lassus et al., 1989; Bricelj et al., 1990; and unpublished results). In nature, episodic blooms of PSP toxin-producing dinoflagellates often last up to several weeks. Therefore, peak toxicities of mussels and other species that are relatively insensitive to PSP toxins are likely to reflect time-saturating conditions and to be primarily influenced by differences in water column toxin concentration (pg STXeq L⁻¹), thereby facilitating global comparisons (Figure 1). *Mytilus edulis* can also maintain a relatively constant feeding rate over a wide range of acclimation temperatures (ca. 10 to 20°C) (Bayne et al., 1977); therefore, toxin uptake in this species is likely to be less affected by geographic or seasonal differences in temperature than in species in which clearance rate is strongly influenced by seasonal temperature.

Toxin accumulation rates of up to 550 to 757 µg STXeq 100 g⁻¹ day⁻¹ have been reported for field populations of *Mytilus* spp. (Hurst and Gilfillan, 1977; Price et al., 1991). Furthermore, laboratory studies show that *M. edulis* can exceed the regulatory level within < 1 h of exposure to a highly toxic *Alexandrium* strain (Bricelj et al., 1990). These findings have important implications for PSP monitoring programs, which are routinely conducted on a weekly basis during seasons of high PSP incidence, and less frequently at other times of the year. Such high toxin uptake rates could preclude early warning of a PSP outbreak during highly toxic blooms and in areas where mussels are targeted for consumption, thus justifying the use of a conservative regulatory level (80 µg STXeq 100 g⁻¹), well below that likely to cause human illness.

In relatively insensitive species, toxin accumulation rate and peak toxicity are expected to be an increasing function of dinoflagellate cell toxicity (assuming a constant cell density or regulation of ingestion rates with density). Thus, *M. edulis* and *S. solidissima* achieved a higher toxin body burden when exposed to high-toxicity *Alexandrium* isolates (GtCA29 or PR18b) than to low-toxicity isolates (LI22, or AL1V) at comparable cell densities (Table 2). A more complex and less predictable relationship between cell toxicity and peak shellfish toxicity is expected in sensitive species that experience feeding inhibition in the presence of highly toxic dinoflagellate cells (Figure 8). Few studies have investigated the effect of cell toxicity on bivalve feeding rates, taking advantage of the wide variation in specific toxicity among *Alexandrium* isolates. Significant inhibition in clearance rate was demonstrated in *M. edulis*, *C. gigas*, and *Mya arenaria* above a threshold of cell toxicity (Figure 8, and Lee, 1993). Both the threshold toxicity required to depress feeding and the magnitude of feeding inhibition varied among these studies. However, because both total cell toxicity and toxin composition vary among the isolates used in these studies, it is difficult to make direct comparisons among species. In sensitive bivalves, toxin accumulation is also strongly influenced by the presence of nontoxic cells in a mixed phytoplankton assemblage, which may stimulate feeding on toxic cells. For example, *Mya arenaria* attained a higher ingestion rate of toxic cells and tissue toxicity when offered a high-toxicity strain of *Alexandrium* in a mixed suspension with nontoxic diatoms, than when it was offered alone (Bricelj et al., 1996, Figure 8). Similarly, *M. mercenaria* individuals were only induced to open their valves and initiate feeding when a highly toxic isolate was offered in combination with nontoxic cells (Bricelj et al., 1991).

Variation in toxin ingestion rate (cell ingestion $\times$ toxin concentration) appears to be more important in explaining differences in toxin accumulation within and among species than the efficiency with which bivalves absorb ingested toxic cells. Comparable maximal absorption efficiencies (ca. 60% of organic matter) were obtained for *M. mercenaria* fed a low-toxicity isolate of *Alexandrium* (Bricelj et al., 1991) and *M. edulis* fed either a low- or high-toxicity isolate (Lee, 1993), suggesting that absorption efficiency does not vary with dinoflagellate specific toxicity. Despite these moderate absorption efficiencies, bivalves are capable of relatively high accumulation efficiencies for PSP toxins [(cumulative toxin ingested/toxin incorporated in tissues) $\times 100$]: 72 to 96% in *M. californianus* (Dupuy 1968) and 78% in *M. edulis* (Bricelj et al., 1990).

2. Prior History of

Twarog (1974) suggest affect bivalve PSP tox repeatedly exposed to accumulate higher tox. Very little is known a toxin accumulation rate adaptation, responsible and Cucci (1987) found different histories of feeding response to to planted from a toxin-fr toxin during a mid-su estuary than mussels f sure to PSP toxins (Che populations were no l suggesting that they di between the two pop related to the consider the second bloom. In not differ among three with varying histories that high toxin resist after toxin exposure (high levels of STX in tl effective deterrent to (1991) to speculate t species as a chemical d nipping fish. However siphons has not been f PSP-affected areas arg

3. Sources of Intra

Variation in sensitivity to sampling location (and to the quality of the data documented recently by [Pascarella et al. 2005](#)). The fact that the same substitutions in the same species can have different binding capacity in different species is a genetic basis for such

ulation rate and peak
of dinoflagellate cell
ation of ingestion rates
chieved a higher toxin
alexandrium isolates (GtCA29
1V) at comparable cell
redictable relationship
expected in sensitive
resence of highly toxic
vestigated the effect of
vantage of the wide
m isolates. Significant
A. edulis, *C. gigas*, and
gure 8, and Lee, 1993).
eding and the magni-
ies. However, because
ry among the isolates
t comparisons among
is also strongly influ-
mixed phytoplankton
ic cells. For example,
toxic cells and tissue
alexandrium in a mixed
it was offered alone
arenaria individuals were
eding when a highly
ntoxic cells (Bricelj et

x toxin concentration)
nces in toxin accumu-
y with which bivalves
bsorption efficiencies
arenaria fed a low-
91) and *M. edulis* fed
ggesting that absorp-
ecific toxicity. Despite
e capable of relatively
cumulative toxin in-
% in *M. californianus*
, 1990).

2. Prior History of Exposure to PSP Toxins

Twarog (1974) suggested that prior history of exposure to PSP may also affect bivalve PSP toxin accumulation, such that bivalve populations repeatedly exposed to PSP toxins might become more resistant and accumulate higher toxin levels than those with no prior contamination. Very little is known about inter- or intrapopulation variability in PSP toxin accumulation rate, or the mechanisms, either acclimation or genetic adaptation, responsible for maintaining any existing variation. Shumway and Cucci (1987) found that mussels, *M. edulis*, from populations with different histories of exposure to PSP differed significantly in their feeding response to toxic cells (Figure 6). Additionally, *M. edulis* transplanted from a toxin-free area were found to accumulate about 50% less toxin during a mid-summer *Alexandrium* bloom in the St. Lawrence estuary than mussels from populations with a chronic history of exposure to PSP toxins (Chebib et al., 1993). These initial differences between populations were no longer apparent during a second summer bloom, suggesting that they did not have a genetic basis. Lack of differentiation between the two populations during reexposure may, however, be related to the considerably lower water column toxicity associated with the second bloom. In contrast, the level of nerve sensitivity to STX did not differ among three populations of butter clams, *Saxidomus giganteus*, with varying histories of exposure to PSP toxins (Table 3A), suggesting that high toxin resistance in this species is innate rather than acquired after toxin exposure (Kvitek and Beitler, 1991). Long-term retention of high levels of STX in the siphons of *S. giganteus* was shown to act as an effective deterrent to predation (Kvitek, 1991, 1993), leading Kvitek (1991) to speculate that toxin compartmentalization evolved in this species as a chemical defense against sublethal predators such as siphon-nipping fish. However, the fact that selective toxin accumulation in the siphons has not been found in any other siphonate bivalve species from PSP-affected areas argues against this hypothesis.

3. Sources of Intrapopulation Variability

Variation in sensitivity to PSP toxins among individuals from the same sampling location (and thus same history of exposure to PSP toxins) was documented recently in *Mya arenaria* (Bricelj et al., 1996 and unpublished data). The fact that mutations resulting in single amino acid substitutions in the sodium channel pore region may change the STX-binding capacity in rats (Kontis and Goldin, 1993) suggests a possible genetic basis for such intrapopulation differences in toxin sensitivity.

Variation in body mass may also influence toxin accumulation rates. Based on allometric considerations, we can predict that during toxification, weight-specific toxicity of bivalves (in $\mu\text{g STXeq } 100 \text{ g}^{-1}$) will vary inversely with body size, because smaller individuals have a higher cell ingestion rate per unit biomass than larger ones. Thus, Aalvik and Framstad (1981) found that small (3 to 4 cm in shell length) field-collected *M. edulis* attained peak toxicities twice as high as those of larger (>6 cm) mussels. Similarly, juvenile (1.7 g wet tissue weight) surfclams, *S. solidissima*, became twice as toxic as adults (8 g) after 2 weeks of laboratory toxification under identical conditions of exposure to toxic cells (Bricelj et al., unpublished Table 2). The standard AOAC bioassay often requires pooling of individuals in order to attain the specified 100 g of shellfish meats for toxin extraction. These results argue for the need to sample individuals of a relatively uniform body size in order to eliminate the confounding effects of body size when the goal is to reduce overall biological variability or more accurately depict the risk posed by a particular size class.

An understanding of the sources of high intrapopulation variation in toxin levels, exceeding that accounted for by imprecision in the mouse bioassay (ca. $\pm 20\%$, Adams and Furfari, 1984), is crucial in the sampling design of regional toxin-monitoring programs. Factors other than variation in body size may contribute to this high individual variability: differences in feeding rates or reproductive condition among individuals, microgeographic variation in exposure to toxic cells due to bloom patchiness in subtidal populations, and differences in height and thus immersion or feeding time in the intertidal zone. Thus, Quayle (1969) found no clear correlation between individual toxicity of butter clams, *Saxidomus giganteus*, from natural populations and body size (50 to 90 mm in shell length), but described a gradient of decreasing toxicities with increasing tidal height during the period of toxification. Differences in toxicity of *Mya arenaria* from Cap Chat in the lower St. Lawrence estuary were also found to be positively related to tidal submergence time (Cembella and Frechette, unpublished data). Tenfold differences in toxicity of softshell clams were obtained over a 1.2-km distance in Lepreau Basin, Bay of Fundy, along the longitudinal axis of this estuary (Prakash et al., 1971). White et al. (1993b) reported significant variation in PSP toxin levels among Georges Bank *S. solidissima* within sampling stations in which surfclams occurred over a range of several hundred meters (mean coefficient of variation, CV, among sampling stations = 49%), but found no correlation between toxicity and shell length (11 to 17 cm). Their study also suggested an inverse relationship between the CV and shellfish toxicity determined by mouse bioassay.

However, a detailed analysis of PSP toxin levels, determined from the Gulf of Maine, is limited by analytical error, thus making it difficult to assess the true toxicities approaching 100 $\mu\text{g STXeq } 100 \text{ g}^{-1}$.

Even under continuous exposure, there is a vertical gradient in the distribution of toxins in the distribution of toxins in bivalve toxicity. In the Bay of Gaspé, exposed mussels collected nearshore and placed subtidally nearshore showed higher levels of mussels suspended in the water column. Therefore, as concluded by Cembella, mussels collected nearshore are more relevant to assess the risk of PSP toxin accumulation. A vertical gradient in abundance of *Alexandrium* in the Bay of Gaspé, Canada, was observed when suspended at the surface and at the bottom. Desbiens and Cembella (1994) found that PSP toxin accumulation in *Patinopecten yessoensis* was related to water column depth.

VI. ANATOMICAL

Paralytic shellfish toxin (PST) is found in the tissues, thus resulting in paralytic shellfish poisoning (PSP) (in $\mu\text{g STXeq g}^{-1}$) of individuals (Shumway et al., 1994; Shumway et al., 1994). This is of particular interest because PST is of particular interest because organs are marketed for consumption (e.g., scallops (Pectinidae)). Inter-PSP toxins (e.g., PSP toxins) are useful in developing a marketing tool to reduce the risk of PSP pathways and rates of PSP. Silver and Cembella (1994) found that PST is useful in developing a marketing tool to reduce the risk of PSP pathways and rates of PSP.

The capacity for PST is related to the selective retention of PST in the tissues of individuals. The capacity for PST is related to the selective retention of PST in the tissues of individuals.

in accumulation rates. That during toxification, (eq 100 g⁻¹) will vary. Mussels have a higher cell density. Thus, Aalvik and others (shell length) field-collected as high as those of mussels (g wet tissue weight) as adults (8 g) after 14 conditions of exposure. The standard AOAC method in order to attain the goal. These results argue for uniform body size in the size when the goal is to accurately depict the population variation in the precision in the mouse bioassay. In the sampling factors other than variability: individual variability: among individuals, cells due to bloom density in height and thus. Thus, Quayle (1969) toxicity of butter clams, and body size (50 to 90 increasing toxicities with cation. Differences in the St. Lawrence estuary at submergence time and fold differences in a 1.2-km distance in the longitudinal axis of this) reported significant in *S. solidissima* within a range of several CV, among sampling on toxicity and shell an inverse relationship by mouse bioassay.

However, a detailed analysis of tissue-specific individual variability in PSP toxin levels, determined by HPLC-FD, in surfclams and sea scallops from the Gulf of Maine, did not substantiate this pattern, and attributed it to analytical error, that is, lower precision of the mouse bioassay at low toxicities approaching the limit of detection (Cembella et al., 1993).

Even under continuous immersion, vertical and horizontal gradients in the distribution of toxic cells may lead to substantial spatial variation in bivalve toxicity. In an extreme case, Desbiens et al. (1990) found that in the Bay of Gaspé, eastern Canada, the maximum toxicity of mussels placed subtidally nearshore was two orders of magnitude lower than that of mussels suspended 300 to 600 m offshore in a 15-m water column. Therefore, as concluded in this study, monitoring data based on wild mussels collected nearshore or in the intertidal zone, may not always be relevant to assess the PSP risk of mussels grown in suspended culture. A vertical gradient in shellfish toxicity, clearly related to that in the abundance of *Alexandrium* cells, has been reported in several studies. In the Bay of Gaspé, offshore mussels became 2 to 5 times more toxic when suspended at the surface than near-bottom (Desbiens et al., 1990; Desbiens and Cembella, 1993). Nishihama (1980) found that the rate of PSP toxin accumulation, and timing of peak toxicities of scallops, *Patinopecten yessoensis*, held in suspended culture differed markedly with water column depth over a range of 30 m in Funka Bay, Japan.

VI. ANATOMICAL DISTRIBUTION OF PSP TOXINS

Paralytic shellfish toxins are not evenly distributed throughout bivalve tissues, thus resulting in pronounced differences in the absolute toxicities (µg STXeq g⁻¹) of individual tissues (e.g., Maruyama et al., 1983; Cembella et al., 1994; Shumway et al., 1994). The anatomical partitioning of toxins is of particular interest for those bivalve species in which only some organs are marketed for human consumption, as generally occurs with scallops (Pectinidae). Discarding of those tissues that selectively sequester PSP toxins (e.g., evisceration) may in some cases provide an effective marketing tool to reduce the risk of PSP. An understanding of the pathways and rates of exchange among tissue compartments can also be useful in developing predictive models of toxin kinetics (Lee, 1993; Silvert and Cembella, 1995).

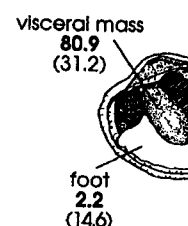
The capacity for *in vivo* biotransformation (Shimizu and Yoshioka, 1981; Sullivan, 1982; Oshima, 1995b; Bricelj and Cembella, 1995) and/or selective retention of individual PSP toxins are major determinants of the differences in toxicity among tissues. However, the contribution of each

tissue to the total toxin body burden is a function of both its absolute toxicity and relative weight contribution. It is well established that during the toxification phase, the digestive gland-stomach complex or viscera, the initial repository of toxic cells following ingestion and absorption of toxic cells, contains by far the greatest proportion of the total toxin body burden. In a number of laboratory- or field-toxified bivalve species, the toxin contribution of the viscera typically ranges from 80 to 98%, despite its relatively modest contribution to total body mass (summarized in Figure 9). Lower values reported for some natural populations (e.g., 50% in *Spisula solidissima* from the Gulf of Maine, Cembella et al., 1993, 60% for *S. giganteus*, Figure 10) probably reflect the fact that peak bloom conditions were missed by the field sampling schedule. Laboratory studies involving northern quahogs, surfclams, softshell clams and mussels indicate that the toxicity of the viscera (in $\mu\text{g STXeq g}^{-1}$) is generally 3 times higher (range = 2.5 to 5.1) than that of whole tissues (including the viscera) (Table 2). Although concomitant field data on both visceral and whole tissue toxicities are scarce, they corroborate this relationship for most bivalves (Table 1). An even higher ratio of viscera to whole body toxicity has been found occasionally in scallop species such as *Placopecten magellanicus* (Table 1) and *Crassadoma gigantea* (Beitler, 1991).

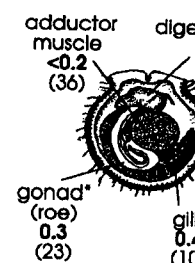
It is also noteworthy that the relative partitioning of PSP toxins among tissues at the peak of toxification appears to be independent of the total toxicity or toxin composition of the dinoflagellate strain that provides the source of toxin. This was shown for *M. mercenaria*, *M. edulis* (Lee and Bricelj, unpublished data), and *S. solidissima* (Bricelj, Laby, and Cembella, unpublished) in laboratory studies in which clams were toxified with low- and high-toxicity isolates of *Alexandrium*.

In contrast to the viscera, locomotory tissue (adductor muscle, pallial muscles, and the muscular foot) contributes substantially to the total weight of soft tissues, but makes a disproportionately low contribution (< 3%) to the toxin body burden (Figure 9, Cembella et al., 1994). The prominent foot of the surfclams *S. solidissima* and *Mactromeris polynyma* is used for sushi in some markets. Therefore, the low toxicity of this tissue (confirmed to date for *Spisula* but not *Mactromeris*) should favor development of this specialized market in areas affected by PSP. The limited capacity for toxin accumulation of the adductor muscle also favors the domestic marketing of scallops within the U.S. and Canada, where traditionally only this tissue is consumed. The toxicity of the adductor muscle in scallops is typically one to three orders of magnitude lower than that of the corresponding digestive gland (see Table 2 in Shumway and Cembella, 1993) and rarely exceeds the regulatory level,

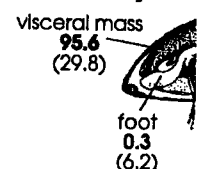
Mercenaria m



Placopecten m



Mytilus



ma
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(18)

FIGURE 9. Anatomical distribution of PSP toxins (boldfaced: relative contribution of tissue to total toxin body burden; italicized: relative (%) contribution of tissue to total body mass) for *M. mercenaria* (Lee and Bricelj, 1990 [juveniles (L)]; E recalculation from Cembella et al., 1990 during peak toxicity periods calculated from Beitler 1992) converted from nmol toxin tissue weight from Table 11 Bricelj et al., 1990 [adults (L); Cembella, 1995 [juveniles (L) laboratory].

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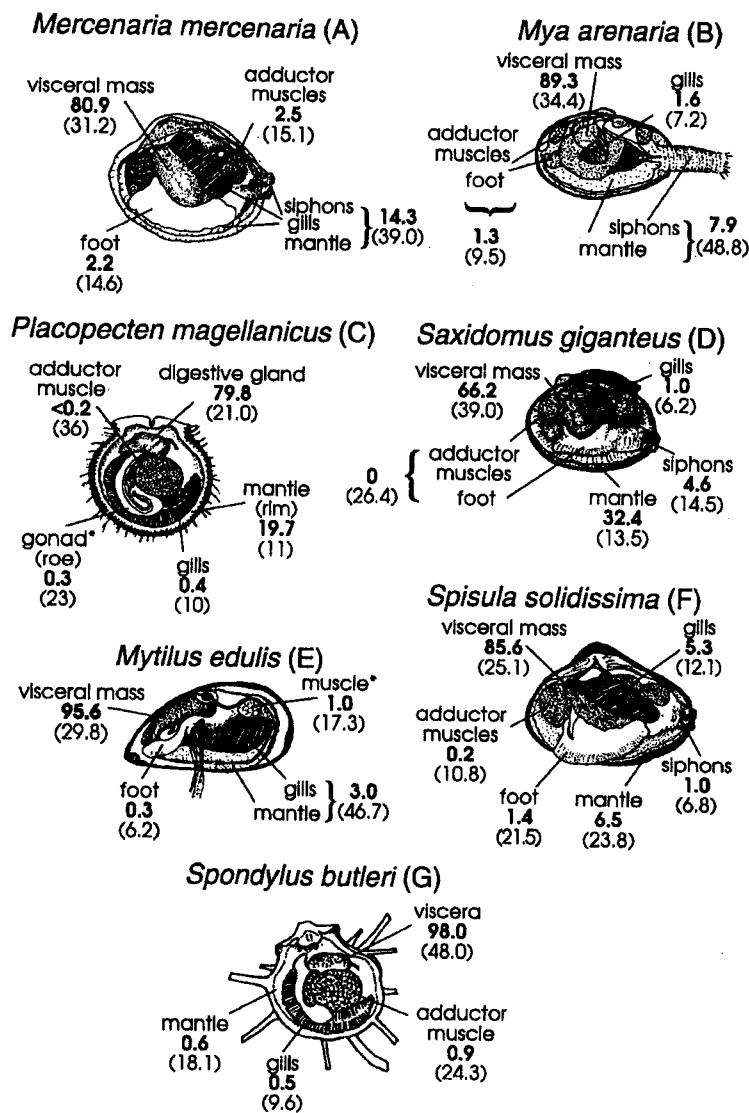


FIGURE 9. Anatomical distribution of PSP toxins in various tissues of bivalves at the peak of toxification (boldfaced: relative toxin load, as % of total toxin body burden, calculated on the basis of toxicity and contribution by weight of each tissue). Values in brackets indicate the relative (%) contribution of each tissue to total wet tissue weight. **References:** (A) Bricelj et al., 1990 [juveniles (L)]; (B) Bricelj, Laby and Cembella, unpublished [juveniles (L)]; (C) recalculated from Cembella et al., 1993 as % of total toxin load, in μg STXeq, averaged during peak toxicity periods in 1988 and 1989 (adults from the Gulf of Maine, U.S.A.); (D) calculated from Beitler 1992 [adults (L), data from Tables 25 and 26 after 15 d of toxification, converted from nmoles toxin to μg STXeq; contribution of adductor muscles and foot to total tissue weight from Table 11); mantle fraction includes the kidney and pallial muscle; (E) Bricelj et al., 1990 [adults (L)]; *includes adductor and pedal retractor muscles; (F) Bricelj and Cembella, 1995 [juveniles (L)]; (G) Harada et al., 1982, Palau. L = animals held in the laboratory.

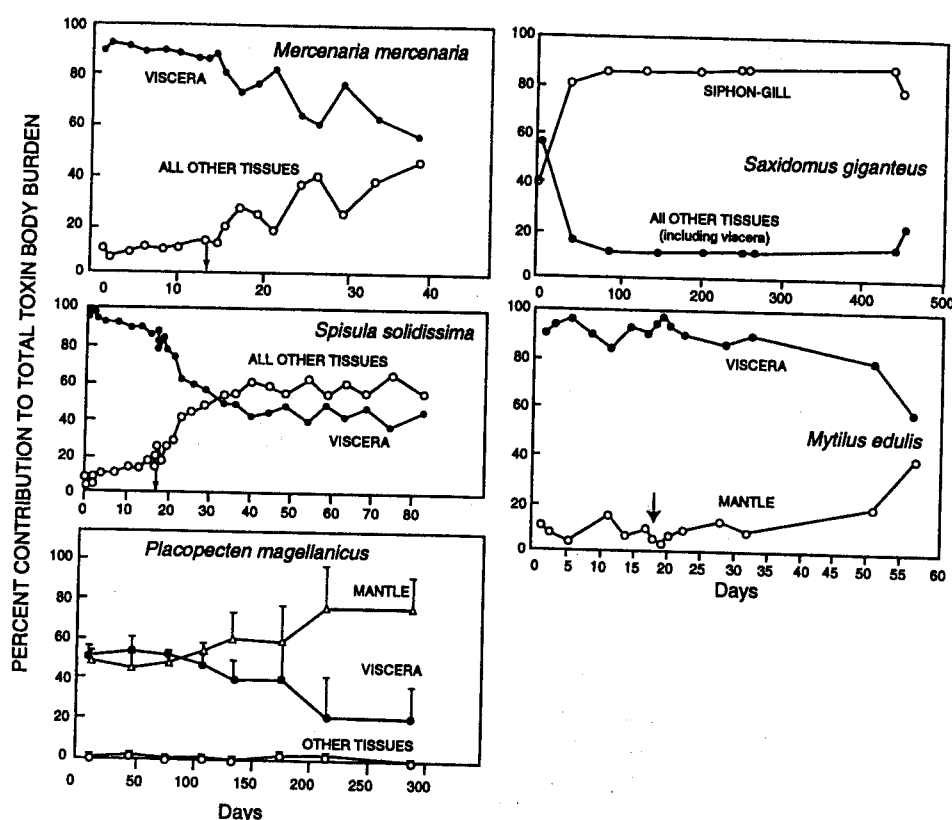
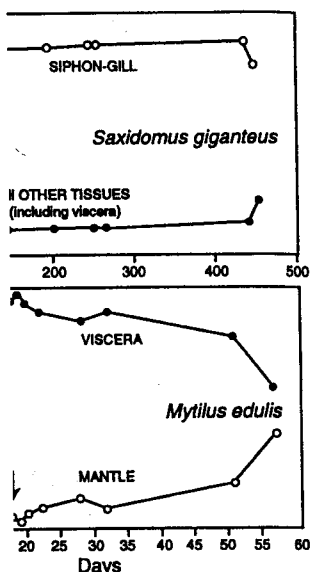


FIGURE 10. Percent contribution of visceral mass (including the digestive gland) and other tissues to total toxin body burden (in μg STX eq) over the course of toxification and/or detoxification in five bivalves species (arrow marks the end of toxification). Laboratory data and toxin analysis by HPLC for northern quahogs, *M. mercenaria* (Bricelj et al., 1991), surfclams, *S. solidissima* (Bricelj and Cembella, unpublished), and mussels, *Mytilus edulis* (Bricelj, Lee and Cembella, unpublished) at 17°C ; day 0 = beginning of toxification. Field-toxified sea scallops, *P. magellanicus*, detoxified in the laboratory (Waiwood et al., 1995). Field data for butter clams, *S. giganteus*, from British Columbia, Canada (Table 8 in Quayle, 1969; day 0 corresponds to the maximum whole clam toxicity recorded on June 3). Toxicity determined by mouse bioassay for the latter two species.

even during dinoflagellate blooms. Maximum levels of approximately $10^2 \mu\text{g}$ STXeq 100 g^{-1} , however, have been reported in *Patinopecten yessoensis* (Noguchi et al., 1978, 1984), *P. magellanicus* (Jamieson and Chandler, 1983) and *C. gigantea* (Beitler, 1991) during severe PSP outbreaks. Lack of a good correlation between toxicity levels in scallop adductor muscles and corresponding viscera indicates that the safety of adductor muscles cannot be assessed from the toxicity of this organ (Beitler, 1991; Cembella et al., 1994).

In countries where Japan, France, and Australia limit the safe maximum and $3200 \mu\text{g}$ STXeq g^{-1} (Hsu et al., 1979) and export of "roe-on" scallops is ongoing, and scallops and Cembella, 1993). I between the toxicity populations of sea scallops Cembella et al., 1992; toxicities reliably from other commercially in costly additional monitoring of the intestinal contain toxic feces, from toxin analysis has delayed in gonadal follicles about the efficiency of *M. edulis*, which under spawned oocytes on weight-specific basis fraction (Bricelj, Lee,

In most bivalves, tissues that attain the toxin load. In *Spisula* (during early detoxification) (Shumway et al., 1994) of the digestive gland which included the digestive gland [= pericardial gland] (Martin et al., 1990), *Saxidomus giganteus* (1992) reported high scallops, *P. yessoensis*; *S. giganteus* is unique of the toxin as STX (Beitler, 1992). Portions (Figure 10). The toxins in this species have suggested that STX is melanin fraction of the work (Beitler, 1992) d



In countries where "roe-on" scallops are consumed, for example, Japan, France, and Australia, high toxicity of the gonad (roe) may at times limit the safe marketing of scallops. Maximum toxicities of 2400 and 3200 $\mu\text{g STXeq g}^{-1}$ were reported in the gonad of *P. magellanicus* (Hsu et al., 1979) and *P. yessoensis* (Maruyama et al., 1983), respectively. Export of "roe-on" scallops from N. America and Japan to Europe is ongoing, and scallops are consumed whole in several countries (Shumway and Cembella, 1993). Unfortunately, no significant correlation was found between the toxicity of the gonad and that of the viscera in wild populations of sea scallops, *P. magellanicus* (Watson-Wright et al., 1989; Cembella et al., 1993). This precludes the ability to predict gonad toxicities reliably from routine PSP toxin monitoring of viscera in this and other commercially important scallop species and imposes a need for costly additional monitoring (Watson-Wright et al., 1993). Careful exclusion of the intestinal loop, which coils through the gonad and may contain toxic feces, from gonad samples of sea scallops prepared for PSP toxin analysis has demonstrated that these toxins are indeed accumulated in gonadal follicles (Cembella et al., 1993). Although little is known about the efficiency of transfer of PSP toxins into the gonad, mussels, *M. edulis*, which underwent gonadal growth during laboratory toxification spawned oocytes containing high toxicity levels, comparable on a weight-specific basis to those of the combined mantle and gill tissue fraction (Bricelj, Lee, and Cembella, unpublished results).

In most bivalves, the mantle (rims) and gills are the nonvisceral tissues that attain the highest toxicities as well as the highest relative toxin load. In *Spisula solidissima* the toxicity of the gills may at times (during early detoxification) exceed that of all other individual tissues (Shumway et al., 1994; Cembella et al., 1993). Toxicities exceeding those of the digestive gland or viscera were also found in the tissue fraction which included the heart and excretory organs (kidney and Keber's gland [= pericardial gland]) of *Mya arenaria* from the Bay of Fundy (Martin et al., 1990), and in the pericardial gland of the butter clam, *Saxidomus giganteus* (Beitler and Liston 1990). Similarly, Lassus et al., (1992) reported high toxicities in the kidney associated with "roe-on" scallops, *P. yessoensis*, imported from Japan. As mentioned earlier, *S. giganteus* is unique among bivalves in that it rapidly concentrates most of the toxin as STX (Beitler, 1992) in the siphons, especially in their distal portions (Figure 10). The mechanism of tissue-specific retention of PSP toxins in this species remains to be elucidated. Price and Lee (1971, 1972) suggested that STX was electrostatically and reversibly bound in the melanin fraction of the butter clam's pigmented siphon, but subsequent work (Beitler, 1992) does not validate this hypothesis. The muscular foot

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of other clam species typically contains a relatively low toxin load: 1% in the purple clam *Soletellina diphos* (Hwang et al., 1987) and in *S. solidissima* toxified in the laboratory (Figure 9).

The distribution of toxin among tissues, however, does not remain constant over time. During toxification of *S. giganteus* a greater proportion of the toxin body burden is initially contained in the body or visceral mass (60 to 66%, Figures 10 and 9, respectively), but complete reversal in the toxin distribution occurs within ≤ 6 weeks (i.e., 15% in body:85% in siphon-gill, Figure 10). In at least three other bivalve species in which the time course of anatomical partitioning of PSP toxins has been studied, *M. mercenaria*, *S. solidissima*, and *P. magellanicus*, the proportion of toxin contained in the viscera remained fairly constant during the toxification phase, but decreased steadily during detoxification (Figure 10). A reversal in the toxin content of the digestive gland and other tissues was also observed during detoxification of Bay of Fundy populations of *Mya arenaria* (Martin et al., 1990). This characteristic pattern is largely attributed to the fact that detoxification of the viscera, accomplished via fecal production (egestion) as well as exchange with other tissue pools, occurs at a faster rate than that of other tissues (Bricelj and Cembella, 1995). The crossover point, or time from initial detoxification when the viscera and other tissues contain equal toxin loads, varies among species and is presumably dependent on environmental conditions. These results suggest that the relative toxin content of the viscera and remaining tissues (or body and siphon in the case of butter clams) may provide a useful indicator to determine whether toxification or detoxification is occurring in natural populations (as noted by Quayle [1969]), and even estimate the timing of toxic bloom termination. In *M. edulis*, in contrast to other species, the viscera retained the bulk of the toxin body burden throughout depuration (Bricelj et al., unpublished, Figure 10), suggesting that modeling of toxin kinetics of whole bivalves, based exclusively on the toxicity of the viscera, as attempted by Silvert and Cembella (1995), may be justified for this species but inappropriate for others.

VII. DETOXIFICATION KINETICS

Bivalve species exposed to the same bloom conditions are known to vary markedly in their ability to detoxify accumulated PSP toxins, as measured by the time (t_{80}) required to detoxify below the regulatory level ($RL = 80 \mu\text{g STXeq } 100 \text{ g}^{-1}$) (Figure 6, Table 4). However, interspecific differences in this parameter (t_{80}) may reflect differences in the peak

TABLE 4
Detoxification of PSP toxins by various bivalve species (adults unless indicated), classified as relatively fast (A) and slow (B) detoxifiers, as measured by the time required to achieve the regulatory level ($RL, 80 \mu\text{g STXeq } 100 \text{ g}^{-1}$), and detoxification rate (% loss of toxin day^{-1}), calculated from an exponential decay equation: $T_t = T_0 e^{-kt}$ fitted to empirical data ($T = \text{toxicity}$; $t = \text{detoxification time}$). Detoxification determined for whole tissues and toxicity determined by mouse bioassay unless specified

Species	Peak toxicity ^d ($\mu\text{g STXeq } 100 \text{ g}^{-1}$)	Time to RL (weeks) ^b	Detox. rate (% day^{-1})	Location/detoxification conditions	Source
(A) Fast Detoxifiers:					
<i>T. truncatula</i>	2500	5.2 - 11.4 ^a	—	Thamesia Inlet BC, Canada	Quayle (1969)

y low toxin load: 1% et al., 1987) and in ver, does not remain eus a greater propor- n the body or visceral out complete reversal i.e., 15% in body:85% alve species in which PSP toxins has been allanicus, the propor- y constant during the detoxification (Figure tive gland and other Bay of Fundy popu- characteristic pattern of the viscera, accom- exchange with other er tissues (Bricelj and initial detoxification l toxin loads, varies environmental condi- content of the viscera case of butter clams) ether toxification or (as noted by Quayle oom termination. In tained the bulk of the et al., unpublished, cs of whole bivalves, attempted by Silvert ies but inappropriate

litions are known to lated PSP toxins, as v the regulatory level owever, interspecific erences in the peak

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Detoxification of PSP toxins by various bivalve species (adults unless indicated), classified as relatively fast (A) and slow (B) detoxifiers, as measured by the time required to achieve the regulatory level (RL, 80 µg STXeq 100 g⁻¹), and detoxification rate (% loss of toxin day⁻¹), calculated from an exponential decay equation: $T_t = T_0 e^{-kt}$ fitted to empirical data (T_t = toxicity; t = detoxification time). Detoxification determined for whole tissues and toxicity determined by mouse bioassay unless specified

Species	Peak toxicity ^a (µg STXeq 100 g ⁻¹)	Time to RL (weeks) ^b	Detox. rate (% day ⁻¹)	Location/detoxification conditions	Source
(A) Fast Detoxifiers:					
<i>Tresus capax</i>	3520	5.2 < t < 11.6 ^a	—	Theodosia Inlet, BC, Canada	Quayle (1969)
<i>Mercenaria mercenaria</i>	2150 ^a (GtL122)	>3.6 (3.4)	9.5	Laboratory, 17°C, fed juveniles	Bricelj et al. (1991)
	10543 ^b (GtCA29)	>2.6 (6.1)	>0.8 d = 9.3 ^c	Kumbie estuary, India	Karunasagar et al. (1984)
	3787	4.4	—	Maine, USA (fall)	Hurst and Gilfillan (1977)
	110–1425	1.0 – 4.0	9.8	Gulf St. Lawrence, floats, 4 – 17°C	Larocque and Cembella (1991)
<i>Meretrix casta</i>	200–1200	7.1 – 5.0	—		
<i>Mytilus edulis</i>	~ 270–470	3.3 – 4.0	—	Bay of Fundy, Canada	Prakash et al. (1971)
	864	<4.0	—	Theodosia Inlet, BC, Canada	Quayle (1969)
	~ 840	4.7	7.7	Franquelin, St. Lawrence estuary, Canada	Cembella et al. (1988)
	2720 (MOG835)	7.9	5.9	Laboratory, 16°C, fed	Lassus et al. (1989)
	2371 (MOG835)	>2.9 (3.1)	14.2	Laboratory, 16°C, fed	Lassus et al. (1993)
	17490	12.5 < t < 15.6 ^a	—	Nordstraumen, Norway	Alvik and Franstad (1981)
	19259 ^a (GtCA29)	>12.6 (7.6)	9.7	Transferred to toxin-free area	"
		> 5.7 (7.7)	8.9	Laboratory, 17°C	Bricelj, Lee and Cembella, unpublished
	137–1039	1.0 – 4.8	$\bar{X}$ = 15.4	Maine, USA (spring)	Hurst and Gilfillan (1977)
	1575–11180	2.7 – 6.8	$\bar{X}$ = 15.4	Maine, USA (fall)	
	1407–3857	2.9 – 6.0	$\bar{X}$ = 4.8	Spurwink River, Maine, USA	ME Dept. Mar. Res., 1979 to 1993 records
	100–798	0.6 – 4.8	$\bar{X}$ = 5.8		
	1367–9075	2.1 – 9.6	$\bar{X}$ = 12.1	Lumbos Hole, Maine, USA	Price et al. (1991)
	4100	3.0	—	Santa Barbara Channel, California, USA	
	~1100	6.1	—	Bay of Fundy, Canada	Prakash et al. (1971)
	23000	5.1	13.8	10 – 11°C	
	~2000	2.1	—	Gaspé Bay, Canada	Desbiens and Cembella (1993)
				Outdoor tanks, no toxic cells	Oshima et al. (1982)

TABLE 4 (continued)

Detoxification of PSP toxins by various bivalve species (adults unless indicated), classified as relatively fast (A) and slow (B) detoxifiers, as measured by the time required to achieve the regulatory level (RL, 80 $\mu\text{g STXeq } 100 \text{ g}^{-1}$), and detoxification rate (% loss of toxin day^{-1}), calculated from an exponential decay equation: $T_t = T_0 e^{-\lambda t}$ fitted to empirical data (T = toxicity; t = detoxification time). Detoxification determined for whole tissues and toxicity determined by mouse bioassay unless specified

Species	Peak toxicity ^d ($\mu\text{g STXeq } 100 \text{ g}^{-1}$)	Time to RL (weeks) ^b	Detox. rate (% day^{-1})	Location/detoxification conditions	Source
<i>Mytilus californianus</i>	2100–5300 900–1900 240–790 1100–1456 685–768 353–444	6.8–7.5 2.8–6.4 3.0–9.0 3.1–3.4 3.2–8.9 3.8–3.9	$\bar{X} = 8.9$ $\bar{X} = 8.1$ $\bar{X} = 5.3$ $\bar{X} = 8.1$ $\bar{X} = 6.6$ $\bar{X} = 5.1$	BC, Canada Sequim Bay, WA, USA, floats, 13–16°C Clallam Bay, WA, USA, floats, 9–11°C Crescent Bay/Agate Beach, USA, floats, 11–13°C Oaxaca, Mexico	DFO 1992 records Sribhithadh (1963) Cortés-Altamirano et al. (1993)
<i>Choromytilus</i> <i>pallidopunctatus</i> <i>Perna viridis</i> <i>Modiolus modiolus</i> <i>Crassostrea gigas</i>	540 ~240* — 209–379 710 ~620–810 1336* 1000 2700	1.2 < t < 7.0* (1.4) 1.7 0.9–9.2 0.6–1.5 2.0 1.8–3.8 6.9 6.4 >6.3 (6.4)	17.2 9.3 $\bar{X} = 7.0$ — — 8.9–18.1 5.5 4.0 7.4	Laboratory, fed, 26–28°C Maine, USA Juan de Fuca St., WA, USA floats, 12–15°C San Mateo Bay, BC, Canada Oaxaca, Mexico Kumbale estuary, India Harpwell, Maine, USA Laboratory, 16°C, fed	Gacutan et al. (1989) Hurst and Giffillan (1977) Sribhithadh (1963) DFO 1987 Cortés-Altamirano et al. (1993) Karnasagar et al. (1984) Shumway et al. (1990) Lassus et al. (1989)
(B) Slow Detoxifiers: <i>Saxidomus giganteus</i>	8640 3174 278 14000 2800 (Siphon) 620 (Siphon) 30429* (PR18B) 16810* (AL1V)	>90* (159) 114* (111) >14 >73 (85) >42 (118) 39 (40) > 8 (24) > 9* (13)	< 83 d = 1.2° > 83 d = 0.3 > 47 d = 0.6° — 0.9 0.4 0.8 3.4 < 6 d = 24.3° > 6 d = 4.2	Theodosia Inlet, BC, Canada Little River, BC, Canada Laboratory, 7.5–16.5°C, fed Bodega Harbor, CA, USA Laboratory, fed, juveniles 16–17°C Laboratory, fed, juveniles 16°C	Quayle (1969) Madenwald (1985) Price et al. (1991) Bricelj, Laby and Cembella, unpublished Bricelj and Cembella (1995) and unpublished
<i>Spisula</i> <i>solidissima</i>	4514 1752 6000 3900 4010–4510 1705 1140 (Viscera) 1624 (Mantle)	100* (81) 96 (59) > 100 (48) (132)* >69* (73) > 70 (51) > 26 > 13	< 32d = 1.9° > 32 d = 0.5 0.8 1.3 < 42d = 1.2° > 42 d = 0.4 < 30d = 1.6° > 30 d = 0.6 1.1 — —	Head Beach, ME, USA ME Dept. Mar. Res. Scarborough Beach, ME, USA Georges Bank, USA, Station 3 Georges Bank, USA, Station 1 Georges Bank, USA, St. 2 & 4 Laboratory, 15°C, 20 μm -filtered ambient water Laboratory, fed	Shumway et al. (1988) and Shumway et al. (1994) Bogoslawski and Stewart (1978)

<i>Pecten maximus</i>	2700	>6.3 (6.4)	7.4	Laboratory, 16°C, fed	Lassus et al. (1989)
(B) Slow Detoxifiers:					
<i>Saxidomus giganteus</i>	8640	>90° (159)	< 83 d = 1.2° > 83 d = 0.3 > 47 d = 0.6°	Theodosia Inlet, BC, Canada	Quayle (1969)
	3174	114° (111)	—	Little River, BC, Canada	Madenwald (1985)
	278	>14	0.9	Laboratory, 7.5–16.5°C, fed	Price et al. (1991)
<i>Saxidomus nuttalli</i>	14000	>73 (85)	0.4	Laboratory, fed, juveniles	Briceij, Laby and Cembella, unpublished
	2800 (Siphon)	>42 (118)	0.8	16–17°C	Briceij and Cembella (1995) and unpublished
	620 (Siphon)	39 (40)	3.4	Laboratory, fed, juveniles	
<i>Spisula solidissima</i>	30429* (PR18B)	> 8 (24)	< 6 d = 24.3° > 6 d = 4.2	16°C	
	16810* (AL1V)	> 9° (13)			
	4514	100° (81)	< 32d = 1.9° > 32 d = 0.5	Head Beach, ME, USA	Shumway et al. (1988) and
	1752	96 (59)	0.8	ME Dept. Mar. Res.	
	6000	> 100 (48)	1.3	Scarborough Beach, ME, USA	
		(132)*	< 42d = 1.2° > 42 d = 0.4 < 30d = 1.6° > 30 d = 0.6	Georges Bank, USA, Station 3	Shumway et al. (1994)
	3900	>69° (73)	1.1	Georges Bank, USA, Station 1	
	4010–4510	> 70 (51)	—	Georges Bank, USA, St. 2 & 4	
	1705	> 26	—	Laboratory, 15°C, 20 µm-filtered ambient water	
<i>Cardium edule</i>	1140 (Viscera)	> 13	—	Laboratory, fed	Blogoslawski and Stewart (1978)
	1624 (Mantle)	11.2	3.3	Lagune d'Óbidos, Portugal	De Sousa and Silva (1963)
	3100*		<14 d = 3.6° >14 d = 1.2	11–15°C	
<i>Soletellina diplos</i>	40000 (Dig.)*	> 51° (31)	0.6	Culture pond, Tungkan, Taiwan	Hwang et al. (1990)
	~1674 (Dig.+Mantle+Gill)	> 28 (78)	0.2	Laboratory, starved	Shumway et al. (1988)
<i>Placopecten magellanicus</i>	809 (–Adductors)	> 52 (135)	0.6	Laboratory, –0.2 to +14°C	Waiwood et al. (1995)
	6179 (Dig.)	> 52 (104)	—	Mascarene, Bay of Fundy, Canada	Bourne (1965)
	2720 (Dig.)	> 17	—	Whitehouse, Bay of Fundy	
	1440 (Mantle)	> 8.7	—	Canada	
	4000 (Dig.)	> 8.7	—	Canada	
<i>Patinopecten yessoensis</i>	1248	> 30 (60)	1.2	Funka Bay, Japan	Nishihama (1980) (Figure 4)
	15000 (Dig.)*	> 16.7 (31)	2.2	Funka Bay, Japan, 10 m	Nishihama (1980) (Figure 10)
	~11000 (Dig.)*	>12 (10)	2.5	Funka Bay, Japan, 25 m	
	~11100 (Dig.)*	~24 (38)	1.6	Funka Bay, Japan, 1981	
	6380 (Dig.)*	23 (16)	4.1	Funka Bay, Japan, 1986	
	7920 (Dig.)*	> 27 (22)	3.1	Funka Bay, Japan, 1984	
	10880 (Dig.)*	> 19 (15)	3.8	Ofunato Bay, Japan	Ogata et al. (1982)
	6000 (Dig.)*	> 17	—	Ofunato Bay, Japan	
	14500 (Dig.)*	> 21° (20)	< 8 d = 11.7° > 8 d = 1.3	Outdoor tanks, no toxic cells	Oshima et al. (1982)
	340000 (Dig.)*				

^a Toxicity determined by HPLC.
^b obtained by linear interpolation. From empirical data; value in brackets calculated from a fitted exponential decay equation.
^c Calculated assuming a biphasic detoxification pattern, with initial linear toxin loss and subsequent exponential loss.
^d Where a range is reported, values correspond to different locations or years; the dinoflagellate isolate used in laboratory toxication studies is indicated in brackets.
^e Calculated using a conversion factor of 0.2 µg STXeq MU⁻¹.
^f Sampling interval too large to allow interpolation.

toxicity achieved, rather than intrinsic, species-specific differences in the rate of toxin elimination (% toxin loss day⁻¹). Thus, Hurst and Gilfillan (1977) found a significant positive correlation between log peak toxicity and the time required to attain the RL (t_{80}) in *M. edulis*, *Mya arenaria*, and the horse mussel *Modiolus modiolus* from coastal waters in Maine. Therefore, peak toxicity values are reported with their corresponding t_{80} in Table 4, which attempts a broad classification of bivalves in terms of their detoxification kinetics. The toxin loss rate was calculated by fitting a negative exponential function to toxicity data (nonlinear curve fitting) derived from selected laboratory or field depuration studies. Only detoxification series consisting of ≥ 5 data points were included for this calculation. Field data were excluded where there was evidence of retoxification, based on either the presence of putatively toxic cells in the plankton, or of secondary peaks in shellfish toxicity. Laboratory studies in which detoxification occurred in a controlled, toxin-free environment, and field studies in which bivalves were transplanted to certifiably toxin-free waters (e.g., Aalvik and Framstad, 1981) obviously provide the most reliable information on detoxification rates. Tabulated toxicity data were used where possible. Alternatively, where only plotted data were available, mean toxicity and time values were generated by digitizing (using a FORTRAN program, Digitize01, and a Summagraphics Microgrid II digitizing table).

As illustrated in Table 4, bivalve species fall into two general categories in terms of their detoxification capacity. Rapid to moderate detoxifiers such as the blue mussel, *M. edulis*, for which an extensive database is available, take only a few weeks (1 to 10 weeks) to reach the RL and average a toxin elimination rate of 10.6% day⁻¹. Slow detoxifiers, most notably *Saxidomus giganteus* and *Spisula solidissima*, typically take several months to years to detoxify below the RL, and exhibit average detoxification rates of 0.7% in *S. giganteus* and *S. nuttalli*, 0.5% in *Placopecten magellanicus* and 1 to 4% in *Patinopecten yessoensis*. Preliminary experiments by Lassus et al. (1993) suggested that the rate of toxin loss in *M. edulis* was positively correlated with peak or initial toxicity. However, although *M. californianus* from British Columbia show a similar trend, that is, highest detoxification rates are associated with the most toxic mussels, this relationship is not apparent for *M. edulis* from Maine waters (Table 4).

In most cases, detoxification patterns could be adequately fit by a single-compartment, negative exponential model. The suitability of this model was assessed from the coefficient of determination and visual inspection of fitted curves superimposed on empirical data to determine whether the model adequately simulated two critical points, the maxi-

mum toxicity and the management context. Underestimated the time required to reach the RL in *S. solidissima*. A better fit of the t_{80} was provided by a model consisting of an initial rapid (exponential) phase followed by a slower (exponential) phase. For *S. solidissima* from Georgia, the initial detoxification rate was rapid, whereas the second phase, referred to these as late phase, provided a better fit to toxicity data from an estuary using a two-compartment model. Toxicities simulated by rapid detoxification followed the *Alexandrium* clone AL1V (Brice et al., 1995) pattern was described by a single compartment model, other, non-visceral tissues.

In conclusion, it may be more appropriate to use a single compartment model. However, the underlying detoxification of PSP (paralytic shellfish poisoning) (defecation), (detected) compounds, and direct evidence exists of elimination. Albeit gut known to contribute to the viscera (Bricelj et al., 1995) contribution remains to be determined.

Patterns of toxin uptake are often asymmetrical (Skjott et al., 1988; Figures 3 and 4). Rates of detoxification are several studies have shown to be at a faster rate than other studies (et al., 1995; Bricelj and Shumway, 1995) rank order of various tissues was as follows: viscera, muscle (Bricelj and

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two general catego-
moderate detoxifiers
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S. nuttalli, 0.5% in
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mum toxicity and the t_{80} , given the importance of this value in a management context. However, this simple model often markedly underestimated the time required to reach the RL, especially in *S. giganteus* and *S. solidissima*. A better fit to the data, and more accurate prediction of the t_{80} , was provided in some cases by a biphasic detoxification model, consisting of an initial more rapid detoxification phase and subsequent slower (exponential) phase of toxin elimination (illustrated in Table 4 for *S. solidissima* from Georges Bank, Station 3). It has been suggested that the initial detoxification phase represents gut evacuation of unassimilated toxin, whereas the second phase represents the release of toxins assimilated and incorporated in tissues (Lee, 1993). Silvert and Cembella (1995) referred to these as labile and bound toxin compartments and obtained a better fit to toxicity data for the viscera of *M. edulis* from the St. Lawrence estuary using a two-compartment model. In agreement with our findings, toxicities simulated by a one-compartment model tended to fall off more rapidly during detoxification than the empirical data. In *M. mercenaria* fed the *Alexandrium* clone GtCA29 (Bricelj et al., 1990) and *S. solidissima* fed clone ALIV (Bricelj and Cembella, 1995), a biphasic detoxification pattern was described for the viscera (and whole tissues), but not for other, non-visceral tissues.

In conclusion, it appears that a biphasic, two-compartment model may be more appropriate to model bivalve detoxification kinetics than a single compartment model in at least some bivalve populations. However, the underlying mechanisms operating in bivalves for the detoxification of PSP toxins are poorly understood. They may involve egestion (defecation), excretion, degradation to nontoxic (hence undetected) compounds, and biotransformation among individual toxins, but direct evidence exists only for the first and last of these pathways of toxin elimination. Albeit gut evacuation of intact and fragmented toxic cells is known to contribute partly to the initial, more rapid detoxification phase in the viscera (Bricelj and Cembella, 1995), the magnitude of this contribution remains to be quantified.

Patterns of toxin uptake and loss observed in natural populations are often asymmetrical (skewed to the right) (e.g., Nishihama, 1980; Tazawa et al., 1988; Figures 3 and 9 in Shumway et al., 1988), suggesting that the rates of toxification are frequently greater than those of toxin elimination. Several studies have shown that the digestive gland (viscera) detoxifies at a faster rate than other body tissues (e.g., Bricelj et al., 1991; Waiwood et al., 1995; Bricelj and Cembella, 1995). In surfclams, *S. solidissima*, the rank order of various tissue pools in terms of their detoxification rates was as follows: viscera >> gill > mantle $\approx$ siphon $\approx$ foot > adductor muscle (Bricelj and Cembella 1995). Detoxification rates are also

expected to vary with age/body size, especially in species, such as *S. solidissima*, characterized by high growth rates and a low intrinsic rate of detoxification relative to other bivalve species. Rapid growth of younger/smaller animals during long-term detoxification will tend to "dilute" the residual toxin load, and thereby effectively accelerate detoxification. Thus, toxin dilution ascribed to growth of juvenile surfclams was estimated to account for 54% of the toxin loss over 2 months of depuration (Bricelj and Cembella, 1995). Allometric (differential) growth of individual tissues that vary in their capacity for toxin retention may further complicate prediction of whole-body detoxification rates in these species. It is noteworthy that shorter t_{80s} (13 to 24 weeks) were calculated for juvenile *S. solidissima* in laboratory studies than for field-collected adults of this species (51 to 132 weeks) (Table 4), albeit these differences may be partly attributable to differences in water temperature and toxin composition of ingested dinoflagellates among these studies.

Although detoxification rates of PSP toxins are expected to decrease concomitantly with other physiological rates, with a reduction in ambient temperature, surprisingly little is known about the effect of temperature on toxin elimination in bivalves. Madenwald (1985) found no significant effect of temperature on detoxification of *S. giganteus* over the range 7.5 to 16.5°C, but this species is unique in its ability to sequester PSP toxins and did not detoxify at either temperature over a 14-week depuration period. Preliminary data by Prakash et al. (1971) suggested that *M. edulis* detoxified faster at 21 than 12°C in the laboratory, but results of this study were inconclusive. Additionally, animals that are fed nontoxic algae during the detoxification phase may be able to detoxify faster than those held in filtered, particle-free seawater, because active feeding is likely to accelerate gut evacuation rates and overall metabolism (degradation, excretion) of toxins. However, biotransformation of individual PSP toxins from less potent to more potent derivatives (see following section) may result in an apparent reduction in the overall rate of detoxification.

No effective method has as yet been developed to accelerate the detoxification of live bivalves contaminated with PSP toxins. Ozonation, commonly used to depurate bacterially contaminated shellfish, can inactivate PSP toxins from crude extracts of dinoflagellates or shellfish (Thurberg, 1975). Detoxification of PSP-contaminated bivalves (e.g., *M. arenaria* and *S. solidissima*) in flowing seawater supplied with ozone gas was promoted by Blogoslawski and Stewart (1978) and Blogoslawski et al. (1979) as a means of accelerating the rate of toxin loss, but the ineffectiveness of this method in detoxifying bivalves was subsequently demonstrated by White et al. (1985). The main limitation

in the *in vivo* use of ozone can inhibit the bivalve contact with toxins in

VIII. TOXIN BIOTRANSFORMATION

It is well known that detoxification rates differ significantly from those reported in field studies (Bricelj et al., 1995) and in controlled laboratory studies (Bricelj et al., 1990, 1993) in their capacity for detoxification. For example, among species with different toxin profiles are *Protothaca staminea* (Figures 11 and 12) and *Ostrea edulis* (Martin et al., 1990; Bricelj et al., 1981; Oshima et al., 1981) and *Mytilus edulis* (Bricelj et al., 1990). A limited capacity for detoxification of the dinoflagellate isolates that undergo extensive biotransformation and timing and duration of changes is important, but also because biotransformation of toxicity in bivalve tissues.

Changes in the selective retention or elimination of toxins from a variety of biotransformation in the presence of natural biotransformation (Figure 12) (Bricelj et al., 1993, 1994; Oshima et al., 1981) and biotransformation by the appearance of dinoflagellate cells using toxins with a hydrophobic epimerization through 13, which is accelerated (GTX_{3,4} and C_{2,4}) are the late cells, and the presence of C_{1,3} increases gradually until it reaches

ly in species, such as and a low intrinsic rate of clearance. Rapid growth of bivalves will tend to actively accelerate detoxification rates in these weeks) were calculated than for field-collected bivalves, albeit these differences in temperature and toxin retention may be expected to decrease with a reduction in ambient temperature (Oshima 1995) found no significant difference in *Saxidomus giganteus* over the range of temperatures. The ability to sequester PSP toxins in the laboratory, but in the field, animals that are fed may be able to detoxify faster, because active metabolism and overall metabolic rate, biotransformation of potent derivatives (see Table 1) in the overall rate

needed to accelerate the PSP toxins. Ozonation, of contaminated shellfish, can destroy dinoflagellates or shellfish contaminated bivalves in seawater supplied with oxygen (Stewart 1978) and increasing the rate of toxin detoxifying bivalves was suggested. The main limitation

in the *in vivo* use of strong oxidants or other chemical agents is that they can inhibit the bivalves' pumping rates and may not come into direct contact with toxins incorporated in tissues.

VIII. TOXIN BIOTRANSFORMATIONS

It is well known that the toxin composition in bivalve tissues can differ significantly from that of the toxic dinoflagellates ingested, as demonstrated in field studies (e.g., Oshima et al., 1976, 1990; Asakawa et al., 1995) and in controlled laboratory experiments (Beitler and Liston, 1990; Bricelj et al., 1990, 1991, 1996). Bivalve species, however, differ markedly in their capacity for transformation of PSP toxins (Figure 11). For example, among species occurring in North America, changes in the toxin profile are pronounced in *Saxidomus giganteus* (Beitler 1992), *Protothaca staminea* (Sullivan et al., 1983) and *Spisula solidissima* (Figures 11 and 12) and occur to a lesser degree in *Mya arenaria* (Figure 11, Martin et al., 1990; Bricelj et al., 1996), *Crassostrea gigas* (Onoue et al., 1981; Oshima et al., 1987), *Mercenaria mercenaria* (Bricelj et al., 1991), and *Mytilus edulis* (Bricelj et al., 1990; Chebib et al., 1993). Species with a limited capacity for toxin biotransformation are best suited to identify the dinoflagellate isolate acting as toxin vector in the field, whereas those that undergo extensive toxin metabolism can be useful to predict the timing and duration of blooms. Understanding toxin compositional changes is important, not only as a means of predicting bloom dynamics, but also because biotransformations are a major determinant of net toxicity in bivalve tissues.

Changes in the toxin profile of shellfish tissues may arise from selective retention or elimination of individual toxins, epimerization, or from a variety of biotransformation processes: reductive conversion in the presence of natural reductants, hydrolysis at low pH, or enzymatic conversion (Figure 13, also see reviews on this subject by Cembella et al., 1993, 1994; Oshima, 1995b). Strong evidence supporting the metabolic interconversion of toxins rather than selective kinetics is provided by the appearance of toxins in bivalve tissues that were not detected in dinoflagellate cells using sensitive analytical methods. In all bivalves, PSP toxins with a hydroxysulfate group at the C11 position undergo epimerization through thermodynamic equilibration (reaction 1 in Figure 13, which is accelerated at high pH and temperature). The β -epimers (GTX_{3,4} and C_{2,4}) are the favored configuration synthesized in dinoflagellate cells, and the proportion of the more stable α -epimers (GTX_{1,2} and C_{1,3}) increases gradually in bivalve tissues following ingestion of toxic cells, until it reaches a β : α ratio of ca. 1:3 (Oshima, 1995b). Thus, the

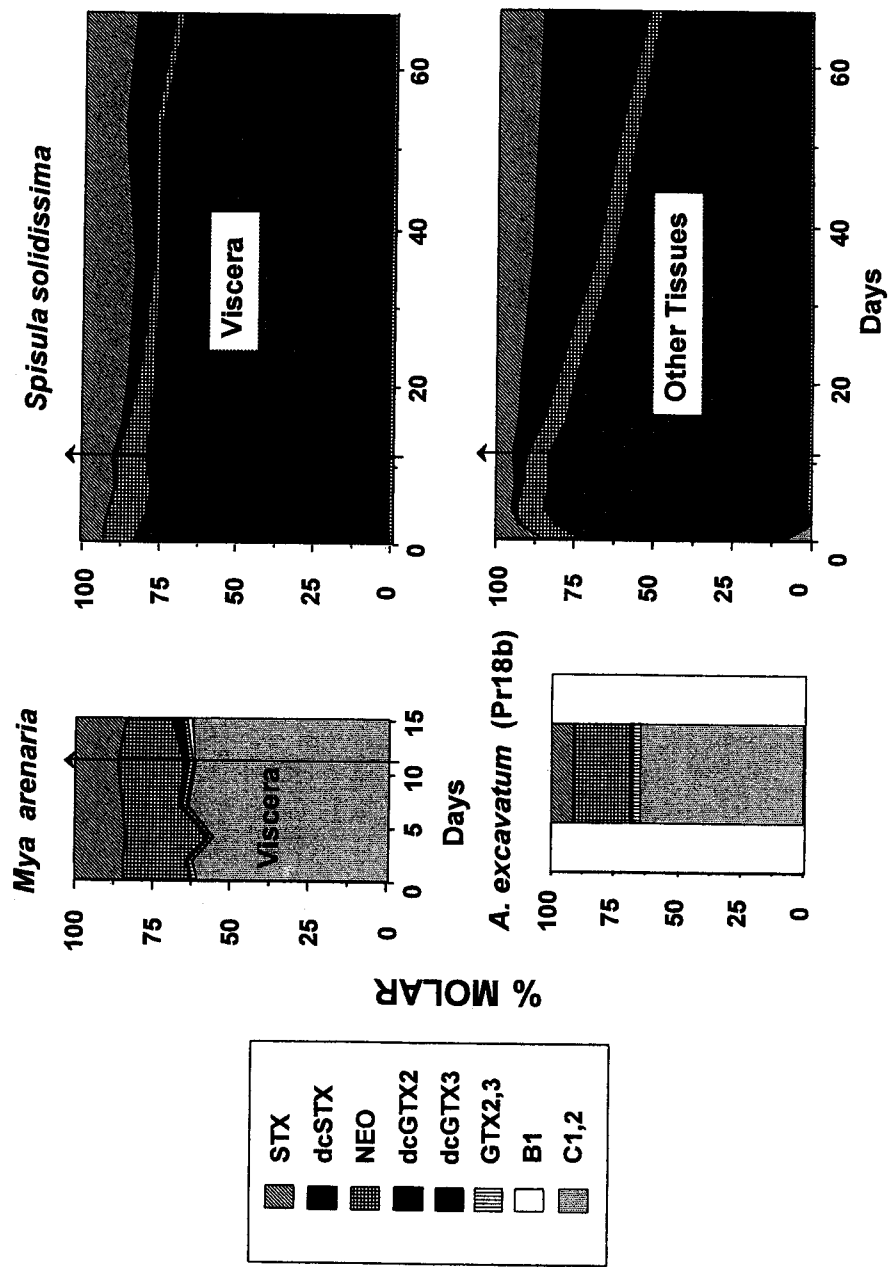
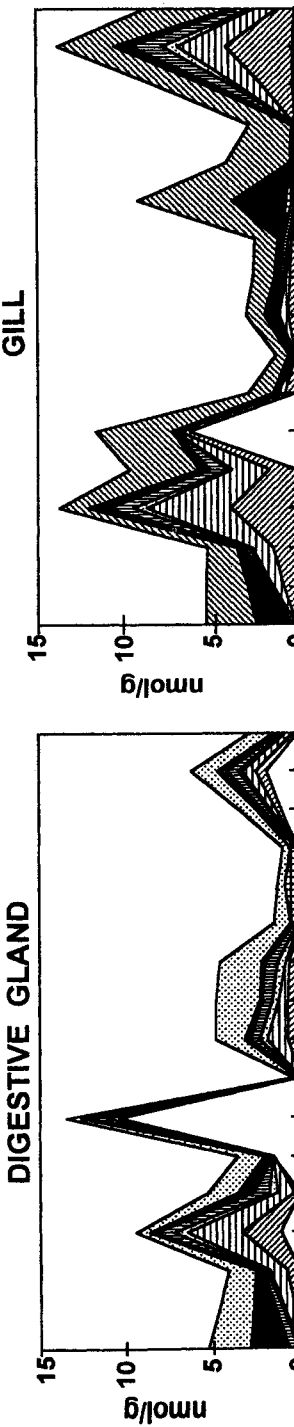


FIGURE 11. Composition (% molar) of PSP toxins in viscera and other tissues of surfclams, and in viscera of softshell clams during experimental toxification with *Alexandrium tamarense* cf. *excavatum*, strain PR18b (toxin composition of dinoflagellate cells is shown for comparison), and subsequent depuration on a nontoxic algal diet (Bricelj, Laby, and Cembella, unpublished). Arrows mark the beginning of depuration; PSP toxins as in Figure 3.



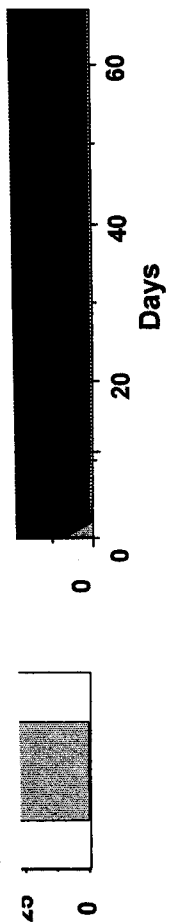


FIGURE 11. Composition (% molar) of PSP toxins in viscera and other tissues of surfclams, and in viscera of softshell clams during experimental toxification with *Alexandrium tamarense* cf. *excavatum*, strain PR18b (toxin composition of dinoflagellate cells is shown for comparison), and subsequent depuration on a nontoxic algal diet (Bricelj, Laby, and Cembella, unpublished). Arrows mark the beginning of depuration; PSP toxins as in Figure 3.

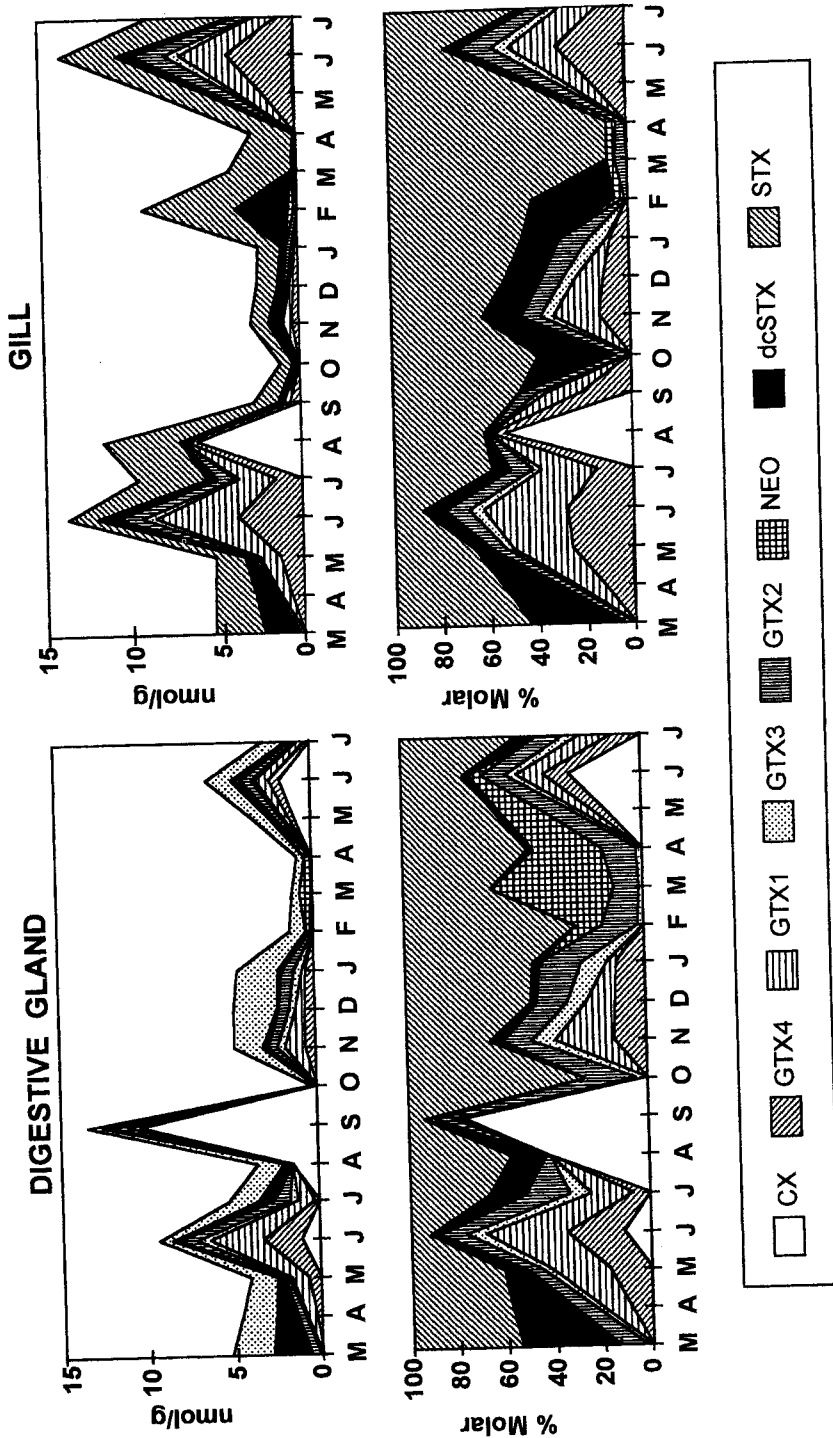


FIGURE 12. Seasonal variation in the composition of PSP toxins in two representative tissues of adult surfclams, *Spisula solidissima*, from inshore populations in the Gulf of Maine, U.S.A.. Upper graphs: Absolute toxin concentrations in nmol g^{-1} (Cembella and Shumway, unpublished). Lower graphs: relative toxin concentration (% molar). Toxins as in Figure 3. (Redrawn from Cembella and Shumway, 1995.)

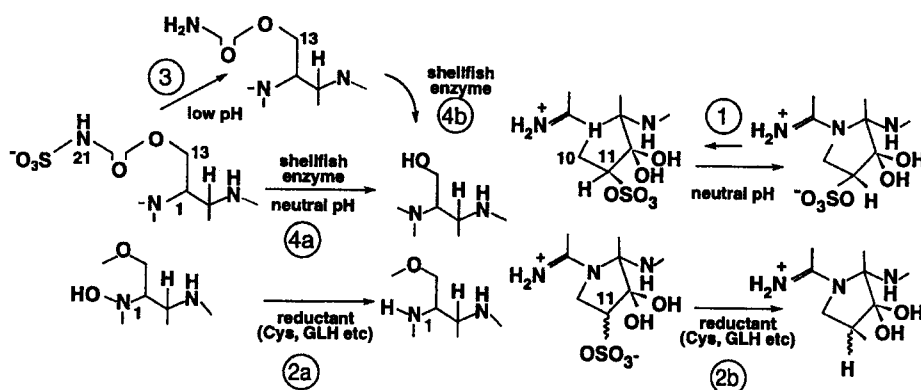


FIGURE 13. Schematic of *in vivo* transformations of PSP toxins occurring in marine bivalves (modified from Oshima 1995; see Figure 3 for structure and designations of individual toxins). 1. Epimerization from β to α epimers; 2. Reduction: (a) from GTX_{1,4} and NEO to GTX_{2,3} and STX, (b) from GTX_{1,2,3,4} to STX and NEO, in the presence of sulfhydryl reductants such as cysteine and glutathione; 3. Acidic hydrolysis; 4. Enzymatic hydrolysis (decarbamoylation): conversion from *N*-sulfocarbamoyl (4a) or carbamate (4b) toxins to decarbamoyl derivatives.

epimer ratio provides a potential index of retention time of toxins in bivalves since their initial accumulation from suspension. Epimerization occurs over a relatively short time scale, typically during toxin uptake and early detoxification (Bricelj et al., 1991; Cembella et al., 1994), such that equilibrium levels in the $\beta:\alpha$ ratio are achieved within about 2 to 5 weeks from the start of toxification.

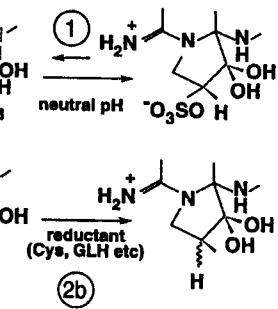
Conversions among carbamate toxins occur in many bivalve species, probably caused by natural reductants (Oshima, 1995), via reduction of the N1 hydroxyl, or reductive cleavage of C11-hydroxysulfate (Figures 13, 2a, and b, respectively). Both transformations were reported in homogenates of the scallops *Placopecten magellanicus* (Shimizu and Yoshioka 1981) and *Patinopecten yessoensis* (Oshima, 1995b), and conversion of GTX₂₊₃ to STX by bacteria isolated from *M. edulis* was reported by Kotaki (1989). These conversions tend to occur *in vivo* over slower time scales than epimerization. However, no STX or NEO were detected in juvenile *Spisula solidissima* fed an *Alexandrium* strain that produces only gonyautoxins, suggesting that this species may lack the capacity for reductive desulfation at C11.

The greatest differences between bivalve and dinoflagellate toxin profiles occur when the algae ingested are rich in *N*-sulfo-carbamoyl toxins, because these are more labile than other toxins. High proportions of C toxins (> 60 to 90% molar) are characteristic of

Gymnodinium catenatum (1993), *Alexandrium* (1990), *A. tamarense* (1994), several North America (Cembella et al., 1994). *N*-sulfocarbamoyl toxin (molar basis) in *Pyrodinium* does not produce 11- β -hydroxy (1989). The dominant isolates is of considerable toxins are readily converted to derivatives in shellfish (1994). Shift in the dominance of toxins may explain (e.g., *Placodictyon magnum* seasons (Cembella et al., 1994). Detoxification rates (1994).

Bivalves typically produce toxins and gradual encephalopathy (e.g., Asai, 1990). *N*-sulfocarbamoyl:carbamate is a precursor of new toxin and surfclams from the Guadalupe conversion of *N*-sulfocarbamoyl:carbamate toxin (Figure 13, reaction 1) at high temperature (e.g., Asai, 1990). Therefore, characterization based on HPLC analysis of the method must be interpreted with review. The precise structure of the sulfocarbamoyl and carbamate is not determined.

Enzymatic conversion (Figure 13, 4) is uncommon only in a few Pacific clams (1983), and two Japanese clams out of 20 bivalve species may occur via enzymatic conversion of carbamate toxins (Figure 13, 5) occurs in *P. venulosa*, clams and *M. chinensis*.



toxins occurring in marine
culture and designations of
action: (a) from GTX_{1,4} and
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is; 4. Enzymatic hydrolysis
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Gymnodinium catenatum from Tasmania and Japan (Oshima et al., 1993), *Alexandrium catenella* from Funka Bay, Japan (Oshima et al., 1990), *A. tamarense* from Long Island, U.S.A. (Bricelj et al., 1991; Anderson et al., 1994), several *Alexandrium* strains from the west coast of North America (Cembella et al., 1987), as well as isolates from the St. Lawrence region (Cembella and Destombe, 1996). The N-sulfocarbamoyl toxin B₁ (= GTX₂) is the principal component (on a molar basis) in *Pyrodinium bahamense* var. *compressa*, a species that does not produce 11-hydroxysulfate toxins (GTXs and C₁₋₄) (Oshima, 1989). The dominance of less potent N-sulfocarbamoyl toxins in some isolates is of considerable public health significance, because these toxins are readily converted to more potent and thus more hazardous derivatives in shellfish, leading to an increase in net toxicity. A seasonal shift in the dominance from N-sulfocarbamoyl to carbamate or decarbamoyl toxins may explain the high toxicity retained in some bivalves (e.g., *Placopecten magellanicus* and *Spisula solidissima*) during off-bloom seasons (Cembella et al., 1993, 1994) and partially account for their slow detoxification rates (Table 4).

Bivalves typically show reduced proportions of N-sulfocarbamoyl toxins and gradual enrichment in carbamate toxins relative to dinoflagellate cells (e.g., Asakawa et al., 1995). Thus, an increase in the N-sulfocarbamoyl:carbamate toxin ratio was suggested as a useful indicator of new toxin accumulation and thus toxic bloom occurrence in surfclams from the Gulf of Maine (Cembella et al., 1993). Nonenzymatic conversion of N-sulfocarbamoyl toxins to their corresponding carbamate toxin (Figure 13, reaction 3) is known to readily occur under conditions of high temperature and low pH (Hall and Reichardt, 1984; Hall et al., 1990). Therefore, changes in toxin profiles reported in early studies based on HPLC analysis of toxins extracted by the hot acid AOAC method must be interpreted with caution and are not included in this review. The precise *in vivo* mechanism of conversion between N-sulfocarbamoyl and carbamate toxins in bivalves has not been determined.

Enzymatic conversion of PSP toxins to decarbamoyl (dc) derivatives (Figure 13, 4) is uncommon among bivalves and has been demonstrated only in a few Pacific clam species, *Protothaca staminea* (Sullivan et al., 1983), and two Japanese clams, *Mactra chinensis* and *Peronidia venulosa*, out of 20 bivalve species tested (Oshima, 1995b). Production of dc toxins may occur via enzymatic hydrolysis of N-sulfocarbamoyl toxins and/or carbamate toxins (Figures 13, 4a and b, respectively). Only the latter occurs in *P. venulosa*, whereas both pathways were reported in littleneck clams and *M. chinensis*. However, *in vitro* incubation of purified toxins

with crude homogenates from uncontaminated bivalves has shown that decarbamoylation is highly substrate specific, that is, it occurs at a much faster rate from *N*-sulfocarbamoyl than carbamate toxins, and is faster for β - than α -epimers (Buzy et al., 1994; Oshima, 1995b). Sullivan et al. (1983) found that extracts from nonvisceral tissues of *P. staminea* yielded limited amounts of dc toxins, suggesting that carbamoylase activity may be largely restricted to bivalve viscera. In agreement with these results, Noguchi et al. (1989) found that conversion of *N*-sulfocarbamoyl toxins to dcSTX was only observed in visceral homogenates of the hiogi scallop *Chlamys nobilis*.

Decarbamoyl derivatives are also a major toxin component in the Atlantic surfclam *Spisula solidissima*, both in natural populations (Figure 12; Cembella et al., 1993) and in laboratory-toxified surfclams (Figure 11). Rapid *in vivo* production of dcGTX₂₊₃ from C₂, with complete disappearance of C toxins within a few hours of exposure to *Alexandrium* isolates Gt429 and PR18b (Bricelj et al., 1996), and somewhat slower production of dcGTX₂₊₃ from gonyautoxins present in *A. minutum* (clone ALIV) (Bricelj and Cembella, 1995) were described in *S. solidissima*. However, *in vitro* experiments confirming that these conversions are enzyme mediated have not been conducted for this species. It is noteworthy that dcSTX was the only decarbamoyl product found in surfclams toxified by an unknown source in the field (Figure 12). The absence of dcGTXs, the dominant component in laboratory-toxified *Spisula*, remains to be explained. Small or trace amounts of decarbamoyl toxins reported in a number of other bivalves (*Patinopecten yessoensis*, Oshima et al., 1990; *P. magellanicus*, Cembella et al., 1994; *Perna viridis*, Oshima, 1989; *M. edulis*, Rodriguez-Vazquez, et al., 1989) most likely result from bioaccumulation of algal toxins rather than enzymatic conversion, because dc derivatives have been detected as a minor constituent in several temperate and tropical dinoflagellate isolates (Harada et al., 1982; Oshima et al., 1990, 1993).

Different bivalve tissues are also known to vary greatly in their toxin composition. The toxin profile of the viscera or digestive gland often differs markedly from that of other tissues (Cembella and Shumway, 1995; Cembella et al., 1994), and typically shows the closest resemblance to that of dinoflagellate cells, especially during toxin uptake. This is not surprising because intact dinoflagellate cells in gut contents contribute to the total toxicity of the stomach-digestive gland-intestinal complex, and this is the first organ to accumulate toxins following mechanical, pH- and enzyme-mediated digestion of toxic cells. However, marked differences in toxin profile among non-visceral tissues have also been documented

IX. CONCLUSION AND DIRECTIONS

As illustrated in this review (toxin uptake and elimination and capacity for biotransformation of bivalve species), the ability to accumulate toxins is a biological (feeding) and physiological response, although with many whole-organism responses. The gaps in our knowledge of the potential for biotransformation of suitable species for various regions, and in the field of other species of interest, are highlighted.

Our detailed understanding of the restricted to a few species that are relatively resistant to toxins in *S. solidissima*. This information is needed for species until the understanding of responses observed at different levels of differentiation in the field of *in vivo* mechanisms of capture and rejection of phytoplankton assemblages and flow cytometric techniques.

This study documents the levels as a result of exposure to the distribution of toxins in individual feeding rates, species and physiological condition). The study of which bivalve species can quantify the relative risk of species and eliminate the uncertainty illustrated in this review (by body size, species and pretreatment regional and global of bivalve populations) but remains to be determined for bivalve species that are

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This study documents substantial intraspecific variation in toxin levels as a result of extrinsic factors (microgeography and patchiness in the distribution of toxic cells) and intrinsic factors (differences in individual feeding rates, susceptibility to PSP toxins, body size, and physiological condition). Thus, comparative field or laboratory studies in which bivalve species are similarly exposed to PSP toxins are needed to quantify the relative magnitude of differences in toxin kinetics among species and eliminate the confounding effects of microhabitat variation illustrated in this review. Standardization of toxicity data in bivalves (by body size, species, etc.) will ultimately improve our ability to interpret regional and global patterns of PSP incidence. Genetic adaptation of bivalve populations to PSP toxins has been invoked in several studies but remains to be demonstrated and is most likely to be of interest in bivalve species that are highly susceptible to these toxins. Progressive

incapacitation or acclimation of individuals to PSP toxins have not been demonstrated previously. Testing for these effects is precluded by the fact that previous studies have often neglected to measure individual variability in toxin levels, and that feeding on toxic cells has generally relied on experiments of short duration (hours). The possibility that dinoflagellate cysts, frequently reported in the gut contents of bivalves, provide a source of toxin during off-bloom periods needs to be quantitatively verified by taking into account the availability of cysts, as controlled by the frequency and magnitude of sediment resuspension events in the natural environment and their subsequent digestibility.

Furthermore, in relatively sensitive species toxin levels are not a simple function of cell density and duration of exposure, but were also shown to vary greatly with cell toxicity and the relative abundance of toxic cells. However, information on toxin uptake from mixed, toxic and nontoxic, algal assemblages is extremely scarce, as much of the experimental work so far has relied on feeding bivalves unialgal toxic cultures. Species-specific, predictive relationships between maximum toxin levels in bivalves and peak water column toxin concentration remain to be developed. Direct proportionality between these two parameters is also unlikely to occur in species that are capable of active toxin transformation. These findings point to complex interactions between bivalves and toxigenic dinoflagellates, which preclude the possibility of using phytoplankton monitoring as a substitute for the direct monitoring of bivalves, although both, when practiced in concert, can enhance our understanding of toxin dynamics.

Toxin biotransformations are more prevalent in some species (e.g., *Spisula solidissima* and *Protothaca staminea*) than others (*Mya arenaria* and *Mytilus edulis*). In the former, toxin metabolism may play a significant role in two ways: by causing net changes in bivalve toxicity and by providing a useful tool to predict the source and timing of toxic bloom events in the absence of phytoplankton monitoring. Although some of the pathways of conversion have been elucidated, the complex and diverse toxin profiles of many PSP-producing dinoflagellates have so far made it difficult to obtain accurate estimates of *in vivo* rates of conversion of individual toxins. The temperature dependence and physiological role of the decarbamoylase enzyme/s responsible for the biotransformation of carbamate and *N*-sulfocarbamoyl toxins, described so far in only a few clam species, is of considerable interest, as it does not appear to fulfill a detoxification function. Cellular scavenging or detoxification systems (metallothioneins and mixed-function oxygenases) well known for heavy metals and anthropogenic organic contaminants, respectively, have yet to be identified for PSP toxins. It also remains to be resolved

whether bivalves are individual PSP toxins and detoxification and toxin biotransformation and cellular localization actually lead to the detoxification, which have

Anatomical comparisons have been clearly demonstrated the toxicity of the visceral adductor muscle in scallops and detoxification per the finding that detoxification of viscera generally detoxify contribution to the total detoxification (except PSP toxins from the remain to be determined methods for individual derivatives, may lead to market, it is especially development on the

Prolonged retention is characteristic of some bivalve species (*Patinopecten yessoensis giganteus*), thus rendering tissues are marketed. However, marketing of the foot of some clam muscle of scallops, can PSP.

Quantitative analysis allow comparative ability to manage bivalve of multiphasic frequency sampling and not available from ro this review, such contamination time required to reach reported in the public maximum toxicity at species between toxin mechanisms of toxin

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it in some species o) than others (*Mya* metabolism may play es in bivalve toxicity e and timing of toxic onitoring. Although idated, the complex noflagellates have so of *in vivo* rates of pendence and physi-sible for the biotrans-s, described so far in as it does not appear ing or detoxification enases) well known inants, respectively, nains to be resolved

whether bivalves are capable of selective retention/elimination of individual PSP toxins and whether gut microflora can play a role in detoxification and toxin biotransformation. Advances in our understanding of cellular localization and toxin metabolism in bivalve tissues may eventually lead to the development of artificial methods to accelerate detoxification, which have so far remained elusive.

Anatomical compartmentalization of PSP toxins in bivalve tissues has been clearly demonstrated. Yet, poor correlation is observed between the toxicity of the viscera and that of other organs (e.g., gonad or adductor muscle in scallops) when seasonal data, including toxification and detoxification periods, are pooled. This can be partly attributed to the finding that detoxification rates are highly tissue specific, that is, the viscera generally detoxify faster than other tissues, and therefore their contribution to the total toxin body burden decreases greatly during detoxification (except in *M. edulis*). The efficiency and rate of transfer of PSP toxins from the viscera to other tissue compartments, however, remain to be determined. Development of immunofluorescent detection methods for individual toxins, and the availability of radiolabeled toxin derivatives, may lead to advances in this area. For the roe-on scallop market, it is especially important to determine the effect of gonadal development on the routing of toxins within the organism.

Prolonged retention of toxins (several months to years) is characteristic of some bivalve species (e.g., the scallops *Placopecten magellanicus*, *Patinopecten yessoensis*, and clams *Spisula solidissima* and *Saxidomus giganteus*), thus rendering them unsuitable for harvesting, if whole tissues are marketed, in areas affected by recurrent PSP outbreaks. However, marketing of tissues that accumulate low toxin levels, such as the foot of some clam species for specialized markets, and the adductor muscle of scallops, can allow safe harvesting even in areas affected by PSP.

Quantitative analysis of detoxification data is required in order to allow comparative modeling of detoxification kinetics and improve our ability to manage bivalve stocks in PSP-affected areas. Adequate resolution of multiphasic detoxification patterns will depend on high-frequency sampling and detection of low toxin levels, which typically are not available from routine PSP-monitoring programs. As emphasized in this review, such comparisons cannot be based on measurement of the time required to reach the regulatory level, the parameter typically reported in the published literature, because this is dependent on the maximum toxicity attained, and there is no clear correlation among species between toxification and detoxification rates. The physiological mechanisms of toxin elimination are poorly understood, especially the

excretion of toxins via the dissolved phase and their inactivation or degradation to undetectable compounds in tissues. Development of presently unavailable, highly sensitive analytical methods for detection of PSP toxins in the aqueous phase are needed to make progress in this area. Information on the influence of environmental parameters, especially temperature, on toxin elimination rates is also sorely lacking.

For a few species that are relatively insensitive to PSP toxins, such as *Mytilus edulis* and *Spisula solidissima*, sufficient information has been generated from field and laboratory studies to attempt predictive modeling of toxin kinetics in natural populations. This approach is most likely to prove useful for bivalve populations that are not included in routine monitoring programs, such as those located in offshore waters or those that sustain recreational fisheries. Future modeling efforts should be used to optimize strategies (e.g., sampling intervals) for PSP monitoring, especially given that the financial burden of monitoring is increasingly being shared with government bodies by commercial shellfish growers and fishermen.

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