The biological fate of paralytic shellfish toxins:

Why is toxicity so variable?
Introduction

Toxic effects resulting from paralytic shellfish toxins, particularly saxitoxin (STX) (fig. 1), vary significantly across taxonomic groups, species, and even individuals within populations. It is certain that biological processes occurring in both the toxin producers (marine dinoflagellates and fresh water cyanobacteria, see cover photos) and the contaminated organisms play a role in these observations (Landsberg 2002). My interests lie in the mechanisms responsible for the apparent variations in toxicity. In particular, why are some organisms susceptible to Paralytic Shellfish Poisoning (PSP) while others escape deleterious effects? Also, what processes are involved in the accumulation and depuration of STX? In order to address these questions a basic understanding of the molecular properties of PSP toxins and the mechanism of toxicity is necessary.

Paralytic shellfish toxins are actually a family of water-soluble compounds derived from STX, which include neosaxitoxins and gonyautoxins (Daranas et. al. 2001). Modification of STX, by addition or removal of hydroxyl, carbamyl, and/or hydroxysulfate groups, produces a suite of 21 toxins with a wide range of potencies (Plumley 1997). The most potent of the paralytic shellfish toxins are the carbamate toxins (STX, neoSTX, and gonyautoxins 1-4), followed by the decarbamoyl (dc) toxins (i.e. dc-STX, dc-neoSTX, etc.), and the least potent are the N-sulfocarbamoyl toxins (Bricelj and Shumway 1998). As such, variable toxin biosynthesis in STX-producing species results in unique toxin profiles and differing net potencies (Plumley 1997). In fact, the intraspecific toxicity of each algal strain is thought to fluctuate with environmental and growth conditions (Bricelj and Shumway 1998).
STX and its derivatives (STXs) exert their neurotoxic effects by binding to membrane proteins in neurons and muscle cells. Specifically, STXs bind with high affinity to an extracellular site on voltage-gated sodium channels, effectively blocking the flow of sodium ions into excitable cells (Daranas et al. 2001). Consequently, action potentials are terminated and signal transmission between neurons is inhibited. When administered to mice orally and intraperitoneally, the LD$_{50}$ for these compounds are 263µg/kg (Price et al. 1991) and 10µg/kg (Landsberg 2002), respectively. Symptoms of intoxication include numbness and tingling, weakness, ataxia, inefficient ventilation, and with doses as small as one milligram respiratory paralysis and death may occur (Landsberg 2002). It is important to note that tetrodotoxin (TTX), commonly found in puffer fish, exhibits the same mechanism of toxicity and similar potencies as STX, and the two can co-occur in nature (Sato et al. 2000 and Kodama et al. 1983). Therefore, care must be taken to distinguish between STX and TTX poisoning in situations where an organism may have acquired both. Armed with this basic, yet fundamental, understanding of paralytic shellfish toxins one can begin to explore the biological response of those organisms potentially threatened by PSP.

**Discussion**

In pursuing the biological fate and effects of STXs in marine organisms, both inter- and intra-specific variability can arise from several factors. Differences in exposure, accumulation, defense mechanisms and detoxification rates can be attributed to the toxin producers and/or consumers. To demonstrate this global variability, measurements of maximum toxicity in mussels (*Mytilus* spp.) range from 100 to 127x10$^3$ µg STX equivalents 100g$^{-1}$ of tissue (fig. 2) as reported by Bricelj and Shumway (1998). Furthermore, variable toxin concentrations have been

*Exposure*

The first, and most obvious, source of variability is how much toxin is present in the environment and available to an organism. The majority of variation that arises as a result of toxin exposure can be related to the bloom dynamics of the toxic species, however, spatial differences within a population can be important as well. There are several properties of toxic algal species that can affect toxicity, including cell concentration (i.e. # of cells L⁻¹), bloom duration, cellular toxicity (pg STX equivalents cell⁻¹), and toxin profile.
Exposure is proportional to the size and duration of a bloom, which is influenced by both bottom-up and top-down controls. The larger the toxic bloom and the longer it persists the more toxin is available for uptake. Anderson (1997) reports significant regional differences in bloom toxicity of *Alexandrium* spp. due to circulation patterns and local mixing in the northeastern US. Shellfish in two geographically nearby populations frequently demonstrated different toxicities. In fact, one would be highly toxic while the other contained no measurable toxin. Anderson hypothesizes that these distinct differences are due to a coastal current transporting cells away from the toxin-free population, thus lack of exposure was responsible for the variations. An alternate study in coastal Maine related low *Alexandrium* concentrations (<25 cell L\(^{-1}\)) to downwelling conditions and weak fronts; this event was associated with minimal toxin in zooplankton and none in shellfish (Turner et. al. 2000). Top-down control due to grazing pressure can affect the magnitude and timing of toxic PSP blooms. The impact of grazing, however, is thought to be dependent on which species are the dominant grazers (Turner 1997).

Additionally, exposure can be regulated by variance in cellular toxicities and toxin profiles. Research indicates that the relative toxin composition is characteristic of each microalgal strain or species, while the concentration of STXs varies with growth phase (Landsberg 2002). For example, analysis of Australian strains of the cyanobacterium, *Anabaena circinalis*, and Japanese strains of *Alexandrium tamarense* revealed toxin profiles dominated by the N-sulfocarbamoyl toxins (the least potent of STXs) and carbamate toxins (the most potent of STXs), respectively (Negri et. al. 1997 and Ichimi et. al. 2002). Furthermore, an *A. circinalis* growth experiment demonstrated total toxin concentrations ranged from 570µg g\(^{-1}\) in exponential phase to 3400µg g\(^{-1}\) in late stationary phase (Negri et. al. 1997).
Alternatively, the spatial distribution of a population of exposed organisms can determine toxin variability. This is evident particularly in bivalves in which both horizontal and vertical gradients can result in uneven distribution of STXs. Clearly, intertidal shellfish exposure will change with the ebb and flow of the tide, as some will be submerged longer than others. Furthermore, differences in maximum toxicity have been observed in continuously subtidal mussels in nearshore versus offshore samples, with those offshore being up to 100 times more toxic (Bricelj and Shumway 1998).

Accumulation

The accumulation and tissue distribution of STXs in contaminated animals depends on the life history and physiological responses of the organism. The concentration of toxins in the body can be influenced by trophic level, feeding responses, age and/or size, for instance. The detection of STXs in specific body tissues other than the viscera is more likely due to sequestrations mechanisms, such as STX-binding proteins. It is also worth noting that animals insensitive or tolerant to paralytic shellfish toxins may acquire higher toxin loads.

At the onset of a bloom event most of the toxin is found in the viscera. For most planktivorous and carnivorous organisms consumption of toxic prey during a bloom results in the visceral mass, particularly the gastrointestinal tract, containing the highest concentration of toxins (Bricelj and Shumway 1998 and Lehane 2000). In equally exposed individuals, variability in toxin content is thought to be related to size in the case of bivalves. Small mussels (*Mytilus edulis*) and surfclams (*Spisula solidissima*) tend to acquire more toxin per gram of tissue than larger individuals of the same species because their cell ingestion rate per unit biomass is greater (Bricelj and Shumway 1998). In some cases ingestion of the toxin producer, and thus the
amount of toxin, can be regulated by an organism. Several studies have documented that feeding rates decrease when STX producers or STX contaminated prey are the primary food source (Landsberg 2002). Herbivorous copepods show reduced grazing in response to toxic dinoflagellates (*Alexandrium* spp.), however the extent of the response varies across species (White 1981 and Teegarden and Cembella 1996). Similarly, starlings and sea otters were reported to decrease or stop feeding when toxic clams (*Saxidomus giganteus*) were offered as the only food source (Kvitek and Beitler 1988 and Kvitek et. al. 1991).

Toxin retention and distribution in the body after a bloom, or in the case of a persistent bloom, is more tissue specific (as compared to the whole viscera) and varies significantly across species. This variation has been documented extensively in bivalves. For example, in butter clams (*S. giganteus*), surfclams (*S. solidissima*), mussels (*Mya arenaria*), and scallops (*Patinopecten magellanicus* and *P. yessoensis*), STXs are accumulated in the siphon, gills, kidney, and roe, respectively (Bricelj and Shumway 1998). In Atlantic mackerel, paralytic shellfish toxins were observed to accumulate in their livers, and concentrations were correlated with fish age (Castonguay et. al. 1997).

The mechanisms responsible for the sequestration of STXs are somewhat unclear, and thus, it is currently being researched. Binding of STX to melanin in the siphon of the butter clam (*S. giganteus*) was hypothesized in the 1970’s to be the method of sequestration, however, this has yet to be confirmed or denied (Bricelj and Shumway 1998). More recently, STX-binding proteins have been suggested as a means of transporting and/or sequestering these toxins. The most notable of these proteins, saxiphilin, was first identified in the plasma of bullfrogs and binds to STXs with a very high affinity (STX-saxiphilin binding can be 1000 times stronger than STX-sodium channel binding) (Morabito and Moczydlowski 1994). Since then, numerous
species from around the globe have been analyzed for saxiphilin-like activity, a surprising number of them testing positive. Of those with saxiphilin-like activity, which include species of amphibians, reptiles, bony fish, and arthropods, no biogeographic or taxonomic patterns were observed (Llewellyn et. al. 1997). In support of the hypothesis that saxiphilin is responsible for sequestering STX, Morabito and Moczydlowski (1994) found that saxiphilin binding activity is unevenly distributed in bullfrog tissues, specifically, high activity was identified in the kidney, heart, and ovaries. Puffer fish saxitoxin and tetrodotoxin binding protein (PSTBP), another STX-binding protein found in puffer fish, differs from saxiphilin in its ability to bind tetrodotoxin (Yotsu-Yamashita et. al. 2001). Interestingly, no STX-binding activity has been identified in *S. giganteus* or *M. edulis*, known for accumulating exceptionally high concentrations of STX (Llewellyn et. al. 1997). The significance of these proteins and their role in protecting an organism against PSP and/or in sequestration of toxins has yet to be elucidated.

**Detoxification and Depuration**

Like toxin exposure and accumulation, there are many factors that affect the rate of detoxification and depuration of STXs in an organism. Rates of toxin clearance range from just a few weeks to years, and are species specific. Detoxification can occur through egestion, excretion, or biotransformation. Factors that effect clearance rates include ambient temperature, growth, and the availability of food.

After the initial ingestion and toxification of an animal, biological processes begin eliminating toxin from the body. First to occur is digestion of the stomach contents, which can reduce toxin load by egestion of toxic cellular matter and excretion of STXs in the urine (Bricelj and Shumway 1998). In organisms that retain STXs over long periods of time, growth can
reduce the overall body burden by ‘diluting’ the toxin (i.e. $\mu g\ STX\ g^{-1}$ of tissue decreases over time), and is particularly important in species with high growth rates (Bricelj and Shumway 1998). Conversion of STXs to nontoxic compounds can occur by natural reduction or epimerization, and the rates of these reactions will increase as temperature rises (Smith et. al. 2001). In addition, detoxification rates are thought to increase with continued feeding, particularly on nontoxic prey, as this increases the rate of metabolism and gut evacuation (Bricelj and Shumway 1998).

The other mechanism of detoxification is biotransformation, which relies on enzymatic conversions of STXs. In bivalves, some evidence suggests that bacteria may play a role in the biotransformation of STXs, however, the details are not clear (Smith et. al. 2001). In addition, three species of clam have demonstrated the rare ability to convert potent carbamate toxins (i.e. STX, neoSTX, and gonyautoxins) to their corresponding nontoxic decarbamoyl forms (Landsberg 2002). Interestingly, biotransformation can in some cases result in more toxic compounds; under high temperatures and low pH the N-sulfocarbamoyls are readily converted to more toxic carbamates (Bricelj and Shumway 1998). Perhaps, this transformation is employed in S. giganteus, a species that sequesters only STX in its siphon. One consequence of biotransformation is that toxin profiles of the contaminated organisms differ from that of the producer.

**Conclusion**

There are advantages to understanding the dynamics determining the biological fate of paralytic shellfish toxins. As our knowledge of STXs grows, officials can regulate fisheries more precisely and prevent unnecessary closures. Also, we can predict more accurately which
organisms will be affected in the event of a toxic bloom and which will be more of a threat as toxin vectors. Similarly, the presence of toxin in some species closely reflects the timing of a bloom, thus these species could be used to monitor blooms in the absence of phytoplankton surveys.

While these processes explain how toxin accumulation and depuration can influence toxicity, they only lead to hypotheses of why some animals are able to tolerate paralytic shellfish toxins. Behavioral responses to toxin exposure, STX-binding proteins, and biotransformation are likely important adaptations that allow organisms to survive. However, with these answers come new questions. How is it that an organism can detect the presence of STXs with enough accuracy to prevent PSP? Why are STX-binding proteins present in organisms that are hardly, if ever, exposed to STXs and absent in frequently exposed organisms? Why have some organisms evolved to store these deadly compounds, and how do they do it?

To enhance our understanding of paralytic shellfish toxins research efforts should continue to focus on the many mechanisms involved in PSP. Because of the economic value and potential health threat, most research, and therefore most of what we know, concerns bivalves. As such, one suggestion for future research is to examine PSP in other taxa, such as zooplankton and fish. Other interesting topics related to STXs include the involvement of benthic dinoflagellate cysts in PSP, initiation and transport of toxic blooms in the California current and toxicological effects of chronic exposure. After considering all of the biological factors that contribute to PSP variability observed in marine organisms, it is possible to begin to understand the complex nature of the system.
References


Images taken from the following web pages:

*G. catenatum*: http://dinos.anesc.u-tokyo.ac.jp/Hp2002/Small/HABspecies/006gymno-s.jpg

*Alexandrium* sp.: http://www.uio.no/miljoforum/m_alge/art/alexandrium.htm

*A. circinalis*: http://www.chm.bris.ac.uk/motm/antx/antxj.htm