AN OVERVIEW OF THE OCCURRENCE AND TRANSFER KINETICS OF PARALYTIC SHELLFISH TOXINS IN BIVALVE MOLLUSCS

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ABSTRACT

Bivalve species differ markedly in their ability to accumulate and eliminate paralytic shellfish poisoning (PSP) toxins. Since mussels are often used as sentinel organisms for PSP monitoring, we used their historical toxicities to map the global distribution of PSP, and identify latitudinal patterns and regional foci of high toxicity. Bivalve species are ranked in terms of their sensitivity to PSP toxins and toxin uptake capacity, based on their nerve resistance to saxitoxin (STX), feeding and behavioral responses to toxic dinoflagellates, and toxicity maxima attained in field and laboratory studies. Depending on their detoxification kinetics, bivalves were broadly classified into two groups: slow detoxifiers (e.g. *Saxidomus giganteus*, *Spisula solidissima*, *Placopecten magellanicus* and *Patinopecten yessoensis*) and rapid to moderate detoxifiers (e.g. *Mytilus edulis* and *Mya arenaria*). A biphasic, two-compartment model best describes detoxification kinetics in some species. During toxification, the viscera typically attain toxicities 2-5 fold higher than whole tissues, whereas locomotory tissues (foot and adductor muscle) are least toxic. However, the viscera detoxify faster than other tissues, leading to a steady decline in their contribution to total toxin body burden during detoxification. Biotransformation of toxins in tissues is most pronounced in a few clam species capable of enzymatic decarbamoylation (e.g. *Protothaca staminea*), and more limited in others such as *Mya arenaria* and *Mytilus edulis*. Overall, changes in toxin profile are greatest when ingested dinflagellates are rich in low potency, N-sulfocarbamoyl toxins. We discuss the implications of such changes, and identify future research needs and directions.

INTRODUCTION

Wild and cultured suspension-feeding bivalve molluscs are often a dominant component of the benthic macrofauna in nearshore coastal waters. Due to their ability to filter large volumes of water they are the primary vectors for the transfer of microbial pathogens and algal toxins (paralytic, diarrhetic and amnesic shellfish poisoning toxins) to humans. This summary review focuses on the global occurrence and kinetics of PSP toxins in bivalves, with emphasis on the magnitude and sources of inter- and intraspecific variability in toxin uptake and elimination. Future research directions are suggested in each titled section.

Global distribution of shellfish toxicity

Maximal historical toxicities recorded globally for mussels (*Mytilus*, *Choromytilus* and *Perna* spp.), commonly used as indicator species in PSP monitoring programs, can be used to compare geographic and latitudinal toxicity patterns and identify regions of highest toxicities (Fig. 1). Some regions affected by PSP, but which do not use mussels as the sentinel organism, are not represented in this map (e.g. in Guatemala toxicities are only reported for clams, *Ampichaena kindermanni* [2]). Toxicity values shown in Fig. 1 should be used cautiously, however, because although mussel species generally toxify and detoxify relatively rapidly, differences in toxin kinetics may occur among species. Thus, the green mussel, *Perna viridis*, provides the database in the Indo-Pacific region, whereas *Mytilus* spp. are used as the indicator organism in many other parts of the world. Additionally, although mussels collected for PSP monitoring are usually from wild, intertidal populations, mussels in suspended culture, which typically accumulate higher toxin levels, are used in some areas.

It is apparent, however, that relatively moderate toxicities, typically $\leq 4 \times 10^3$ µg STXeq 100 g^{-1} wet tissue weight, are observed in European waters. In contrast, comparable high maxima occur on the Atlantic and Pacific coasts of North America (3.3×10^4 and 2.8×10^4 µg STXeq $100g^{-1}$ respectively). These values are also similar in magnitude to realized maximum toxicities obtained in laboratory toxification studies (50×10^3 µg STXeq $100g^{-1}$) by *ad libitum* feeding with a high-toxicity (66 pg STXeq $cell^{-1}$) *Alexandrium tamarense* isolate [3]. Highest toxicities have been recorded in southern Chile and Argentina, where the maximum recorded (127×10^3 µg STXeq $100g^{-1}$) coincided with the occurrence of high densities (8×10^5 cells l^{-1}) of a highly toxic *Alexandrium* strain (325 pg STXeq $cell^{-1}$) [4]. It is noteworthy that the occurrence and intensity of PSP appears recently to be spreading northward along the southeastern Pacific. A toxicity maximum of 113×10^3 µg STXeq $100g^{-1}$ was reported in mussels, *Aulacomya ater*, from Chile (latitude 45° 28'S) in 1996 [5]. In the northwestern Atlantic, shellfish toxicities in nearshore waters appear to follow a pattern of increasing toxicity with increasing latitude (Fig. 1), which may be related to the latitudinal distribution of dinoflagellate cell toxicity [6,7]. A latitudinal pattern of mussel toxicity also seems to occur along the southwestern Atlantic.

Toxin uptake and sources of variation in shellfish toxicity

The capacity for PSP toxin uptake is known to vary greatly (up to 100-fold) among bivalve species (reviewed by Bricelj and Shumway [1]). Such differences between co-occurring species are exploited to advantage in regional monitoring programs on the eastern North American coast, where a sentinel species that rapidly accumulates toxins (*Mytilus edulis*) provides early warning for a commercial species characterized by slower toxin uptake (*Mya arenaria*). However, comparative toxin kinetics studies, in which different species are exposed to the same conditions in the laboratory [8, 9] or in the field [10, 11], are rare.

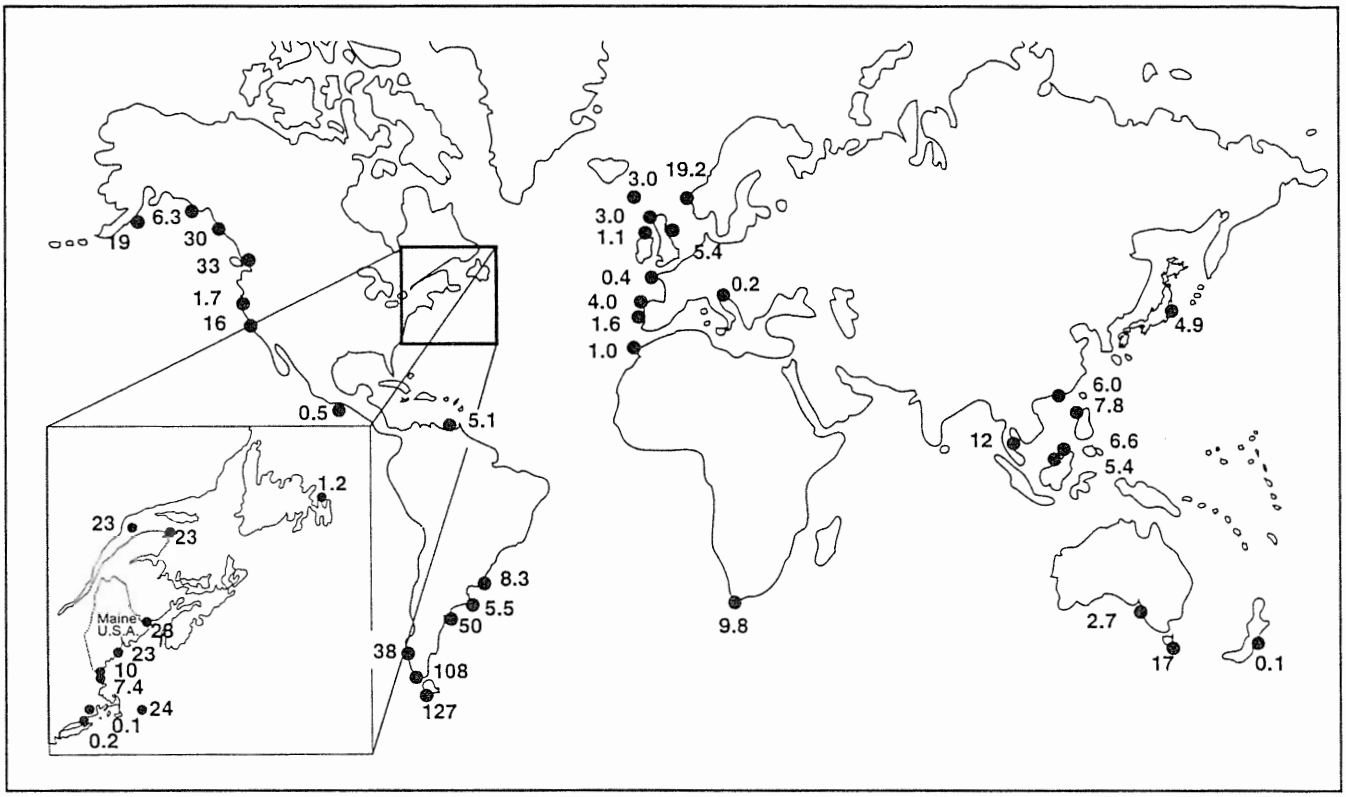

Fig. 1. Maximum historical PSP toxicities (10^3 µg STXeq 100 g^{-1}) generally determined by mouse bioassay throughout the world in mussels [*Mytilus* spp. (*californianus, edulis, galloprovincialis* or *chilensis*), *Perna* spp. (*viridis, perna*), *Choromytilus* spp. (*meridionalis, palliopunctatus*)] [from Bricelj and Shumway (1); see also for detailed sources] A conversion factor of 0.2 µg STXeq per mouse unit (MU) used where values reported in MU.

Interspecific comparisons are often confounded by microhabitat differences, as occurs when intertidal (e.g. mussel) and subtidal (e.g. scallop) populations are compared.

Due to the difficulty in obtaining reliable comparative data, attempts have been made to classify bivalve species in terms of their sensitivity to PSP on the basis of short-term responses, and thereby infer their relative capacity for toxin uptake. Sensitivity in bivalves has been measured using three different responses: a) neurological, or *in vivo* block of the action potential of nerve fibers exposed to STX [12, 13], b) physiological, primarily the inhibition of clearance (feeding) rates elicited by toxic dinoflagellates, and c) behavioral, such as the inhibition of siphon retraction and the ability to burrow in sediments in infaunal bivalves [9]. The first method, practical only for small sample sizes, has so far only been applied towards ranking of North American species. It effectively showed that sensitivity is generally inversely correlated with the ability to accumulate toxins. Thus, some species, e.g. *M. edulis* and *Placopecten magellanicus*, are relatively insensitive to STX and consequently accumulate high toxin levels [historical maximum of 10.8 x 10^3 µg STXeq 100 g^{-1} for the sea scallop (calculated from [14]), while at the opposite end of the spectrum, the Eastern oyster, *Crassostrea virginica*, which attains low toxicities (maximum = 0.2 x 10^3 µg STX $100g^{-1}$ [15]), is highly sensitive to STX. Discrepancies arise, however, with this classification scheme: e.g., the northern quahog, *Mercenaria mercenaria* is ranked as insensitive, but attains only low to moderate toxicities during red tides [12].

Measurement of feeding rates provides a useful index to compare the toxin sensitivity and thus potential for toxin uptake of various species [16], as well as the response within a species to variation in dinoflagellate cell toxicity [9, 17]. The magnitude of inhibition, relative to a non-toxic control diet, and the threshold of specific toxicity that elicits significant feeding inhibition vary among species. Thus, *M. edulis* shows only two-fold reduction of ingestion rate at toxicities ≥ 26 pg STXeq $cell^{-1}$ [17], while the Pacific oyster, *Crassostrea gigas*, a highly sensitive species, exhibits 10-fold reduction with an *Alexandrium* strain of only 7 pg STX eq $cell^{-1}$ [18]. Limitations of some of these studies include the lack of uniformity of experimental design used, which may preclude direct comparisons among studies, failure to characterize dinoflagellate cell toxicity, and lack of control for the confounding effects of differences in cell biomass or biovolume between control and toxic treatments. Nevertheless, previous feeding studies have led to several important findings. For example, they reveal that populations of the same species with different histories of exposure to PSP toxin (e.g. *M. edulis* from Maine and Rhode Island coastal waters) may differ in their feeding response to toxic cells [16]. They also demonstrated that in sensitive species (e.g. *Mya arenaria and M. mercenaria*) feeding response is strongly influenced by the relative abundance of toxic and non-toxic cells in a mixed phytoplankton assemblage [9, 19]. Thus, higher feeding rates and therefore toxin body burden is achieved when a highly toxic *Alexandrium* isolate occurs in a mixed suspension with non-toxic cells, than when offered as a monospecific diet.

Measurements of cell ingestion rate and toxin absorp-

tion efficiency could also be used to determine the maximum potential for toxin incorporation by a suspension-feeding bivalve. The maximum weight-normalized ingestion rate determined experimentally under *ad libitum* feeding conditions equals 2 x 10^6 cells day^{-1} for a standard bivalve 1 g in total wet weight of soft tissues (Table 2 in [1]). Future advances are expected from application of video-endoscopic methods (Bricelj et al., this vol.) and confocal microscopy which should provide new insights on the mechanisms of capture, transport and rejection of toxic dinoflagellate cells by the bivalves' feeding organs. Results of laboratory feeding studies, and concomitant field monitoring of dinoflagellate concentrations and toxicity in bivalve tissues, as implemented in some regions (e.g. the Galician Rías, Spain, [Moroño et al., this vol.]) could in future be used to develop predictive relationships between water column toxin concentrations and peak shellfish toxicities. This relationship is likely to be more complex and difficult to determine in sensitive species, especially since it is not clear from short-term experiments (typically lasting a few hours) whether such species are capable of acclimation to toxins following more prolonged or repeated exposure. Therefore, monitoring of toxic phytoplankton is unlikely to provide an adequate substitute for direct monitoring of bivalve toxicity. The relative merits and efficacy of phytoplankton and shellfish monitoring, even for resistant species, need to be evaluated.

Differential sensitivity among bivalve species is clearly not related to taxonomic status; its basis, at the biochemical and/or molecular level (e.g. different toxin binding characteristics of receptor sites in the sodium channel pore region, or production of toxin binding proteins) also remains to be determined. Further understanding of interspecific differences in toxin kinetics may in future allow increased implementation of species-specific shellfish harvesting closures, a management strategy presently used in Maine, USA.

Significant (two to ten-fold) individual variation in toxicity may also occur within a bivalve population due to differences in microgeography, e.g. tidal height of natural beds [20], or vertical position in the water column of cultured stocks (e.g. [21]). Little is known, however, about the role of intrinsic factors, such as body size, physiological condition and individual genotype in controlling toxin kinetics. Laby [22] found that juvenile *S. solidissima* attain higher toxicities and toxin accumulation rates, on a weight-specific basis, than adults, and determined that the difference in toxin uptake rates between size classes could largely be explained by the allometric relationship between feeding rate and body mass. In contrast, faster detoxification per unit body mass is expected in actively growing, small/young individuals, due to growth dilution of toxins, than in large adults.

Anatomical compartmentalization of toxins

It is well established that in all bivalves examined so far (reviewed in [1]) the digestive gland or visceral mass contains the bulk (80 to 98%) of the total toxin body burden at the peak of toxification. Also, tissues involved in locomotion, such as the muscular foot, and adductor and pallial muscles, invariably attain toxicities 2 to 3 orders of magnitude below those in viscera, and contain a very small proportion, typically < 1% of the total toxin, despite their relatively large mass in some species. This feature has been exploited in the commercialization of scallops for the North American market. It is less evident, however, that the relative toxin contribution of non-visceral tissues increases monotonically and can even exceed that of the viscera during the course of detoxification [1]. This is especially apparent in species that detoxify relatively slowly, such as *Spisula solidissima*, *Saxidomus giganteus* and *P. magellanicus*. This reciprocal pattern is also apparent in species that detoxify rapidly such as *M. mercenaria* and *Mytilus edulis*, but detoxification to levels below the regulatory level may be accomplished before non-visceral tissues can exceed the % toxin contribution of the viscera. Thus in *M. edulis* the viscera retained 80% of the toxin load even after a month of detoxification [1].

This change in the relative toxin contribution of tissue pools over time serves to emphasize that modelling of toxin kinetics in slow-detoxifying species cannot be restricted to the viscera, and must include the non-visceral tissue component. In this context, it is important to note that only the toxicity of the digestive gland is often reported in studies involving the Japanese scallop, *Patinopecten yessoensis*, and the sea scallop, *P. magellanicus*. Additionally, it suggests that the reported lack of correlation between the toxicity of the adductor muscle (or gonad) and that of the viscera in scallops [23, 24] may be due to the fact that seasonal data, pooled over both toxification and detoxification periods, are typically included

Table 1. Classification of various bivalve species as rapid to moderate, and slow detoxifiers based on their rates of detoxification (calculated from original sources and reviewed by Bricelj and Shumway [1], Table 4; see text for explanation).

	FAST/MODERATE DETOXIFIERS	SLOW - DETOXIFIERS
CLAMS	Mercenaria mercenaria	Saxidomus giganteus
	Meretrix casta	Saxidomus nuttalli
	Mya arenaria	Spisula solidissima
		Soletellina diphos
SCALLOPS	Pecten maximus	Placopecten magellanicus
		Patinopecten yessoensis
MUSSELS	Mytilus edulis	
	Mytilus californianus	
	Choromytilus palliopunctatus	
	Perna viridis	
	Modiolus modiolus	
OYSTERS	Crassostrea gigas	
	Crassostrea iridescens	
	Crassostrea cucullata	
	Ostrea edulis	

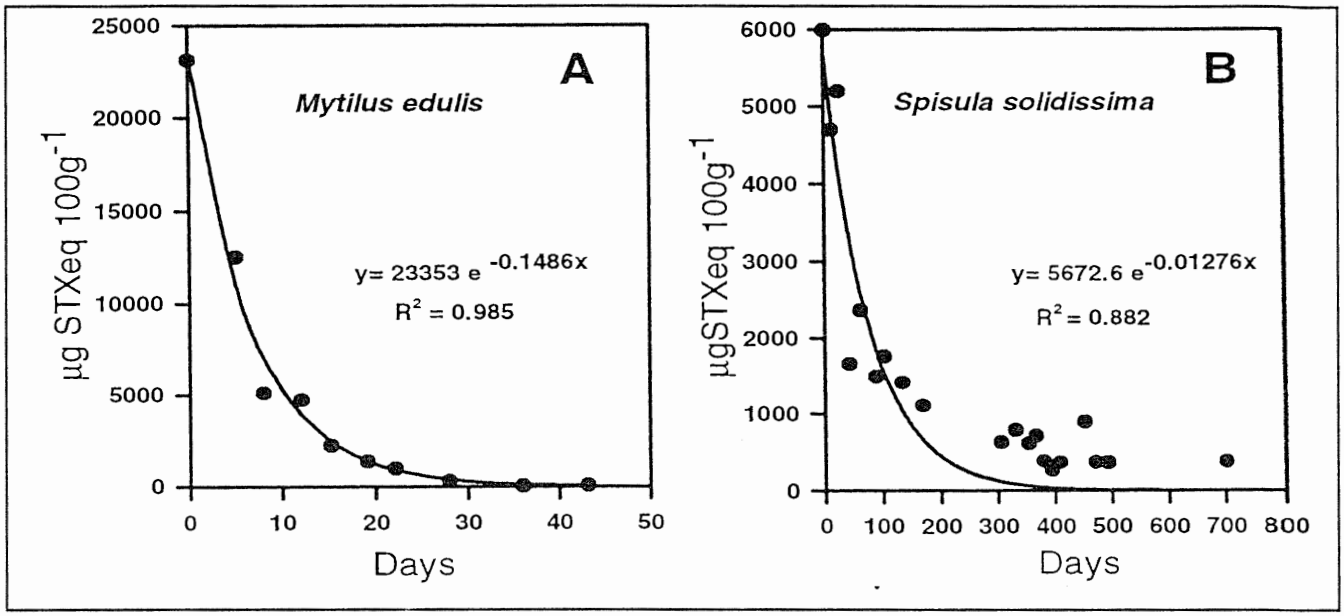

Fig. 2. Representative detoxification trajectories fitted by monophasic exponential curves of the form $y = ae^{-bx}$ (y = toxicity of whole tissues, x = detoxification time, where to = time when peak toxicity is attained (see text) for a fast (A) and slow (B) detoxifying bivalve species. **A.** Blue mussels, *M. edulis* from Gasp, Bay, Canada (calculated from [21]). Estimated time to regulatory level (t_{80}) = 5.1 wks; detoxification rate = 13.8 % toxin loss day^{-1}. **B.** Surfclams, *S. solidissima*, from Georges Bank, USA (calculated from [26] and unpubl. data, Station 3). Measured t_{80} = > 100 wks; estimated using a monophasic, single exponential fit (shown in Fig. 2B) = 48 wks; estimated using a biphasic or two-compartment model, second exponential phase assumed to start 42 days from initial detoxification = 132 wks (not shown). Note that the former poorly fits the data during late detoxification, and greatly underestimates the measured t_{80}. Daily detoxification rate estimated from monophasic model = 1.3%; from biphasic model = 1.2 % for initial rapid phase (< 42 days) and 0.4% for the slower detoxification phase.

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Although existing data are sufficient to allow broad generalization on the bulk compartmentalization of toxins in bivalves, little is known about the exchange rates between tissues, or the localization and binding mechanisms for PSP toxins at cellular and subcellular levels. The production of specific antibodies to PSP toxins may lead to future advances in this area.

Detoxification kinetics

Bivalve species also differ markedly in their capacity for elimination of PSP toxins, which has typically been inferred from the time (t_{80}) required to detoxify to the regulatory level (RL = 80 µg STXeq $100g^{-1}$). However, this parameter does not adequately characterize detoxification kinetics, since it can be correlated with the maximum toxicity achieved [25], and there is no clear correlation between toxin uptake and elimination rates. Therefore, Bricelj and Shumway [1] made an attempt to classify bivalves in terms of their detoxification kinetics, on the basis of their detoxification rates, or % loss of toxin day^{-1} (Table 1). This parameter, when not provided in the original source, was estimated by fitting two-parameter exponential decay equations ($y = a e^{-bx}$, where x = time and y = toxicity) to published tabular data or values determined by digitizing published graphs. Non-linear curve fitting (DeltaGraph user-defined curve option) used an iterative least-squares algorithm. Only laboratory and field data sets were selected in which the absence of toxic cells during detoxification was confirmed, where bivalves were transplanted to toxin-free areas, or there was no evidence of re-toxification, i.e. shellfish toxicity distributions were unimodal. Whole tissues were generally considered, although only the toxicity of viscera was available in some cases (e.g. for *P. yessoensis*). It is important to note that detoxification kinetics is well characterized in some species, which have been extensively monitored, such as *M. edulis*, *M. californianus* and *Mya arenaria*, whereas limited data are available for other species such as *O. edulis*, thus affecting the reliability of calculated parameters. Within a species, detoxification rate did not appear to consistently correlate with peak toxicity [1]. This observation, however, is based on a limited data set, and needs to be verified in future studies specifically designed to test this hypothesis.

Species classified as fast to moderate detoxifiers in Table 1 were found to require ca. 1 to 10 weeks to attain the RL, and eliminated toxins at a rate of about 6 to 17% day^{-1}. Most mussels and oysters fall into this general category. In contrast, slow detoxifiers take 3 months to 2 yrs to attain the RL, and are characterized by detoxification rates typically ≤ 1% day^{-1} (range = 0.3 to 4%). In most cases reviewed, a single exponential adequately fitted the data (a representative example is shown in Fig. 2A). However, in some instances, especially in species that detoxify relatively slowly, such as *P. magellanicus*, *Saxidomus giganteus* and *S. solidissima*, a one-compartment model grossly underestimated the measured t_{80}, or underestimated the toxicity maximum (illustrated in Fig. 2B). Therefore, two-compartment or biphasic analysis, with an initial phase of rapid detoxification, followed by one of slower detoxification, was adopted. A two-compartment model was also shown to improve the fit to toxicity data in field-toxified

M. edulis [27] and *M. galloprovincialis* (Moroño et al. this vol.) and laboratory-toxified *S. solidissima* (Silvert et al. this vol.).

Although the viscera typically detoxify faster than non-visceral tissues, the two compartments identified above do not necessarily correspond to anatomical compartments *per se*, since two-compartment kinetics has been suggested even where only the toxicity of viscera was considered [27]. They should rather be viewed as representing different, potentially co-occurring physiological processes or pathways of toxin elimination (e.g. of more "labile" and "bound" toxins), although their precise identity and contribution to total toxin loss remain speculative to date. Thus toxins may be eliminated via gut evacuation of unabsorbed toxins associated with intact or fragmented dinoflagellate cells in feces, excretion or metabolic fecal loss of absorbed toxins released during breakdown of digestive gland epithelial cells. In conclusion, we identify a clear need for development of pharmacokinetic or bioenergetic models to aid in the interpretation of toxin exchange rates between compartments, and describe the effect of extrinsic factors (e.g. temperature, seston quantity/quality and dinoflagellate toxin composition) and intrinsic factors (e.g. body size/age) on these parameters.

Toxin composition

Differences between the toxin composition of ingested dinoflagellates and bivalve tissues may arise from epimerization of ingested toxins, chemical or enzymatic transformation and/or selective retention of individual toxins. These differences are greatest when dinoflagellate strains are rich in low-potency but highly labile N-sulfocarbamoyl toxins (e.g. [19]). They are also prominent in a few clam species capable of enzymatic production of decarbamoyl toxins from N-sulfocarbamoyl and in some cases carbamate derivatives: *S. solidissima* [9, 28], *Protothaca staminea* [29], *Mactra chinensis* and *Peronidia venulosa* [30].

The conversion of low-potency derivatives to corresponding carbamate or decarbamoyl toxins of high and intermediate specific toxicity respectively, could potentially result in an increase in net toxicity in bivalve tissues and thus apparent reduction in detoxification rates. The significance of this effect, however, remains to be demonstrated. Changes in toxin profiles between dinoflagellate cells and bivalves are also likely to limit our ability to predict net toxicity in shellfish from measurements of water column toxin concentration and composition, and will require determination of *in vivo* rates of toxin transformation. The role of gut microflora in the interconversion of PSP toxins, proposed by Kotaki [31], and biodegradation to non-toxic derivatives, also needs to be investigated.

Inter-specific differences in the capacity for toxin transformation may ultimately be exploited to provide useful information on toxic bloom dynamics. Species characterized by limited conversion, e.g. *Mya arenaria*, and *Mytilus edulis*, can serve to identify an unknown toxigenic source, whereas species which undergo marked changes in toxin composition (e.g. *S. giganteus*) are likely to be useful in predicting the timing and duration of a toxic bloom.

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