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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 (≤ 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. $ 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 exercized, however, in the interpretation of these global patterns, for a number of reasons. For example,

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 127×10^3 μg saxitoxin equivalents [STXeq] 100 g^{-1}), followed by the Atlantic and Pacific coasts of North America (with comparable maxima of 28 and 30×10^3 μg STXeq 100 $g,^{-1}$ respectively). Lower levels generally occur in the Indo-West Pacific region and in Europe (maxima ranging from 0.2 to 4.0×10^3 μg 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

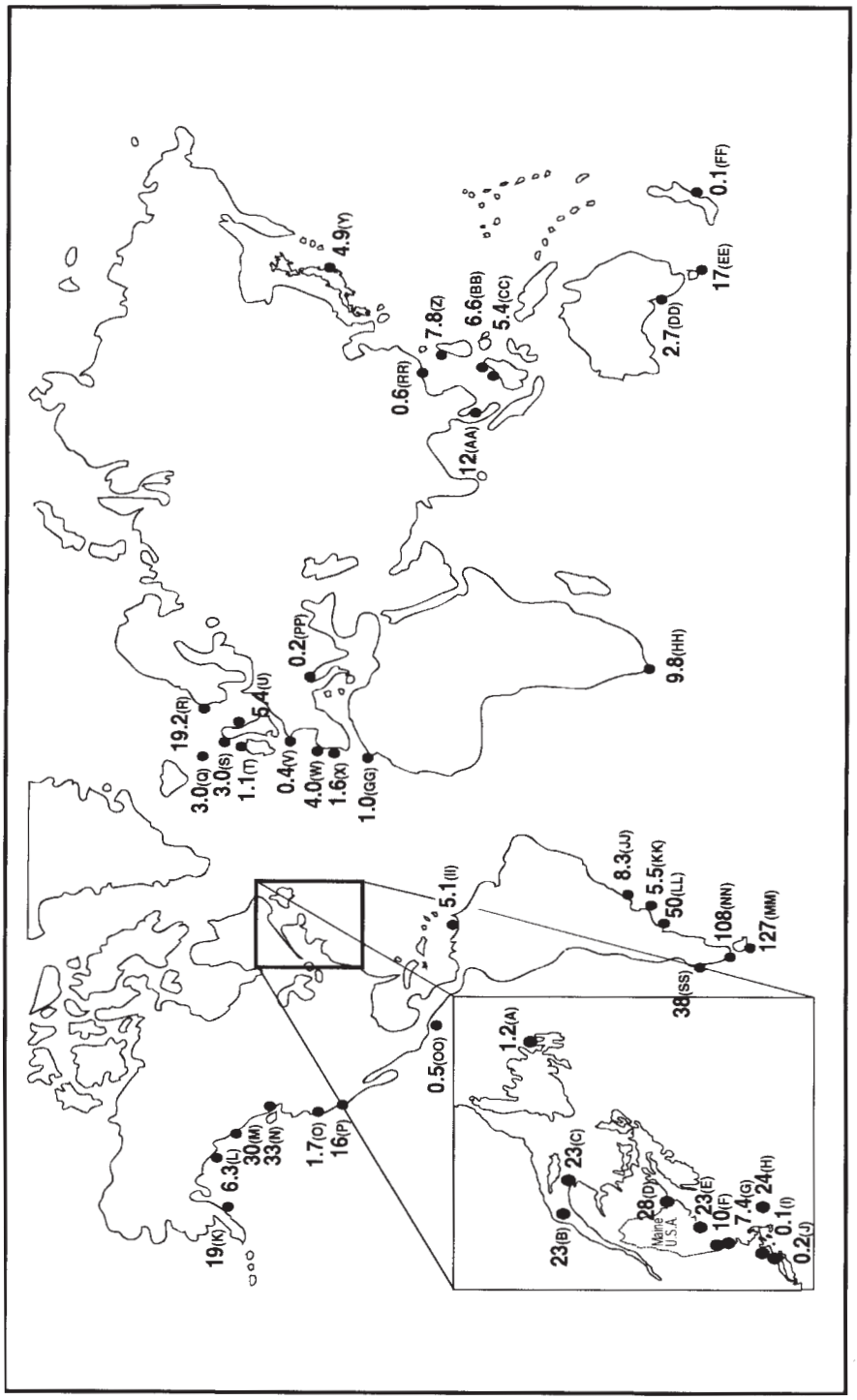

FIGURE 1. Maximum historical PSP toxicities determined by mouse bioassay (plotted values multiplied by 10^3 yield µg STXeq 100 g^{-1}) throughout the world in mussels (*Mytilus* spp. [*californianus, edulis, galloprovincialis* or *chilensis*] unless otherwise indicated). A conversion factor of 0.2 µg 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) Mc Cughey 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; (EE) Hallegraeff 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., 1986; (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 Massachussetts 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 contamined 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.

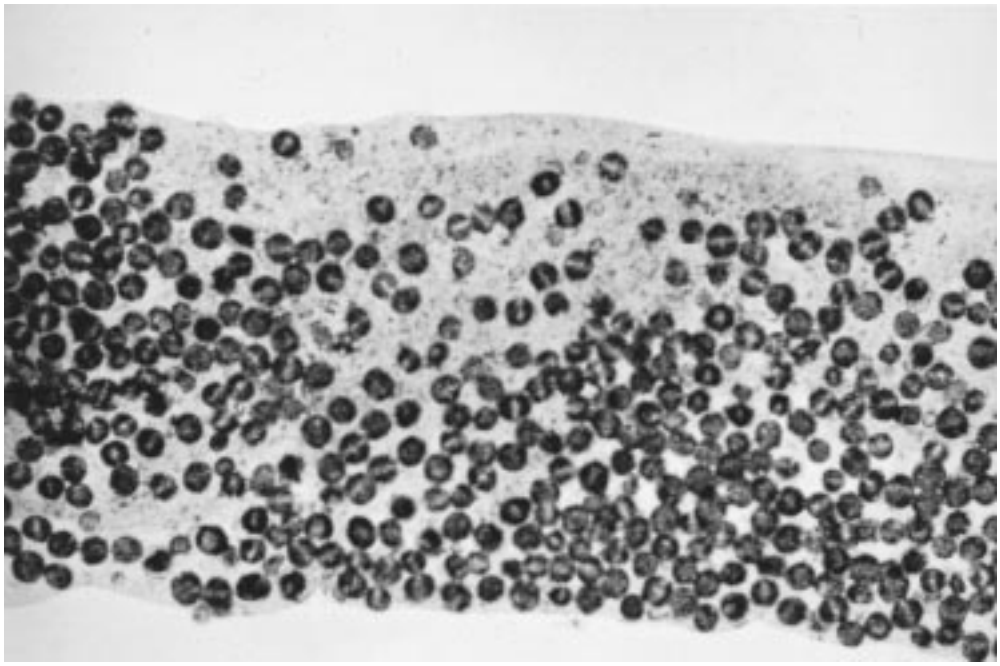

FIGURE 2. Fecal ribbon of juvenile quahogs, *Mercenaria mercenaria*, fed experimentally with a mixed suspension of the dinoflagellate *Alexandrium fundyense* (strain GtCA29 at 100 cells ml^{-1}; toxicity = 96 pg STXeq $cell^{-1}$) and the nontoxic diatom *Thalassiosira weissflogii* (70:30 cell volume 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

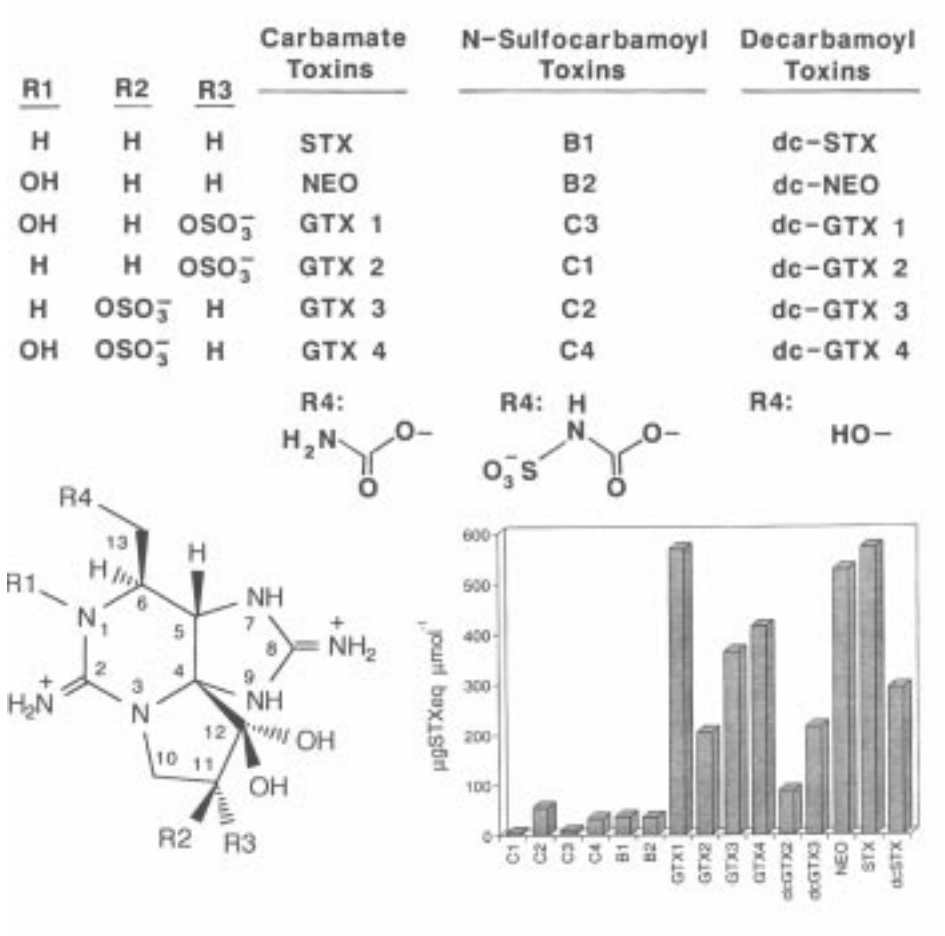

FIGURE 3. Structure of PSP toxins (carbamate: STX = saxitoxin, NEO = neosaxitoxin, $GTX_{1,2,3,4}$ = gonyautoxins 1,2,3,4, *N*-sulfocarbamoyl and decarbamoyl derivatives). Only toxins whose potency has been determined are included. Insert shows the relative potency of individual PSP toxins, as measured 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 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 pg STXeq $cell^{-1}$ in Long Island waters to 130 pg 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, $GTX_{2,3}$, whereas southern U.S. low-toxicity isolates have relatively higher levels of less potent toxins $C_{1,2}$. However, high relative amounts of $C_{1,2}$ toxins are also characteristic of high-toxicity *Alexandrium* isolates from the Gulf of St. Lawrence region in northeastern Canada, indicating that the relationship betwen 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,* toxicites 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 toxicites 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, metereological, 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,

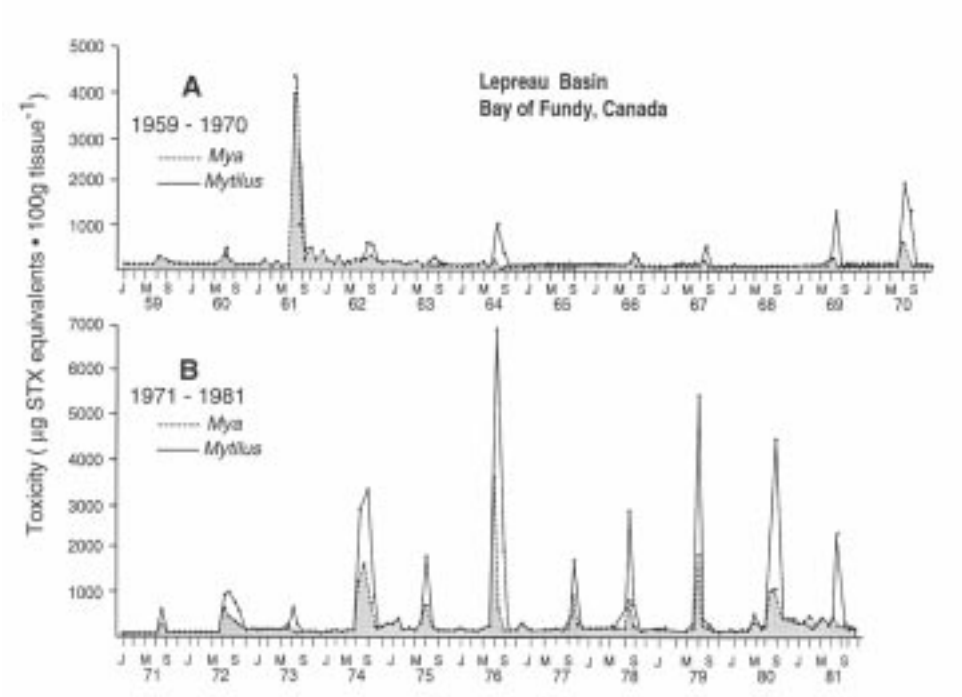

FIGURE 4. Temporal patterns in PSP shellfish toxicity, 1959–1981: mean monthly toxicity (in µg STXeq 100 g^{-1} wet tissue weight) in blue mussels, *Mytilus edulis*, and softshell clams, *Mya arenaria*, at Lepreau Basin, southwestern Bay of Fundy, New Brunswick, Canada (redrawn from White, 1982). Extended 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 exercized, 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 µg STXeq 100 g^{-1} appear to occur every 1 to 4 years, but intensification of PSP outbreaks

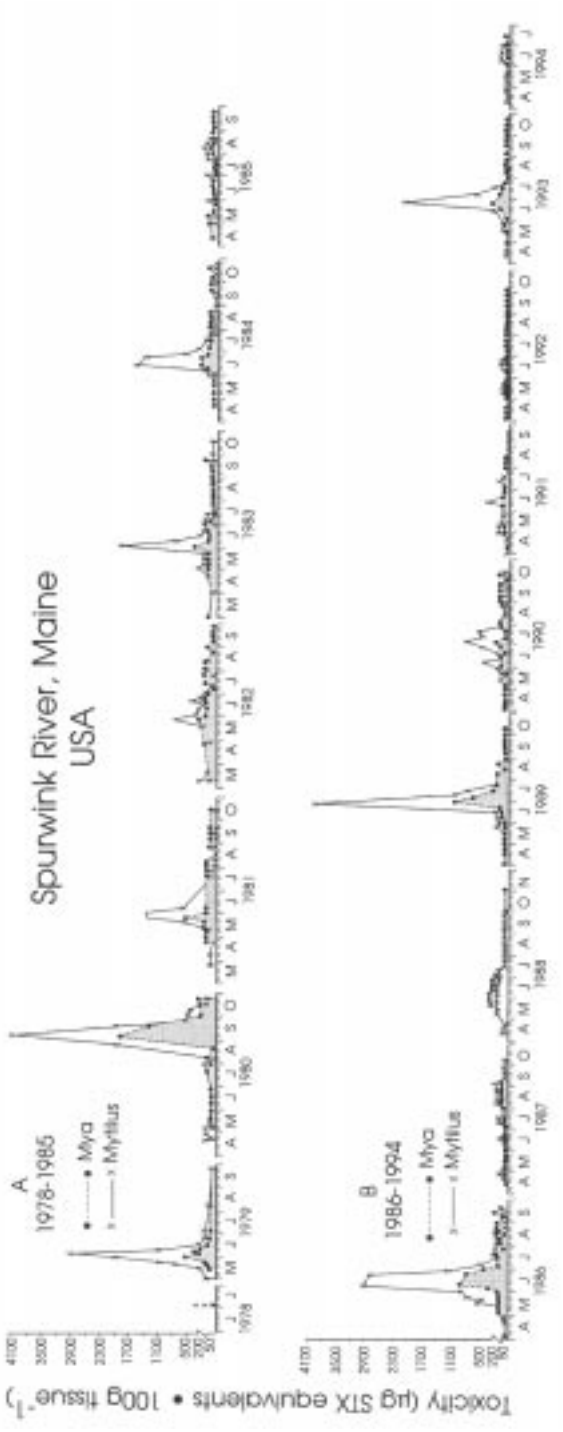

FIGURE 5. Temporal patterns in PSP shellfish toxicity from 1978 to 1994 (in µg STXeq 100 g^{-1}) in mussels, *Mytilus edulis*, and softshell clams, *Mya arenaria*, from the Spurwink River, Maine, U.S.A (State of Maine Dept. of Marine Resources PSP monitoring records, courtesy of J. Hurst).

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 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 exercized 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 8×10^3 μg STXeq 100 g^{-1} (Table 1), whereas experimentally toxified adults attained 19×10^3 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}) Whole animal	Visceral mass	Location and year of sample collection	Ref.
CLAMS, COCKLES AND ARKS				
Scapharca broughtonii (blood ark shell)		40600[a]	Senzaki Bay, Japan (1986)	Ikeda et al. (1989)
Saxidomus nuttalli (Washington clam)	14000		Campbell Cove, California USA (1980)	Price et al. (1991)
Saxidomus giganteus	9600		Gilford Island, northern BC, Canada (1985)	Chiang (1988)
(butter clam)	8640		Theodosia Inlet, southern BC Canada (1965)	Quayle (1969)
	2299		Sequim Bay, Washington, USA (1990)	WA Dept. Health (L. Hanson, personal communication)
Mya arenaria	9600		Merrimack estuary, Massachusetts USA (1972)	Twarog (1974)
(softshell)	9000		Lepreau Basin, Bay of Fundy, Canada (1976)	Martin and Richard (1996)
	4900[b]	10000[b]	Crow Harbour, Bay of Fundy, Canada (1986)	Martin et al. (1990)
	2854		Wells, Maine, USA (1972)	Yentsch et al. (1975)
Soletellina diphos (purple clam)	9100[a]	40000[a]	Tungkang, South Taiwan (1986)	Hwang et al. (1987)
Schizothaerus (=Tresus) nuttalli (Pacific gaper)	7283		Campbell Cove, California, USA (1980)	Price et al. (1991)
Donax gracilis (coquina)	7640		Mazatlán Bay, Gulf of California, Mexico (1979)	Mee et al. (1986)
Clinocardium nuttallii	6700		Charter Creek, BC, Canada (1992)	DFO (1992)
(Nuttall cockle)	1860		San Juan Is., Washington, USA (1981)	Erickson and Nishitani (1985)
Spisula solidissima	6423	21000	Georges Bank, USA (1990)	Shumway et al. (1994)
(Atlantic surfclam)	7934		Head Beach, Maine, USA (1981)	Shumway et al. (1994)
	5104		Sagadahoc Bay, Maine, USA (1980)	
	3740		Lepreau Basin, Bay of Fundy, Canada (1961)	Quayle and Bourne (1972)
	2939		Hampton, New Hampshire, USA (1972)	Sasner et al. (1975)

Tapes (= Venerupis = Ruditapes) philippinarum (= japonica) (Japanese littleneck)	6086		Okeover Inlet, BC, Canada (1986)	R. Chiang, DFO, (personal communication)
		29000[a]	Senzaki Bay, Yamaguchi Prefecture, Japan (?[a]1986)	Ikeda et al. (1989)
	2000[a]		Ise Bay, Japan (1976)	Noguchi et al. (1978)
	1710		Portage Bay, Washington, USA (1992)	WA Dept. Health (L. Hanson, personal communication)
Amphichaena kindermanni (Kindermann's false donax)	6000[a]		Champerico, Guatemala (1987)	Rosales-Loessener (1989)
Cardium edule (common European cockle)	5300[a]		Laguna d'Obidos, Portugal (1959)	DeSousa and Silva (1963)
Protothaca staminea (Pacific littleneck)	5053		Water Bay, BC, Canada (1986)	DFO (R. Chiang personal communication)
	1055		Portage Bay, Washington, USA (1990)	WA Dept. Health (L. Hanson, personal communication)
Meretrix casta (chaste venus or backwater clam)	3787[a]		Kumble Estuary, Mangalore, West India (1983)	Karunasagar et al. (1984)
Tresus capax (fat gaper)	3520		Theodosia Inlet, BC, Canada (1965)	Quayle (1969)
Donax serra (saw donax; white mussel)	3230		Elands Bay, South Africa (1980)	Horstman (1981; personal communication)
Tridacna crocea (crocus clam)	1900[a]		Arumizu Bay, Koror Island, Palau (1981)	Harada et al. (1982)
Arctica islandica (ocean quahog)	1900[a]		Machias Bay, Maine, USA (1985)	Shumway et al. (1988)
	1218		Georges Bank, USA (1990)	White et al. (1993a)
	1010		Plum Island, Mass., USA (1972)	Sasner et al. (1975)
Saxidomus purpuratus (purple Washington clam)		7200[a]	Senzaki Bay, Japan (1987)	Ikeda et al. (1989)
Siliqua costata (Atlantic razor)	1727		Hampton, New Hampshire, USA (1972)	Sasner et al. (1975)
Acanthocardia (=Rudicardia) tuberculata (Mediterranean cockle)	1457		La Linea, Mediterranean Sea, Spain (1989)	Berenguer et al. (1993)
Mercenaria mercenaria (northern quahog)	1113		Monhegan Island, Maine, USA (1975)	Maine Dept. Marine Resources (J. Hurst, personal communication)
Ensis directus (Atlantic jackknife)	735		Hampton, New Hampshire, USA (1972)	Sasner et al. (1975)
	500[c]		Pocologan, NB, Bay of Fundy, Canada	Medcof et al. (1947)
Siliqua patula (Pacific razor)	720	3480	Long Beach, Washington, USA (1992)	WA Dept. Health (L. Hanson, personal communication)

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}) Whole animal	Visceral mass	Location and year of sample collection	Ref.
Panopea generosa (=abrupta) (Pacific geoduck)		2200	British Columbia, Canada (1989)	DFO (1989) in Beitler (1992)
Paphies subtriangulata (tuatua clam)	412		Bay of Plenty, New Zealand (1993)	Cawthron Institute (L. MacKenzie, personal communication)
Anadara granosa (granular ark; tembayangan cockle)	188		Brunei Bay, Brunei (1980)	Jaafar and Subramaniam (1984)
OYSTERS				
Spondylus butleri (thorny oyster)	22000[a]	46000[a]	Arumizu Bay, Koror Island, Palau (1981)	Harada et al. (1982)
Crassostrea gigas = (iredalei) (Pacific oyster)	9929		Okeover Inlet, BC, Canada (1986)	DFO (R. Chiang, personal communication)
	5500		Drakes Estero, California, USA (1980)	Conte (1984)
	2400[a]		Senzaki Bay, Yamaguchi Prefecture, Japan (1979)	Onoue et al. (1980)
		22600[a]	Senzaki Bay, Yamaguchi Prefecture, Japan (1986)	Ikeda et al. (1989)
	2376		Minter Bay, Washington, USA (1988)	WA Dept. Health (L. Hanson, personal communication)
	236		Aber Wrach'h estuary, Brittany, France (1988)	Nezan and Piclet (1991)
	1500		Sabah, Malaysia (1987)	Ting and Wong (1989)
Lopha cristagalli (cock's comb oyster)	2600[a]		Arumizu Bay, Toror Island, Palau (1981)	Harada et al. (1982)
Crassostrea iridescens (rock oyster)	1720		Mazatlán Bay, Gulf of California, Mexico (1979)	Mee et al. (1986)
	810		Bays of La Ventosa, Gulf of Tehuantepec, Mexico (1989)	Cortés-Altamirano et al. (1993)
Saccostrea (=Crassostrea) mordax (=cucculata) (hooded or pink oyster; teritip)	1336[a]		Kumble Estuary, Mangalore, W. India (1983)	Karunasagar et al. (1984)
	487		Serasa, Brunei (1988)	Jaafar et al. (1989)
		4200[a]	Arumizu Bay, Koror Island, Palau (1981)	Harada et al. (1982)
Ostrea edulis (edible oyster)	1300		Long Point Creek, Maine, USA (1986)	Shumway et al. (1990)
Crassostrea virginica (eastern oyster)	214		Acadian peninsula, SE Gulf of St. Lawrence, Canada (1988)	Worms et al. (1993)

Scallops				
Patinopecten yessoensis		220000[a]	Japan (1982)	Noguchi et al. (1984)
(Japanese scallop)		70000[a]	Ofunato Bay, Iwate Prefecture, Japan (1981)	Maruyama et al. (1983)
		15000[a]	Funka Bay, Hokkaido, Japan (1979)	Nishihama (1980)
Placopecten magellanicus		150000	Digby, Bay of Fundy, NS, Canada (1978)	Jamieson and Chandler (1983)
(sea scallop)		65000	Mascarene, Pasamaquoddy Bay, Canada (1980)	Jamieson and Chandler (1983)
	10864		Georges Bank, USA (1990)	calc. from White et al. (1993)
	2200	25000	Mascarene, Pasamaquoddy Bay, Canada (1981)	Jamieson and Chandler (1983)
	1200[c]	7200[c]	Lepreau Basin, Bay of Fundy, Canada (1945)	Medcof et al. (1947)
		4180	Damariscotta River estuary, Maine, USA (1988)	Cembella et al. (1993)
Pecten albicans		30000[a]	Senzaki Bay, Yamaguchi Prefecture, Japan (1987)	Ikeda et al. (1989)
(Japanese baking scallop)				
Crassadoma gigantea	13593	26000	Timber Cove, California, USA (1980)	Beitler (1991)
(=Hinnites multirugosus)	1200		British Columbia, Canada (1989)	Beitler (1991)
(giant rock scallop)				
Chlamys hastata	5900		British Columbia, Canada (1989)	DFO (1989) in Beitler (1992)
(spiny scallop)				
Chlamys rubida	5900		British Columbia, Canada (1989)	DFO (1989) in Beitler (1992)
(reddish scallop)	2363		Lopez Island, Washington, USA (1987)	WA Dept. Health (L. Hanson, personal communication)
Chlamys tehuelcha	5220		San Matias Gulf, Argentina (1991)	El Busto et al. (1993)
(Darwin's or Patagonian scallop)				
Amusium pleuronectes	2000		Cebu, Philippines (1989)	Pastor et al. (1989)
(Asian moon scallop)				
Argopecten irradians	2040		Eastham, Massachusetts, USA (1972)	Twarog (1974)
(bay scallop)				
Pecten maximus	1600[a]		Farne Bank, NE England (1968)	Ingham et al. (1968)
(great scallop)				
Chlamys nipponensis akazara		4000[a]	Ofunato Bay, Japan (1976)	Noguchi et al. (1978)
(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.

[a] *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, $I_{(1g)} = I_x \cdot (W_x^{-b})$, 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) Toxicity of whole tissues	Toxicity of visceral mass	Dinoflagellate strain fed (toxicity; density)[a]	Ingestion rate, I (dinoflagellate cells day^{-1} g^{-1})	Source
Mytilus edulis					
Adults	2,371[b]	—	MOG835 (7;[f] —)	—	Lassus et al., 1993
35–45 mm	1,100[b]	—	MOG835 (—; —)	6.67×10^{5}[e]	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
Spisula solidissima					
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[g]	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[g]	72,316	GtCA29 (14; 196)	—	
Mercenaria mercenaria					
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}	

Mya arenaria					
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
Pecten maximus					
35–45 mm	2,700[b]	—	MOG835 (7[f]; —)	3.98×10^{5e}	Lassus et al., 1989
Ruditapes philippinarum					
35–45 mm	160[b]	—		0.82×10^{5e}	
Crassostrea gigas					
35–45 mm	ca. 80[b]	—		2.62×10^{5e}	

[a] *GtCA29* = A. fundyense. *GtLI22 and MOG835* = A. tamarense; *AL1V* = A. minutum; *PR18b* = A. tamarense *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 (I/W) estimated from Figure 2.*

[f] *Toxicity reported in Lassus et al. (1994).*

[g] *Does not include the toxin contribution of adductor muscles.*

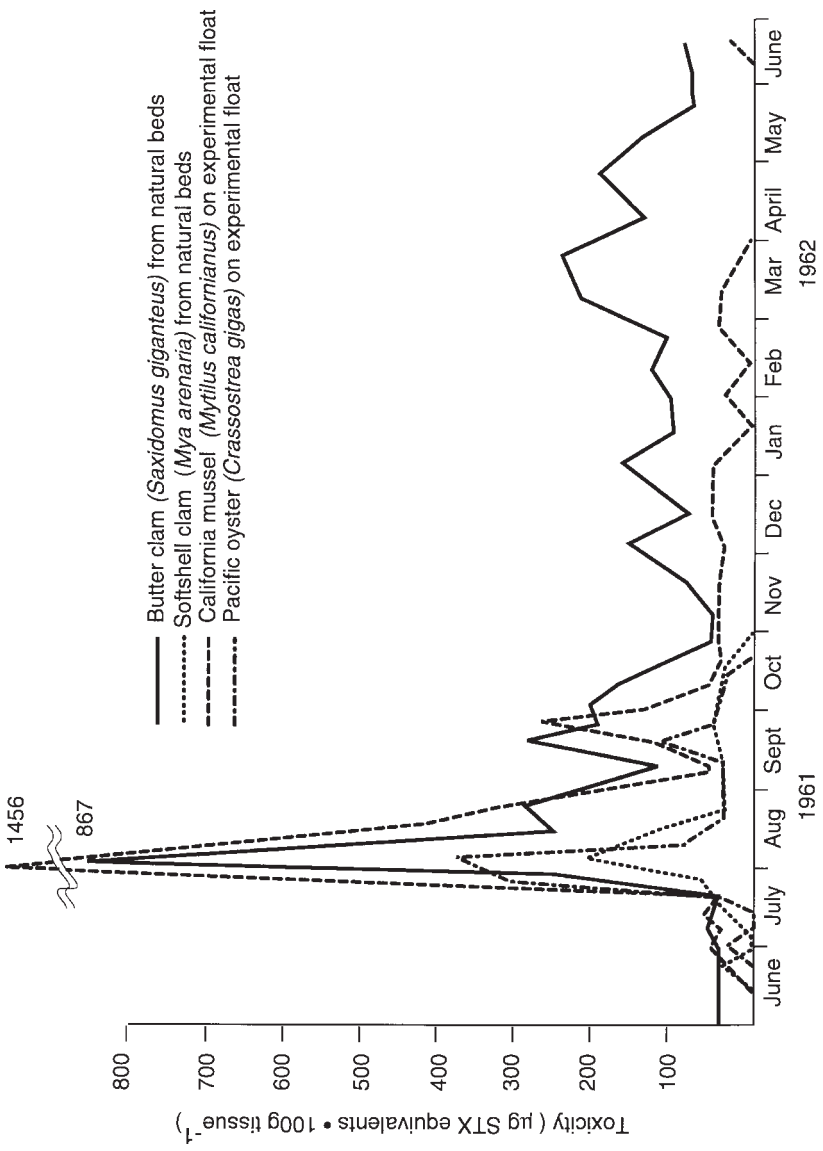

FIGURE 6. Levels of PSP toxins in four species of bivalves in experimental floats and natural beds in Sequim Bay, Washington, U.S.A. (After Sparks et al. [1962]).

measured for different 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}
Mytilus edulis[a]	Blue mussel	0	0	0	0	0	nd
Placopecten magellanicus[a]	Sea scallop	0	0	0	0	0	nd
Humilaria kennerleyi[b]	Kennerly venus	0	0	0	0	0	(+)
Mercenaria mercenaria[a]	Northern quahog	0	0	0	0	(+)	nd
Saxidomus giganteus[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	(+)	+
Saxidomus nuttalli[b]	Washington clam	0	0	0	0	(+)	+
Modiolus demissus[a]		0	0	0	0	(+)	+
(=Geukensia demissa)	Ribbed Mussel	0	0	0	0	+	nd
Mya truncata[b]	Truncate softshell	0	0	0	(+)	+	+
Pecten irradians[a]							
(=Argopecten irradians)	Bay scallop	0	0	0	(+)	(+)	nd
Mya arenaria[a,b]	Softshell	0	0	(+)	+	+	+
Tresus capax[b]	Fat gaper	0	0	(+)	+	+	+
Protothaca staminea[b]	Pacific littleneck	0	(+)	(+)	+	+	+
Crassostrea virginica[a]	Eastern oyster	0	+	+	+	+	nd

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

(B) Species	Common name	Block of action potential by TTX (g ml^{-1})			
		10^{-8}	10^{-7}	10^{-6}	10^{-5}
Mytilus edulis (Massachusetts)	Blue mussel	0	0	0	0
Mytilus californianus	California mussel	0	0	0	0
Placopecten magellanicus	Sea Scallop	0	0	0	0
Saxidomus nuttalli	Washington clam	0	0	0	0
Mya arenaria	Softshell	0	0	0	0
Modiolus modiolus	Northern horsemussel	0	0	0	+
Modiolus (=Geukensia) demissus	Ribbed mussel	0	0	(+)	+
Mercenaria mercenaria	Northern quahog	0	0	(+)	+
Schizothaerus (=Tresus) nuttalli	Pacific gaper	0	0	+	+
Petricola pholadiformis	False angelwing	0	0	+	+
Ensis directus	Atlantic jackknife	0	0	+	+
Zirfaea pilsbryi	Pilsbry piddock	0	0	+	+
Hinnites multirugosus (=Crassadoma gigantea)	Giant Rock Scallop	0	(+)	(+)	+
Pecten (=Argopecten) irradians	Bay scallop	0	(+)	+	+
Tagelus gibbus (=divisus)	Purplish tagelus	0	+	+	+
Spisula solidissima	Atlantic surfclam	(+)	(+)	+	+
Crassostrea virginica	Eastern oyster	(+)	+	+	+
Protothaca staminea	Pacific littleneck	(+)	+	+	+

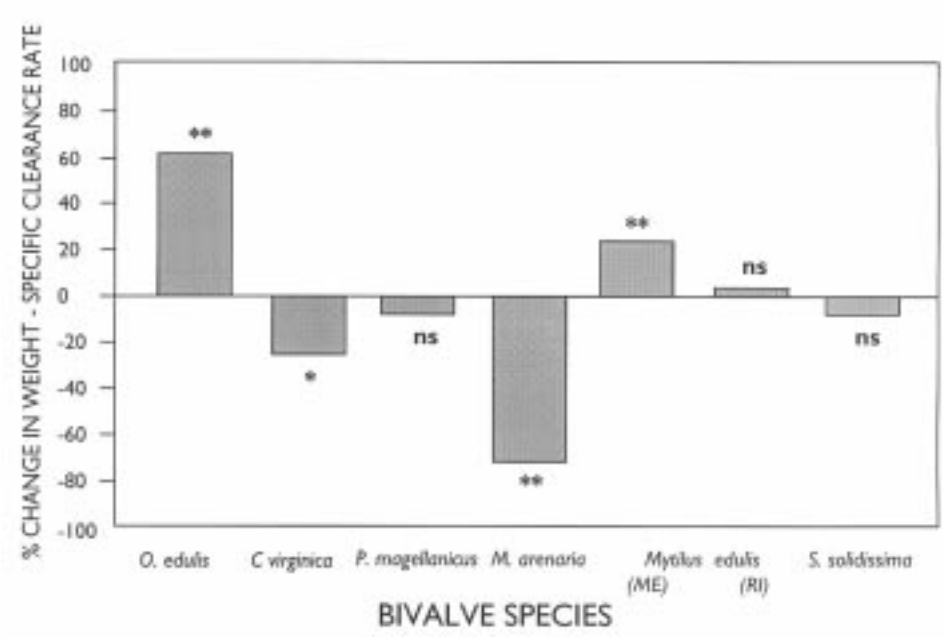

FIGURE 7. Percent change (increase or decrease) in weight-specific clearance rate of various bivalve species in response to addition of 500 cells ml^{-1} of toxic *Alexandrium tamarense*, clone Gt429, to a nontoxic, control algal diet (mixed suspension of *Thalassiosira pseudonana, Chroomonas salina* 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. tamarense* 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).

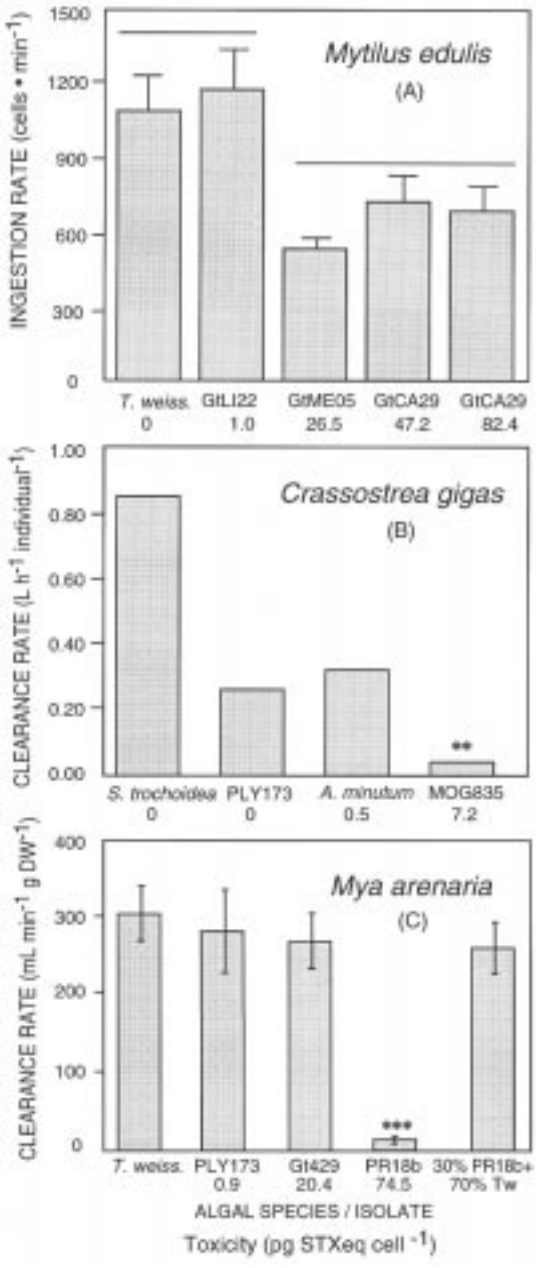

FIGURE 8. Effect of dinoflagellate PSP cell toxicity on bivalve feeding (algal species/clone, and toxicities, in pg STXeq cell^{-1}, indicated below each bar): **A**. Mean weight-normalized ingestion rate, in cells min^{-1}, for a standard animal 1 g in total body wet weight (± standard error, SE), of 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 (±SE) of juvenile (33 mm) sofshell 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., 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 10^5 to 10^6 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. 10^5 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 20×10^5 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 × 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] × 100): 72 to 96% in *M. californianus* (Dupuy 1968) and 78% in *M. edulis* (Bricelj et al., 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 μg STXeq 100 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. ± 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 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 μg 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,

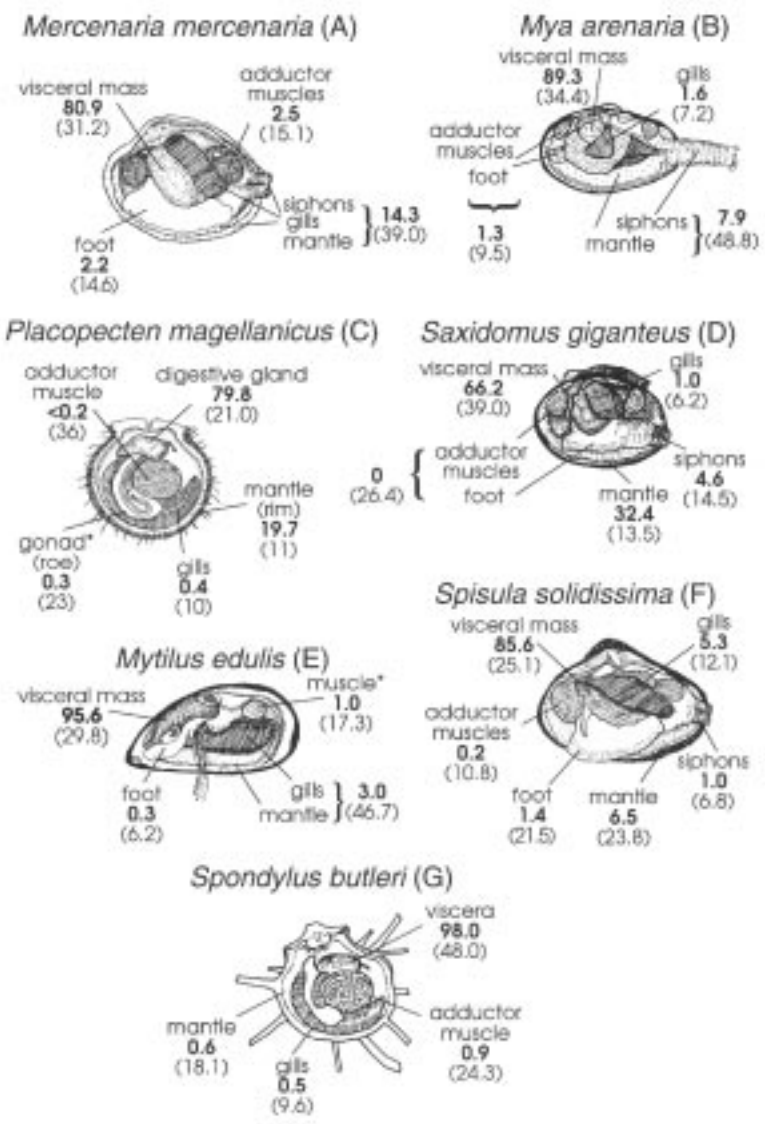

FIGURE 9. Anatomical distribution of PSP toxins in various tissues of bivalves at the peak of toxification (boldfaced: relative toxin load, as % of total toxin body burden, calculated on the basis of toxicity and contribution by weight of each tissue). Values in brackets indicate the relative (%) contribution 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.

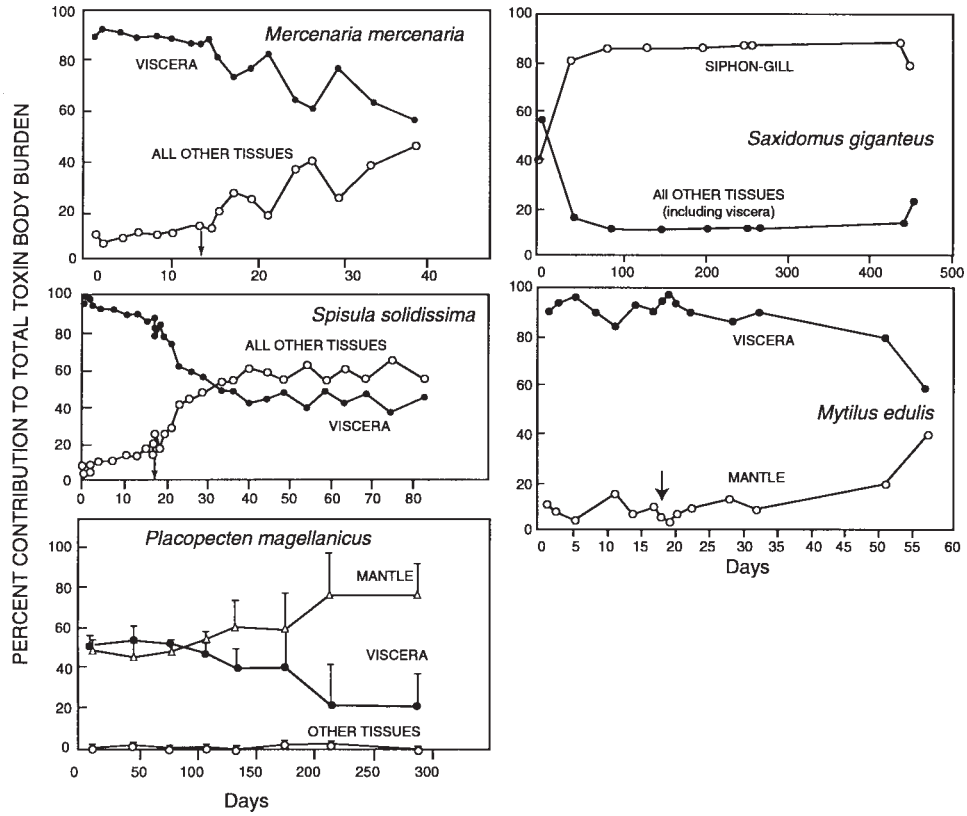

FIGURE 10. Percent contribution of visceral mass (including the digestive gland) and other tissues to total toxin body burden (in μg STX eq) over the course of toxification and/or detoxification in five bivalves species (arrow marks the end of toxification). Laboratory data and toxin analysis by HPLC 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 μg 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 "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 μg 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 know 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

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 μg STXeq 100 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 μg STXeq 100 g^{-1}), and detoxification rate (% loss of toxin day^{-1}), calculated from an exponential decay equation: $T_t = T_o e^{-\lambda t}$ 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] (μg STXeq 100 g^{-1})	Time to RL (weeks)[b]	Detox. rate (% day^{-1})	Location/detoxification conditions	Source
(A) Fast Detoxifiers:					
Tresus capax	3520	5.2 < t < 11.6[e]	—	Theodosia Inlet, BC, Canada	Quayle (1969)
Mercenaria mercenaria	2150[a] (GtLI22)	>3.6 (3.4)	9.5	Laboratory, 17°C, fed	Bricelj et al. (1991)
	10543[b] (GtCA29)	>2.6 (6.1)	>0.8 d = 9.3[c]	juveniles	
Meretrix casta	3787	4.4	—	Kumble estuary, India	Karunasagar et al. (1984)
Mya arenaria	110–1425	1.0 – 4.0	9.8	Maine, USA (fall)	Hurst and Gilfillan (1977)
	200–1200	7.1 – 5.0	—	Gulf St. Lawrence, floats, 4 –17°C	Larocque and Cembella (1991)
	~ 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)
Mytilus edulis	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[e]	—	Nordasstraumen, Norway	Alvik and Framstad (1981)
		>12.6 (7.6)	9.7	Transferred to toxin-free area	"
	19259[a] (GtCA29)	> 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.,
	100–798	0.6 – 4.8	$\bar{X}$ = 5.8		1979 to 1993 records
	1367–9075	2.1 – 9.6	$\bar{X}$ = 12.1	Lumbos Hole, Maine, USA	
	4100	3.0	—	Santa Barbera Channel, California, USA	Price et al. (1991)
	~1100	6.1	—	Bay of Fundy, Canada 10 – 11°C	Prakash et al. (1971)
	23000	5.1	13.8	Gaspé Bay, Canada	Desbiens and Cembella (1993)
	~2000	2.1	—	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 μg STXeq 100 g^{-1}), and detoxification rate (% loss of toxin day^{-1}), calculated from an exponential decay equation: $T_t = T_o e^{-\lambda t}$ 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] (μg STXeq 100 g^{-1})	Time to RL (weeks)[b]	Detox. rate (% day^{-1})	Location/detoxification conditions	Source
Mytilus californianus	2100–5300	6.8 – 7.5	$\bar{X}$ = 8.9	BC, Canada	DFO 1992 records
	900–1900	2.8 – 6.4	$\bar{X}$ = 8.1		
	240–790	3.0 – 9.0	$\bar{X}$ = 5.3		
	1100–1456	3.1 – 3.4	$\bar{X}$ = 8.1	Sequim Bay, WA, USA, floats, 13–16°C	Sribhibhadh (1963)
	685–768	3.2 – 8.9	$\bar{X}$ = 6.6	Clallam Bay, WA, USA, floats, 9–11°C	
	353–444	3.8 – 3.9	$\bar{X}$ = 5.1	Crescent Bay/Agate Beach, USA, floats, 11–13°C	
Choromytilus palliopunctatus	540	1.2 < t < 7.0[e] (1.4)	17.2	Oaxaca, Mexico	Cortés-Altamirano et al. (1993)
Perna viridis	~240*	1.7	9.3	Laboratory, fed, 26–28°C	Gacutan et al. (1989)
Modiolus modiolus	—	0.9 – 9.2	$\bar{X}$ = 7.0	Maine, USA	Hurst and Gilfillan (1977)
Crassostrea gigas	209–379	0.6 – 1.5	—	Juan de Fuca St., WA, USA floats, 12–15°C	Sribhibhadh (1963)
	710	2.0	—	San Mateo Bay, BC, Canada	DFO 1987
Crassostrea iridescens	~620–810	1.8 – 3.8	8.9 – 18.1	Oaxaca, Mexico	Cortés-Altamirano et al. (1993)
Crassostrea cucullata	1336*	6.9	5.5	Kumble estuary, India	Karunasagar et al. (1984)
Ostrea edulis	1000	6.4	4.0	Harpswell, Maine, USA	Shumway et al. (1990)
Pecten maximus	2700	>6.3 (6.4)	7.4	Laboratory, 16°C, fed	Lassus et al. (1989)
(B) Slow Detoxifiers:					
Saxidomus giganteus	8640	>90[c] (159)	< 83 d = 1.2[c] > 83 d = 0.3	Theodosia Inlet, BC, Canada	Quayle (1969)
	3174	114[c] (111)	> 47 d = 0.6[c]	Little River, BC, Canada	
	278	>14	—	Laboratory, 7.5–16.5°C, fed	Madenwald (1985)
Saxidomus nuttalli	14000	>73 (85)	0.9	Bodega Harbor, CA, USA	Price et al. (1991)
	2800 (Siphon)	>42 (118)	0.4		
	620 (Siphon)	39 (40)	0.8		
Spisula solidissima	30429[a] (PR18B)	> 8 (24)	3.4	Laboratory, fed, juveniles 16–17°C	Bricelj, Laby and Cembella, unpublished
	16810[a] (AL1V)	> 9[c](13)	< 6 d = 24.3[c] > 6 d = 4.2	Laboratory, fed, juveniles 16°C	Bricelj and Cembella (1995) and unpublished

	4514	100[c] (81)	< 32d = 1.9[e]	Head Beach, ME, USA	Shumway et al. (1988) and
			> 32 d = 0.5	ME Dept. Mar. Res.	
	1752	96 (59)	0.8	Scarborough Beach, ME, USA	
	6000	> 100 (48) (132)[c]	1.3	Georges Bank, USA, Station 3	Shumway et al. (1994)
			< 42d = 1.2[e]		
			> 42 d = 0.4		
	3900	>69[c](73)	< 30d = 1.6[e]	Georges Bank, USA, Station 1	
			> 30 d = 0.6		
	4010–4510	> 70 (51)	1.1	Georges Bank, USA, St. 2 & 4	
	1705	> 26	—	Laboratory, 15°C, 20 µm-filtered ambient water	
	1140 (Viscera)	> 13	—	Laboratory, fed	Blogoslawski and Stewart (1978)
	1624 (Mantle)				
Cardium edule	3100*	11.2	3.3	Lagune d'Óbidos, Portugal 11–15°C	De Sousa and Silva (1963)
Soletellina diphos	40000 (Dig.)*	> 51[c](31)	<14 d = 3.6[e]	Culture pond, Tungkang, Taiwan	Hwang et al. (1990)
			>14 d = 1.2		
Placopecten magellanicus	–1674 (Dig.+Mantle+Gill)	> 28 (78)	0.6	Laboratory, starved	Shumway et al. (1988)
	809 (–Adductors)	> 52 (135)	0.2	Laboratory, –0.2 to +14°C	Waiwood et al. (1995)
	6179 (Dig.)	> 52 (104)	0.6		
	2720 (Dig.)	> 17	—	Mascarene, Bay of Fundy, Canada	Bourne (1965)
	1440 (Mantle)	> 17	—		
	4000 (Dig.)	> 8.7	—	Whitehouse, Bay of Fundy Canada	
	1248	> 8.7	—		
Patinopecten yessoensis	15000 (Dig.)*	> 30 (60)	1.2	Funka Bay, Japan	Nishihama (1980) (Figure 4)
	~ 11000 (Dig.)*	> 16.7 (31)	2.2	Funka Bay, Japan, 10 m	Nishihama (1980) (Figure 10)
	~11100 (Dig.)*	>12 (10)	2.5	Funka Bay, Japan, 25 m	
	6380 (Dig.)*	~24 (38)	1.6	Funka Bay, Japan, 1981	Tazawa et al. (1988)
	7920 (Dig.)*	23 (16)	4.1	Funka Bay, Japan, 1986	
	10880 (Dig.)*	> 27 (22)	3.1	Funka Bay, Japan, 1984	
	6000 (Dig.)*	> 19 (15)	3.8	Ofunato Bay, Japan	Ogata et al. (1982)
	14500 (Dig.)*	> 17	—	Ofunato Bay, Japan	
	340000 (Dig.)*	> 21[c](20)	< 8 d = 11.7[c]	Outdoor tanks, no toxic cells	Oshima et al. (1982)
			> 8 d = 1.3		

[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 toxification studies is indicated in brackets.*

* *Calculated using a conversion factor of 0.2 µg STXeq MU^{-1}.*

[e] *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 horsemussel *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 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 AL1V (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 $\simeq$ siphon $\simeq$ foot > adductor muscle (Bricelj and Cembella 1995). Detoxification rates are also

expected to vary with age/body size, especially in species, such as *S. soldissima,* 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_{80}s (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 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

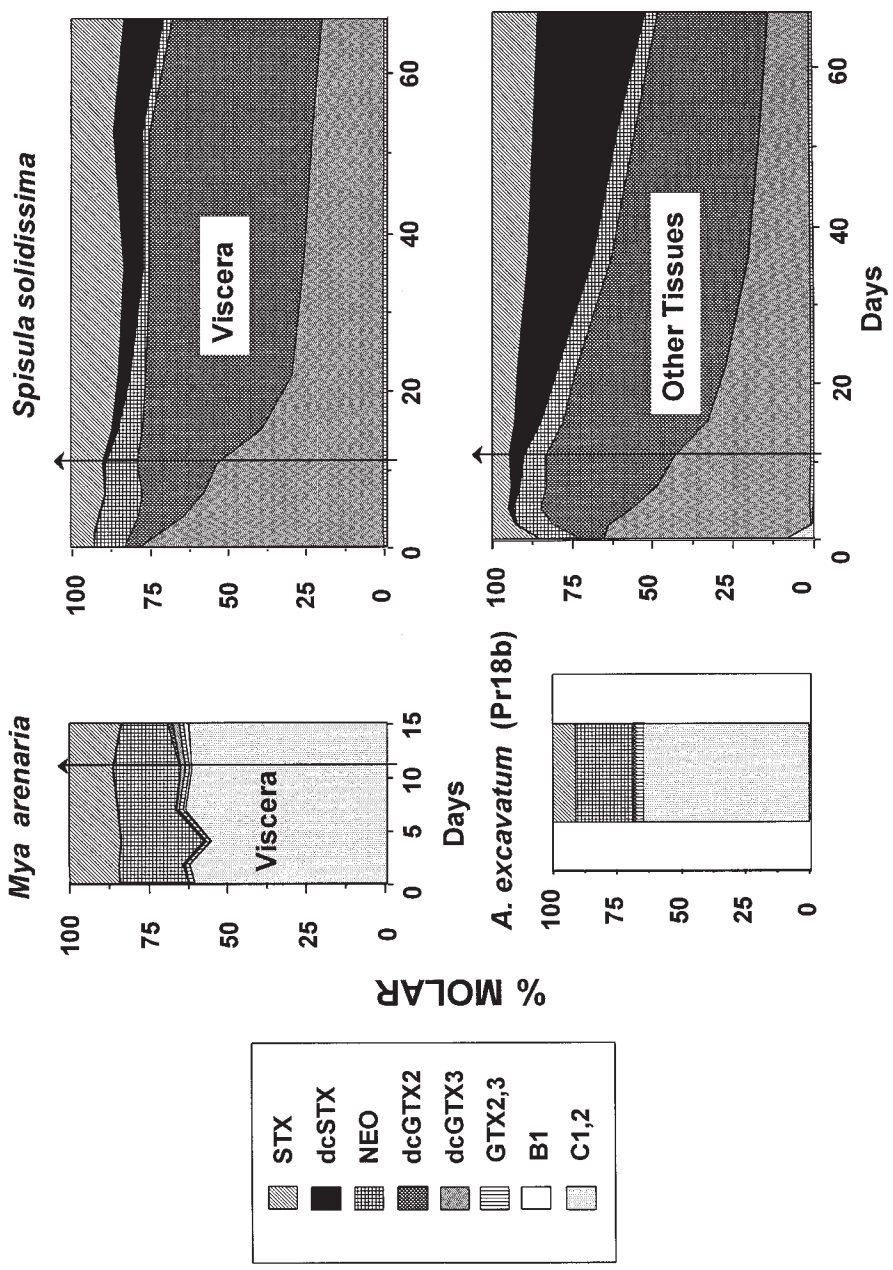

FIGURE 11. Composition (% molar) of PSP toxins in viscera and other tissues of surfclams, and in viscera of softshell clams during experimental toxification with *Alexandrium tamarense* cf. *excavatum*, strain PR18b (toxin composition of dinoflagellate cells is shown for comparison), and subsequent depuration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

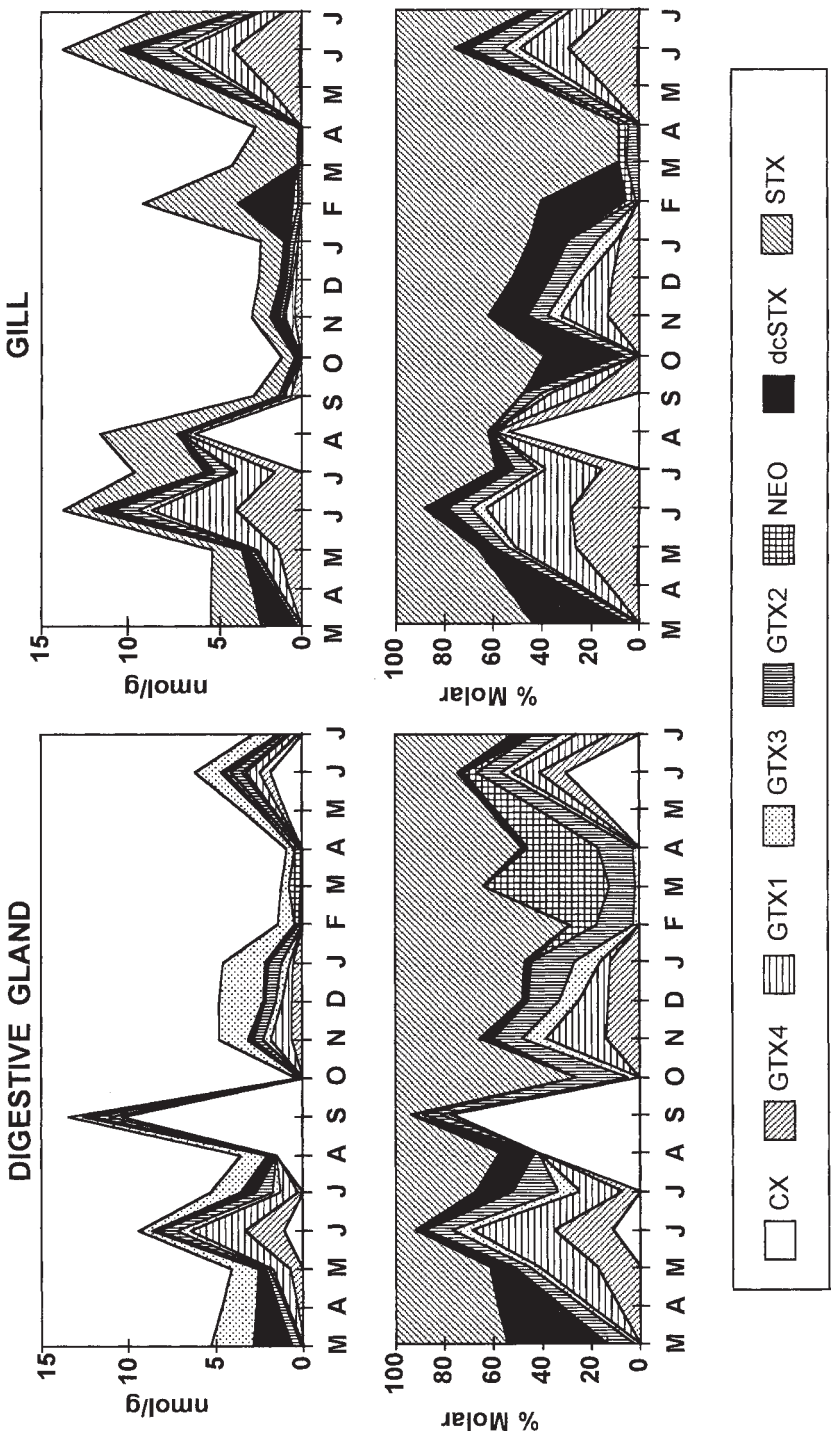

FIGURE 12. Seasonal variation in the composition of PSP toxins in two representative tissues of adult surfclams, *Spisula solidissima*, from inshore populations in the Gulf of Maine, U.S.A.. Upper graphs: Absolute toxin concentrations in nmol g^{-1} (Cembella and Shumway, unpublished). Lower graphs: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

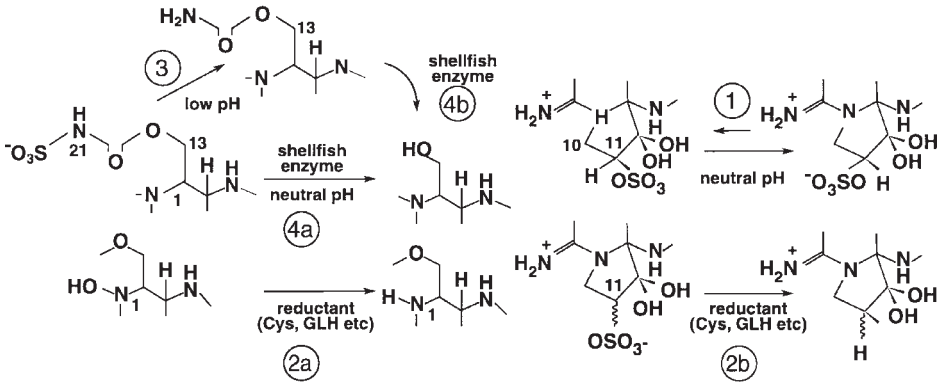

FIGURE 13. Schematic of *in vivo* transformations of PSP toxins occurring in marine bivalves (modified from Oshima 1995; see Figure 3 for structure and designations of individual toxins). 1. Epimerization from β to α epimers; 2. Reduction: (a) from $GTX_{1,4}$ and NEO to $GTX_{2,3}$ and STX, (b) from $GTX_{1,2,3,4}$ 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 β:α 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 GTX_{2+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*-sulfocarbamoyl toxins, because these are more labile than other toxins. High proportions of C toxins (> 60 to 90% molar) are characteristic of

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 B_1 (= GTX_5) is the principal component (on a molar basis) in *Pyrodinium bahamense* var. *compressa,* a species that does not produce 11-hydroxysulfate toxins (GTXs and $C_{1–4}$) (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 $dcGTX_{2+3}$ from C_2, 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 $dcGTX_{2+3}$ 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 in the above studies, suggesting that the flux of toxins from the viscera may be tissue specific.

IX. CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

As illustrated in this review, bivalve species differ greatly in the kinetics (toxin uptake and elimination rate constants), anatomical distribution, and capacity for biotransformation of PSP toxins. The differential sensitivity of bivalve species to PSP toxins, which generally correlates with the ability to accumulate toxins, can be determined on the basis of physiological (feeding) and behavioral (e.g., burrowing) and neurological responses, although we note some discrepancies between *in vitro* and whole-organism responses. By integrating these responses, and despite gaps in our knowledge for many species, an overall classification scheme emerges on the potential for toxin accumulation of commercially exploited species worldwide. This ranking provides a basis for the selection of suitable species for culture as well as monitoring of PSP in different regions, and in the future can be modified and expanded to include other species of interest as additional information becomes available.

Our detailed understanding of PSP toxin kinetics, however, is largely restricted to a few commercially important bivalve species, which are relatively resistant to the toxins, such as *Mytilus edulis* and *Spisula solidissima*. This information cannot be generalized to include other species until the underlying mechanisms responsible for the differential responses observed among species are identified. Molecular studies of differentiation in the sodium channel region, and physiological studies of *in vivo* mechanisms of pre- and post-ingestive toxin detection, and capture and rejection of toxic and nontoxic dinoflagellates from mixed phytoplankton assemblages, for example, using video endoscopy and flow cytometric techniques, may serve to elucidate these mechanisms.

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. Standarization 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 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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