Relative insensitivity of butter clam neurons to saxitoxin: a pre-adaptation for sequestering paralytic shellfish poisoning toxins as a chemical defense

Rikk G. Kvitek, Mark K. Beitler

1 Department of Zoology, University of Washington, Seattle, Washington 98195, USA
2 Division of Aquaculture and Food Science, University of Washington, Seattle, Washington 98195, USA

ABSTRACT: Neurons from the butter clam Saxidomus giganteus and its congener the Washington clam S. nuttalli were shown to be 10 to 100 times more resistant to the dinoflagellate toxin saxitoxin (STX) than those from 4 co-occurring infaunal bivalves, Mya arenaria, M. truncata, Tresus capax and Protobrachia staminea. Only neurons from the venerid clam Humilana kennerlyi proved more resistant than those of Saxidomus spp. No difference in sensitivity to STX was found between butter clams from 3 sites with different histories of toxin contamination (chronically, occasionally or never toxic) suggesting that the resistance of this species to STX is innate rather than acquired with increased exposure. The resistance of butter clam neurons to STX may have permitted the evolution of this species' unique ability to sequester high concentrations of STX, especially in the siphon, for long periods (> 2 yr). Neither of the 2 other venerid clams analyzed, H. kennerlyi and P. staminea, were found to concentrate STX or derivatives in their siphons. Because predators have been shown to reject bivalve prey contaminated with STX, long-term retention of this toxin may have been favored in the butter clam as an acquired chemical defense.

INTRODUCTION

Although many marine species are known to employ algal toxins sequestered from their diets as defensive agents (Faulkner & Ghiselin 1983, Paul & van Alstyne 1988, Scheuer 1990), relatively little is known regarding the means by which these species avoid autotoxicity (van Alstyne & Paul 1988). One source of toxins is dinoflagellates of the genus Protogonyaulax (see Halstead 1978). Global in distribution, this group has been identified as the source of paralytic shellfish poisoning toxins (PSPT), commonly known as 'red tide'. Suspension feeders, especially bivalve molluscs, frequently become contaminated with PSPT when exposed to Protogonyaulax blooms, presenting a significant risk to public health. Contrary to popular belief most bivalves contaminated with PSPT, the toxins are restricted to the digestive gland or gills (Prakash 1971) and are usually lost within 5 to 11 wk (Shumway 1990).

The butter clam Saxidomus giganteus is unique among bivalves with respect to PSPT toxicology. Butter clams have the longest known PSPT retention time (> 2 yr; Shumway 1990), and the majority of the toxins are sequestered in the siphon (Neal 1967, Quayle 1969, Kvitek et al. unpubl.). Furthermore, butter clams appear to preferentially sequester saxitoxin (STX) (Beitel 1988, Beitler & Liston 1990), one of the 2 most lethal PSPT (all STX derivatives) produced by toxic dinoflagellates (Boyer et al. 1986). Recent work also has demonstrated that PSPT sequestered by butter clams can function as an effective feeding deterrent to sea otter (Kvitek et al. unpubl.), avian (Kvitek & Beitler 1988, Kvitek unpubl.) and fish predators (Kvitek et al. unpubl.). These findings are all consistent with the hypothesis that S. giganteus sequesters PSPT as a chemical defense (Kvitek & Beitler 1988, Kvitek et al. unpubl.) in a manner analogous to the monarch butter-
fly's use of milkweed toxins as a deterrent to avian predation (Brower & Fink 1985).

Saxitoxin is a highly lethal neurotoxin, capable of blocking voltage-gated sodium channels at extremely low concentrations, and therefore the propagation of action potentials along invertebrate neurons (Twarog et al. 1972, Strichartz & Castle 1990). If *Saxidomus giganteus* has evolved to utilize ingested STX as a chemical defense, it is likely that they also have evolved or been pre-adapted (i.e. exapted, sensu Gould & Vrba 1982) to avoid autotoxicity. The first objective of this study was to determine if butter clams were more resistant to STX than other members of its 'guild' (Root 1967) of infaunal suspension-feeding bivalves. The second objective was to determine whether the level of sensitivity to STX in butter clams is innate or varies with history of exposure to PSPT. The final objective was to determine to what extent the butter clam's unusual toxicology (STX resistance if demonstrated, and the ability to sequester high levels of PSPT, especially in its siphon) is shared by other venerid clams within its guild. These included (1) *S. nuttalli*, the butter clam's California congener which also sequesters the majority of PSPT in the siphon (Whitefleét et al. 1985) and appears to retain it for many months (D. Price, Environmental Planning and Local Health Services, Department of Health Services, Santa Rosa, California, unpubl.); (2) *Protothaca staminea*, which appears to enzymatically transform the carbamate PSPT into less toxic derivatives (Sullivan et al. 1983) and to retain them for < 5 wk (Shumway 1990); and (3) *Humilaria kennerlyi*, about which nothing is known regarding toxin retention or sensitivity to PSPT.

As an assay for the relative sensitivity of bivalves to STX, we chose to determine the concentration of STX required to block neuronal action potentials (Twarog et al. 1972). This is because the principal action of STX is to block sodium channels and thereby, in invertebrates, to prevent neural transmission via the propagation of action potentials. In the study by Twarog et al. (1972) most of the species were tested with the puffer fish toxin, tetrodotoxin (TTX), rather than STX. Although similar in action to STX, TTX is not a component of PSPT, nor did bivalve nerves tested with both toxins show comparable sensitivities. Of the 8 species Twarog et al. (1972) did test with STX, only 2 were infaunal clams, and of these only *Mya arenaria* is indigenous to the Pacific and co-occurs with *Saxidomus giganteus*.

**METHODS**

**STX sensitivity.** The level of STX required to block neuronal action potential transmission in each of 7 species of infaunal bivalves was determined via extracellular recordings of nerve preparations serially exposed to increasing STX concentrations.

**Bivalves:** The clams used in this study were all collected between March and November 1989, and held at 10 °C in recirculating seawater aquaria prior to testing. *Saxidomus giganteus* were taken from 3 sites with different histories of PSPT exposure. Butter clams at the Sequim Bay, Washington, USA, collection site (Middle Ground) have been chronically toxic for many years, and consistently well above harvest closure levels (80 µg STX equivalents per 100 g tissue) for at least the last 14 yr (G. Skow, Washington State Department of Social and Health Services, unpubl.). Middle Ground is a low, sand and gravel intertidal island near the mouth of the bay. Toxin levels in intertidal butter clams at the Mukilteo, Washington, site have been below detectable concentrations since 1983, and have not exceeded closure level since 1979 (G. Skow, Washington State Department of Social and Health Services, unpubl.). In Hood Canal, Washington, butter clams as well as other monitored bivalve species have not been above closure level for > 30 yr of monitoring and there is no history of PSP in this area (Nishitani & Chew 1988). Butter clams collected at all sites had shell lengths ≥ 80 mm with > 6 annual growth rings.

*Saxidomus nuttalli* were collected from Elkhorn Slough, Monterey Bay, California, USA, where no history of PSPT contamination is known. All other species were collected from Puget Sound, Washington, sites, and are species that commonly co-occur with *S. giganteus*. *Humilaria kennerlyi* and *Mya truncata* were collected near Hall Island (toxic history unknown). *M. arenaria* were taken in Carr Inlet (first shellfish closure due to PSPT occurred in 1988). *Tresus capax* were collected from Sequim Bay, Mukilteo, and Carr Inlet. *Protothaca staminea* came from Sequim Bay and Carr Inlet.

**Action potential recordings:** The methods used were modified from Twarog et al. (1972). Extracellular recordings were made of compound action potentials evoked in sections of the commissure connecting the cerebral and pedal ganglia (C-P nerve) removed from the visceral mass of each test individual. Three or more individuals of each species were tested except *S. nuttalli* for which only 2 clams were available. Lengths of up to 2 cm of the C-P nerve were placed in a 6-cell lucite test chamber (Fig. 1). Cells were isolated with a mixture of petroleum jelly and heavy mineral oil, and each filled with physiological saline solution (Twarog 1967). All electrical connections with the saline cells were via chlorided silver wire inserted into agar bridges. The bridges were made from bent glass 50 µl micropipets filled with a 1 to 2 % agar and saline solution.

Action potentials were evoked by single supramaxi-
mal shocks at intervals of 1.3 s and recorded on a storage oscilloscope. Serial STX test solutions (saline plus toxin) were made up from 3.84 mg STX provided by Sherwood Hall, United States Food and Drug Administration (US FDA), Washington, D.C. Test solutions of $10^{-3}$ g STX ml$^{-1}$ were stored at $-10^\circ$C and $+10^\circ$C for 6 mo and quantified by HPLC analysis to evaluate chemical stability. Concentrations were increased from $10^{-8}$ g ml$^{-1}$ in the toxin cell of the nerve chamber (Fig. 1) until a total block of the action potential was achieved within 20 min, or until a maximum concentration of $10^{-3}$ g ml$^{-1}$ was reached. Unless the block was reversible by washing off toxin with saline, the results were discarded.

**Venerid toxicity.** The toxicological comparison of *Saxidomus giganteus*, *Humilana kennerlyi* and *Protothaca staminea* was based on 2 parameters: (1) whole body toxin concentrations in STX equivalents of co-occurring populations of each species, and (2) the proportion of total toxin in STX equivalents sequestered in the siphon of each species. Toxin analysis involved both high performance liquid chromatography (HPLC) (Sullivan & Weckel 1986) and the mouse bioassay (AOAC 1984, analyses courtesy of the Shellfish Program, Washington State Department of Health, Seattle, Washington, and the Shellfish Program, Division of Environmental Health, Alaska Department of Environmental Conservation, Palmer, Alaska).

HPLC analyses were conducted in triplicate employing either a mixed paralytic shellfish toxin standard coded MS-33, diluted 1 : 20 in 0.05 N HOAc (US FDA, Seattle) or a STX secondary standard containing 0.90 µM STX in HCl (pH 3.5) prepared from a STX primary standard (100 µg STX ml$^{-1}$ HCl, pH 3.5; US FDA, Cincin-
nati, Ohio). The concentrations of decarbamoyl gonyautoxins (GTX) II and III were calculated by assuming the fluorescence of decarbamoyl and corresponding carba-
mate toxins were identical (Sullivan et al. 1983). This assumption was made because no standards for the decarbamoyl toxins were available. Toxicities for carba-
mate and decarbamoyl toxins were calculated using appropriate conversion factors from Boyer et al. (1986) and Sullivan et al. (1985) respectively. Whole clams, siphons and remaining bodies were analytically weighted and homogenized in 0.10 N HoAc (1 : 2, w/v). Homogenates were centrifuged and resulting suspens-
ants were filtered and frozen until HPLC analysis according to the methods of Kvitek & Beitel (1988).

For the *Saxidomus giganteus* and *Humilana kenner-
lyi* comparison, ≥ 3 clams of each species were collected simultaneously from 9 locations at which they co-occurred in southeastern Alaska during March 1988 and June 1989 (sites and sample sizes are presented with results). All clams were frozen whole immediately following collection, held at $-30^\circ$C, and later thawed and analyzed. Whole clams collected in 1988 and 1989 were analyzed by HPLC and mouse bioassay respectively. Toxic concentrations were compared with a paired t-test, and linear regression. In addition to whole clam analyses, *H. kennerlyi* from 3 of the sites (Sea Otter Sound, N = 1 clam; Porpoise Is., N = 3 clams; The Sisters, N = 5 clams) had their siphons analyzed sepa-
rately from remaining tissues using HPLC. Siphons and bodies were separated after thawing.

*Saxidomus giganteus* and *Protothaca staminea* were collected from Middle Ground, Sequim Bay, on June 8, 1990. *S. giganteus* whole clams (N = 6 clams) and siphons only (N = 11 siphons) were analyzed by mouse bioassay. *P. staminea* siphons (3 groups of 5 siphons) and bodies without siphons (3 groups of 5 corresponding bodies) were analyzed by HPLC. In addition, 31 whole *P. staminea* were pooled and analyzed by mouse bioassay. Comparison of differences in total toxicity between co-occurring populations of *S. giganteus* and *P. staminea* were based on Washington State Department of Health PSP monitoring data (McCallum 1989). Toxicity values for 39 paired samples collected at 4 Washington beaches (Alki Beach; Travis Spit, Sequim Bay; Carkeek Park; and Burly Lagoon) were compared with a paired t-test, and linear regression.

**RESULTS**

**STX sensitivity**

Saxitoxin concentrations required to block action potentials were the same for *Saxidomus giganteus* and its congener, *S. nuttalli* (Fig. 2), and were 10 to 100
times higher than for 4 of the other 5 species tested (Table 1). No relationship was found between frequency or intensity of STX contamination and sensitivity to STX in *S. giganteus*. Action potentials in butter clams from sites with different histories of toxin contamination were blocked at identical concentrations of STX (Table 1). Indeed, at the resolution of this experiment, there was no variation in the STX concentrations required to block and partially block action potentials for all *Saxidomus* individuals tested. This was also true for each of the other species tested.

Although neural sensitivity to STX was consistent within the genus *Saxidomus*, this was not true at the family level in which sensitivity to STX spanned 3 orders of magnitude. *Humilaria kennerlyi* and *Protothaca staminea*, the other 2 venerid species tested, proved to be the most and least resistant respectively (Table 1). Action potentials were only partially blocked in *H. kennerlyi* at $10^{-3}$ g STX ml$^{-1}$, the highest concentration used, whereas action potentials of *P. staminea* were partially blocked at $10^{-7}$ and fully blocked at $5 \times 10^{-6}$ g STX ml$^{-1}$.

The concentration of the $10^{-3}$ g STX ml$^{-1}$ test solutions stored at $-10^\circ C$ and $+10^\circ C$ were equal after 6 mo. Test solution stability was also indicated by the consistent response of butter clam nerves to the test solutions over the entire course of the study.

**Venerid toxicology**

Total toxicity and the proportion of PSPT sequestered in the siphon was consistently higher in *Saxidomus giganteus* than in co-occurring *Humilaria kennerlyi* and *Protothaca staminea*. In paired samples from southeast Alaska, *S. giganteus* was significantly more toxic than *H. kennerlyi* (paired t-test, $t = 5.73$, df = 77, Table 1).

---

**Table 1.** Blockage and partial blockage of the compound action potential in the cerebral-pedal connective occurred at identical saxitoxin (STX) concentrations in butter clams (*Saxidomus giganteus*) from 3 locations in Puget Sound, Washington, and its congener the Washington clam (*S. nuttalli*) from the Elkhorn Slough, Monterey Bay, California. Butter clams from sites with different histories of paralytic shellfish poison contamination did not differ in their sensitivity to STX. Five other infaunal clam species collected from Puget Sound and commonly found with the butter clam throughout its range are listed in order of increasing sensitivity to STX.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample site</th>
<th>$10^{-8}$</th>
<th>$10^{-7}$</th>
<th>$10^{-6}$</th>
<th>$5 \times 10^{-6}$</th>
<th>$10^{-5}$</th>
<th>$5 \times 10^{-5}$</th>
<th>$10^{-4}$</th>
<th>$5 \times 10^{-4}$</th>
<th>$10^{-3}$</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Saxidomus giganteus</em></td>
<td>Sequim (always toxic)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Mukilteo (non-toxic)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Hood Canal (never toxic)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td><em>Saxidomus nuttalli</em></td>
<td>Monterey Bay</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td><em>Humilaria kennerlyi</em></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td><em>Mya truncata</em></td>
<td></td>
<td>0</td>
<td>0</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td><em>Mya arenaria</em></td>
<td></td>
<td>0</td>
<td>0</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td><em>Tresus capax</em></td>
<td></td>
<td>0</td>
<td>0</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td><em>Protothaca staminea</em></td>
<td></td>
<td>0</td>
<td>0</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3</td>
</tr>
</tbody>
</table>

+: Block; (+): partial block; 0: no effect
Kvitik & Beitler: Insensitivity of butter clams to saxitoxin

p = 0.0001), containing on average 360 % (SD = 180) more μg STX equivalents per 100 g (Table 2). Furthermore, PSPT levels in H. kennerlyi did not vary significantly along a spatial gradient of S. giganteus toxicity (Fig. 3a). S. giganteus was also significantly more toxic than P. staminea (paired t-test, t = 4.37, df = 17, p = 0.0004), with butter clams containing 530 % (SD = 330, N = 40) more μg STX equivalents per 100 g (Fig. 3b). However, P. staminea toxicity did increase with that of butter clams especially when the latter contained > 400 μg STX equivalents per 100 g (Fig. 3b).

The siphons of Humilaria kennerlyi analyzed by HPLC contained STX as did the bodies of clams from Porpoise Is. (Table 2). Bodies of clams from The Sisters contained GTX II and STX and those from Sea Otter Sound possessed GTX I–III and STX (Table 2). Toxicities of the siphons of H. kennerlyi were not significantly different from those found in the bodies alone (Mann-Whitney U-test, p = 0.51) (Table 2). This was not the case with P. staminea. HPLC analysis revealed no detectable toxins in the siphons of those clams having whole body toxicity of 35 ± 10 (SD) and 129 μg STX equivalents per 100 g as determined by HPLC and mouse bioassay respectively. The 3 groups of P. staminea bodies contained mainly decarbamoyl GTX II and III (1.37 ± 0.793 nmol g⁻¹), intermediate concentrations of STX or decarbamoyl STX (0.67 ± 0.12 nmol g⁻¹) and trace levels of GTX II (0.057 ± 0.024 nmol g⁻¹).

## DISCUSSION

Our results show that the neurons of the butter clam are 10 to 100 times more resistant to STX than those of most other members of its guild (Table 1). In addition, our results can be extended by comparison with the work of Twarog et al. (1972) who found the neurons of 5 of 8 bivalve species tested to be 5 to 500 times more sensitive than butter clams and only 2 species to be

Table 2. Toxin concentrations in μg STX equivalents per 100 g found in whole bodies of Saxidomus giganteus and Humilaria kennerlyi collected simultaneously at sites in southeastern Alaska. Clams collected in 1988 were analyzed with HPLC, and those collected in 1989 with the mouse bioassay. Toxicities are recorded as the number of gill plates of the mouse killed by the toxins.

<table>
<thead>
<tr>
<th>Date</th>
<th>Site</th>
<th>S. giganteus</th>
<th>H. kennerlyi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole clam N</td>
<td>Whole clam N</td>
<td>Body only N</td>
</tr>
<tr>
<td>March 1988</td>
<td>The Sisters 200</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Porpoise Is. 179</td>
<td>6</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Pt. Frederick 134</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Explorer Basin 46</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>June 1989</td>
<td>Sea Otter Sound 438</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Walker Bay 242</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Shaken Bay 165</td>
<td>3</td>
<td>= 32</td>
</tr>
<tr>
<td></td>
<td>Whale Bay 118</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Thorn Is. 43</td>
<td>3</td>
<td>= 32</td>
</tr>
</tbody>
</table>
more resistant. The butter clams’ resistance appears to be innate, rather than related to the frequency or intensity of PSPT contamination (Table 1). Butter clams taken from a chronically toxic site (Sequim Bay) were no more resistant than those from Mukilteo, where no detectable toxin has been found during the last 6 yr, and toxin levels have not exceeded 68 μg STX equivalents per 100 g in 10 yr. This was also true of butter clams collected in Hood Canal where toxin levels requiring harvest closure have never been reported (Nishitani & Chew 1988).

High neuronal resistance to STX (Table 1), and the ability to sequester high levels of PSPT in the siphon for many months (Quayle 1969, Whitefleet et al. 1985, Shumway 1990, D. Price unpubl.), are traits shared by the butter clam and its congener, Saxidomus nuttalli, but do not extend to other venerid clams within their guild. Although neurons form Humilaria kennerlyi are even more resistant to STX than those from Saxidomus spp. (Table 1), H. kennerlyi was not found to sequester the majority of PSPT in its siphon (Table 2), nor does it become as toxic as co-occurring butter clams (Table 2, Fig. 3); Protogonyaulax staminea was determined to be > 100 times more sensitive to STX than Saxidomus spp. (Table 1), and may therefore cope with carbamate toxins (GTX I–IV, NEO and STX) from ingested Protogonyaulax spp. by decarbamoylating them into less toxic metabolites, a trait not found in butter clams (Sullivan et al. 1983). This is supported by the predominance of decarbamoyl GTX II and III detected in the bodies of P. staminea. This species may also reduce its exposure to PSPT by rapidly depurating the toxins. Sullivan (1982) noted P. staminea removed ingested PSPT in < 4 wk. In contrast, the butter clam sequesters STX for ≥ 4 wk in the siphon after ingesting GTX I–IV and NEO (Beitler & Liston 1990). Thus, it is not surprising that in PSPT-contaminated areas littleneck clams retain much less toxin than butter clams (Fig. 3), and no toxin is detectable in the siphon of the former species.

Of those species for which data are available, Saxidomus giganteus has the longest known toxin retention time (Shumway 1990) and its neurons exhibit one of the highest resistances to STX (Table 1; Twarog et al. 1972). However, the relative resistance of neurons to STX does not necessarily indicate immunity of a bivalve species to PSPT. Gaines & Shumway (1988) have shown that even those species determined by Twarog et al. (1972) to have a neuronal resistance to STX ≥ butter clams (Placopecten magellanicus, Mercenaria mercenaria, and Mytilus edulis) exhibit strong negative responses when exposed to Protogonyaulax spp. P. magellanicus and M. mercenaria increased valve closure and reduced filtration rates. M. edulis from Rhode Island, USA, and Spain which had never been exposed to Protogonyaulax showed reduced filtration and byssus production, however M. edulis from Maine, USA, which had been exposed previously to Protogonyaulax exhibited no response suggesting regional differences based on history of exposure.

Similar experiments have not yet been conducted with Saxidomus spp. However, given the range of negative responses shown by many other less resistant bivalve species (Gaines & Shumway 1988), the ability of butter clam neurons to function even when exposed to high concentrations of STX may well be an adaptation to life in areas of chronic toxic blooms such as southeastern Alaska (Nishitani & Chew 1988). Although other, less resistant, bivalve species are also abundant in these same areas, some may cope with PSPT metabolically (e.g. enzymatic transformation as in Prototitaca staminea; Sullivan et al. 1983) or experience reduced growth rates due to increased valve closure, decreased feeding and other costs associated with exposure to toxic blooms (Gaines & Shumway 1988).

The butter clam therefore, may have been pre-adapted or exapted to sequester and use STX as a chemical defense by virtue of higher neural tolerance. Provided that toxin concentration in the clam’s blood does not exceed the level required to block action potentials, PSPT from ingested dinoflagellates could readily be transported from the digestive gland to siphon epithelium without affecting nerve function. This pathway is consistent with the observation that the proportion of toxin sequestered in the butter clam siphon is highest following the period of initial uptake—often several weeks later (Neal 1967, Beitler & Liston 1990). Butter clams theoretically could transport 10 to 100 times more STX via their blood than most other bivalve species by virtue of their decreased STX sensitivity (Table 1).

In addition, PSPT has been shown to be an effective deterrent to consumption of butter clams by important predators (sea otters, glaucous-wing gulls, and siphon nipping fish; Kvitek et al. unpubl.). However, because sea otter and avian predators kill their prey, group selection must be invoked if they are to be credited for the evolution of PSPT as a defensive agent in butter clams. A more likely evolutionary mechanism is siphon nipping by fish. The vulnerability of fish to PSPT is well documented (White 1981a, b) and recent work has shown fish to have highly sensitive, STX-specific gustatory receptors (Yamamori et al. 1987, 1988). Because it is the siphon of a contaminated butter clam that accounts for the majority of this species’ total toxicity (60 to 80 %) (Neal 1967, Quayle 1969, Price & Lee 1972, Kvitek et al. unpubl.), with the highest toxicities present at the distal end (Quayle 1969) and outer surface of the siphon (Beitler unpubl.), an experienced siphon nipper should be able to distinguish between toxic and
non-toxic prey prior to cropping any tissue, as do some avian predators foraging on chemically defended butter-flies (Brower & Glazier 1975). Furthermore, Peterson & Quammen (1982) reported that the growth rate of Protodraco staminea increased by > 100% when siphon-nipping fish were excluded, suggesting a potent selective force favoring evolution of siphon defense. Once acquired, the ability to sequester these extremely lethal neurotoxins as a defense against siphon croppers could then function as an effective deterrent to total predators such as sea otters and birds, and possibly invertebrates.

Acknowledgements. We are most deeply indebted to D. Baldwin, L. Barlow, J. Coombs-Hahn, L. Dewey-Olsen, K. Graver, L. P. Fink, L. S. Paine, J. Palka, R. Reinstatler, S. Shumway, G. Skow, L. Simonetti, and B. Twarog for their technical support and advice, without which this work would not have been possible. We also thank S. Hall, J. Sullivan, and J. Gilchrist of US FDA for generously providing the toxins for this study, and D. Carney, J. Sullivan, and J. Oliver for their tireless help in the field. Funding was provided by a National Science Foundation grant to J. Oliver (DPP-8619934). National Audubon Graduate Student Research and American Museum of Natural History Lerner-Gray Fund for Marine Research awards to R. Kvitek, University of Washington Graduate School Research Fund and National Science Foundation awards to R. T. Paine, and a Washington Sea Grant to J. Liston.

LITERATURE CITED


This article was presented by Professor K. Banse, Seattle, Washington, USA


Manuscript first received: June 13, 1990
Revised version accepted: September 25, 1990