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SURVEY OF PARALYTIC SHELLFISH POISON AND DOMOIC ACID IN PUGET SOUND PREDATORY GASTROPODS

JOHN C. WEKELL,[1] ROSEANNE M. LORENZANA,[2] MARA HOGAN,[3] AND HAROLD BARNETT[1]

[1]*National Oceanic and Atmospheric Administration*
National Marine Fisheries Service
Northwest Fisheries Science Center
Utilization Research Division
2725 Montlake Blvd. East
Seattle, Washington 98112

[2]United States Environmental Protection Agency
1200 Sixth Ave. ES-098
Seattle, Washington 98101

[3]*Natural Science Division*
Southamptom College, LIU
Southampton, New York 11968

ABSTRACT Two predatory gastropods, moonsnails (*Polinices lewissi*) and frilled dogwinkles (*Nucella lamellosa*), were collected in the Puget Sound basin in the summer and fall of 1994 and in the spring of 1995. Analyses indicated the presence of paralytic shellfish poison (PSP) toxins in these gastropods in all sampling periods; however, no domoic acid was detected in any of the samples. Other species of molluscan shellfish and a species of crustacea, considered possible prey and/or indicators of PSP in the area, were also collected. In September 1994, levels of PSP in moonsnails collected from Agate Passage averaged 145 μg of saxitoxin (STX) equivalents (equiv.)/100 g; butter clams taken from the same area at the same time averaged 73 μg of STX equiv./100 g. In October 1994, when a local monitoring station indicated the presence of PSP in Mystery Bay, a collection and PSP analyses of molluscan shellfish yielded the following average values: dogwinkle (*N. lamellosa*) averaged 72 μg of STX equiv./100 g; blue mussels (*Mytilus edulis*), 652 μg of STX equiv./100 g; Pacific oyster (*Crassostrea gigas*), 45 μg of STX equiv./100 g; northern horse mussel (*Modiolus modiolus*), 48 μg of STX equiv./100 g; and snails (*Searlesia dira*), 50 μg of STX equiv./100 g. In April 1995, another collection and analyses of shellfish from Agate Passage showed that moonsnails averaged 48 μg of STX equiv./100 g, and butter clams (*Saxidomus giganteus*) averaged 35 μg of STX equiv./100 g (range, 0–77 μg of STX equiv./100 g); Pacific littleneck clams (*Protothaca staminea*) and red rock crab (*Cancer productus*) did not contain PSP toxin. Modifications of the sample preparation methods required for the analyses of samples are described.

KEY WORDS: Paralytic shellfish poison, gastropods, domoic acid

INTRODUCTION

In the Puget Sound area of Washington, the consumption of marine invertebrates has increased, in part, because of an increase in ethnically more diverse population and a desire on the part of consumers to try an increased variety of seafoods (Carney and Kvitek 1991). Recent site surveys and beach interviews of shellfish diggers show that predatory marine snails are collected from Puget Sound beaches (Carney and Kvitek 1991). It was found that the moonsnail (*Polinices lewissi*) and other gastropods, such as the dogwinkle (*Nucella lamellosa*), were being noncommercially harvested. In particular, the moonsnail probably because of its size and accessibility to shore gathering, is a popular harvested species. In their 1990 survey, it was estimated that the annual recreational harvests of the moonsnail and dogwinkle, *Nucella* sp., were 21,000 and 119,000 individuals, respectively (Carney and Kvitek 1991).

In the Puget Sound, the Washington Department of Health monitors shellfish from beaches and caged mussel stations for the presence of paralytic shellfish poison (PSP). Beaches are closed to harvesting when PSP toxin levels equal or exceed 80 μg of saxitoxin (STX) equivalent per 100 g (80 μg of STX equiv./100 g) of shellfish tissue. In humans and higher vertebrates, the PSP toxins are potent neurotoxins.

Since predatory marine snails prey on clams that are known to acquire the marine biotoxins associated with PSP, it is not unreasonable to expect that they too could accumulate these toxins. However, little is known about the human risk of PSP intoxication due to the consumption of these animals. Two recent reviews (Matter 1994, Shumway 1995) discussed PSP and its distribution in the marine food web, with emphasis on predatory gastropods. White et al. (1993a and 1993b) reported toxicity at levels near 3,000 μg of STX equiv./100 g tissue in northern moonsnails (*Euspira heros*) and waved whelk (*Buccinum undatum* L.) taken from the Georges Bank. The northern moonsnails were slow to depurate the toxin, and there was a high individual variability of toxicity (White et al. 1993b). Worms (1993) also detected PSP toxins in the northern moonsnails and suggested that their slow depuration rate may be attributed to their slow metabolic rate. He also suggested that predatory snails may be able to maintain detectable toxin levels throughout the year by feeding on bivalve species with a toxin level that falls under the detection limit. The rough whelk (*B. undatum*) was implicated in an outbreak of PSP poisoning in Quebec (Prakash et al. 1971, Medcof 1972). Although 12 people developed symptoms, there were no deaths. Studies following the outbreak showed that the whelk accumulated toxin in its digestive gland after feeding on contaminated bivalve molluscan tissue.

Although no specific large-scale studies have been conducted,

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there are a small number of references to PSP in gastropods from the Puget Sound basin. Quayle (1969) reported the detection of PSP in moonsnails (*P. lewissi*) in nearby British Columbia, above the closure limit of 80 μg of STX equiv./100 g tissue. He also showed that nontoxic moonsnails became toxic when fed butter clams containing PSP toxins. MacDonald (1970) reported that two species of whelks, *Thais* (=*Nucella*) *lima* and *Thais lamellosa*, found at Agate-Crescent Beach area on the Strait of Juan de Fuca, were reported to contain PSP toxins; however, levels of toxin were not given.

In response to the increased consumer demand for marine invertebrates, an interagency meeting was sponsored in May 1994 by the Environmental Protection Agency (EPA) to assess the available risk management data on PSP in Puget Sound predatory gastropods. Participants included the U.S. Food and Drug Administration, the Washington State Department of Health (DOH), the Washington State Department of Fisheries and Wildlife and the National Marine Fisheries Service (NMFS). It was understood that predatory marine snails were reported capable of accumulating significant amounts of PSP toxins at other locations in the world. However, specific data on the PSP content of Puget Sound gastropods were lacking. In a similar manner, the diatom genus *Pseudonitzschia* is frequently observed in Puget Sound (Dr. Rita Horner, University of Washington School of Oceanography, Seattle, WA, personal communication 1994), indicating a possible risk of domoic acid poisoning.

In order to assess the PSP and domoic acid risk within the Puget Sound basin, a limited survey was undertaken of moonsnails, other predatory snails, and clams collected from various sites where routine monitoring by the Washington DOH documented PSP occurrences. Samples were collected by the Region 10 EPA Dive Team and NMFS personnel in the summer and fall of 1994 and the winter of 1995, and the NMFS provided PSP and domoic acid analyses of the samples. The purpose of this analytical survey was to document whether or not moonsnails and other predatory snails become PSP contaminated.

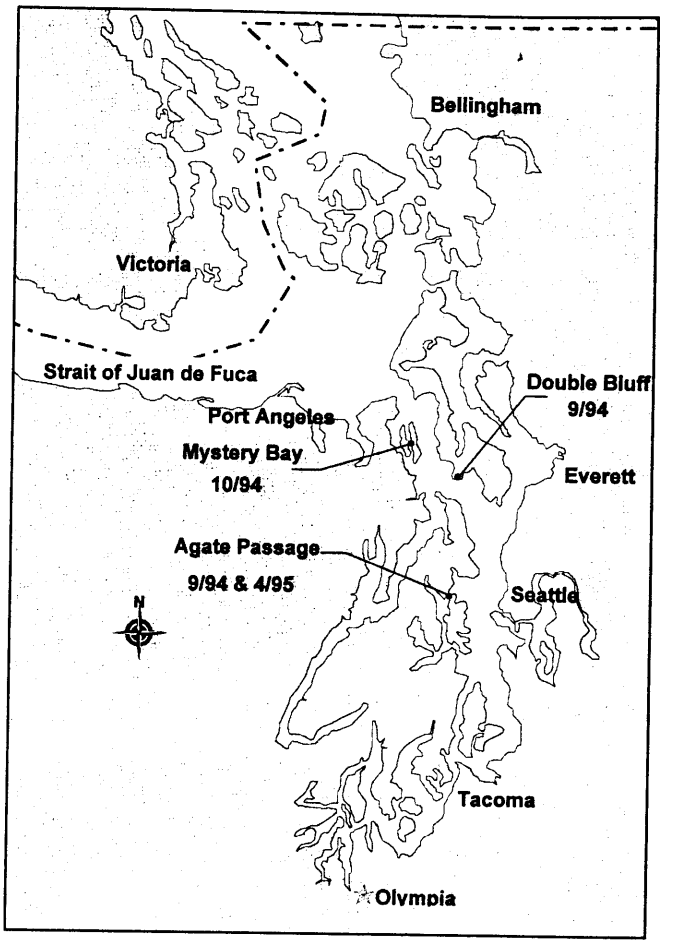

Figure 1. Sample collection sites in Puget Sound, WA.

METHODS

Sample Collection

The collection of moonsnails was accomplished by gleaning from available beaches and by the EPA Region 10 Dive Team. Sampling locations were identified that were expected to be moonsnail habitat and were also in the vicinity of Washington DOH–monitoring sites. Sample collection sites are shown in Figure 1. On September 1, 1994, moonsnails (*P. lewissi*), butter clams (*Saxidomus gigantus*), soft shell clams (*Mya arenaria*), and Pacific little neck clams (*Protothaca stamina*) were collected from the same beach in Agate Pass. On September 2, 1994, six moonsnails were collected from the beach at Double Bluff. On October 28, dogwinkles (*N. lamellosa*), blue mussels (*Mytilus edulis*), Pacific oysters (*Crassostrea gigas*), omnivorous snails (*Searlesia dira*), and horse mussels (*Modiolus modiolus*) were collected from Mystery Bay. On April 13, 1995, moonsnails, Pacific littleneck clams, and red rock crab (*Cancer productus*) were collected at Agate Pass. All animals were stored frozen in the shell until they were processed for PSP and domoic acid analyses. These marine toxins are stable under frozen conditions.

PSP Analyses

PSP analysis was performed by the use of modifications to the AOAC Official Method (1990). All meat was cleaned from the shells. Individual body weights were determined for large specimens. Because of their small size, dogwinkles, blue mussels, and Pacific littlenecks were composited. For crab samples, only hepatopancreas were analyzed for PSP content. Each moonsnail was divided into two samples, the foot tissue and the viscera portion, which included both the digestive gland and gonadal tissue. Moonsnail foot tissue was extremely tough and difficult to homogenize, requiring some modification of the standard procedures. It was necessary to mince the tissue first with a scalpel blade, cutting the tissue into thin strips and then dicing the strips into small pieces. Moonsnail foot tissue samples were combined with an equal amount of water and mixed in a Sorvall Omnimixer-SE (Omni International, 6530 Commerce CT., Gainesville, VA) for 30 s to form smooth homogenates (corrections for PSP content were necessary because of sample dilution, i.e., 1:1). For the PSP analysis, 10 mL of the homogenate was adjusted to pH 4 by the dropwise addition of concentrated 5N HCl, usually one to two drops. Because variations in the bioassay results were small, only two animals were used per sample. Moonsnail samples, collected on April 13, 1995, were divided into two portions: viscera (digestive gland/gonadal tissue) and meat (all other tissue). The viscera portion was identical to that taken in the September survey, whereas the meat portion in this sampling consisted of the foot, musculature, and other edible material. Because of the small sample size and soft tissue, the *Nucella* samples were pooled and homogenized. It was unnecessary to add water to samples of California mussels, blue mussels, and Pacific little necks for homog-

enization, because samples were of adequate size and had soft tissues.

Twenty grams of each 1:1 homogenate was weighed into 50-mL screw-cap glass centrifuge tubes. The pH was determined and adjusted to about pH 4 by the dropwise addition of 5N HCl. Most tissues required only about 1–5 drops of 5N HCl; however, moonsnail viscera took up to 20 drops of the acid solution. Unexpectedly, moonsnail viscera produced an odorless frothing and bubbling homogenate after the addition of the acid, presumably as the result of the release of carbon dioxide. For samples that were not homogenized with water, 10 g of tissue was weighed into a centrifuge tube and 10 mL of 0.1N HCl was added. The pH was then determined and adjusted to the correct range with 5N HCl. All samples were then heated in a constant temperature water bath, containing 50% ethylene glycol, at 105°C for 10 min to denature the proteins. Samples were cooled by either immersion into cold tap water or by air cooling, and the pH was again determined and readjusted if necessary to not higher than pH 4.5. The samples were transferred into polycarbonate centrifuge tubes and spun at 15,000 rpm (27,000 RCF) for 15 min. The separated liquid was decanted and, for extra cleanup, filtered through a Millex-HA 0.45-μm-pore-size filter connected to a plastic disposable syringe into labeled scintillation vials. A Millipore glass fiber prefilter was used for the *Nucella* sample, to avoid clogging the HA filter. The filtrate was then used for the PSP assay according to the AOAC method.

Domoic Acid Analysis

Moonsnail samples were analyzed for domoic acid by the use of the high-performance liquid chromatography method of Hatfield et al. (1994).

RESULTS

September 1994 Sampling

Samples from Double Bluff and Agate Passage were found to contain PSP in the viscera tissue. PSP was detected in every sample collected from Agate Passage in September 1994. Levels averaged 145 μg of STX equiv./100 g of tissue, with a standard deviation of ±45 and a coefficient of variation of 31% (Table 1). PSP levels ranged from nondetectable to a high of 95 μg of STX equiv./100 g of tissue in the Double Bluff samples; however, two of the samples were nontoxic. When PSP was present in the viscera of moonsnails, it appeared lower in the Double Bluff samples than in the Agate Passage samples. In the Double Bluff moonsnail viscera sample, 940902-5, the symptoms demonstrated in the mice were not thought to be consistent with PSP (i.e., respiratory paralysis).

In the September 1994 samplings, foot tissue appeared free of PSP toxins. For example, in the viscera sample containing the highest detected levels of PSP toxins, sample 940901-6-msv (214 μg of STX equiv./100 g) from Agate Passage, no toxin was found in the corresponding foot tissue (940901-6-msf) from the same animal. Because of this lack of toxicity observed in the foot tissue, only viscera tissues were tested for toxicity in the remaining samples.

When moonsnails were collected at the Agate Passage site, a small sampling of other shellfish was also collected. These shellfish included butter clams, soft shell clams, and Pacific little neck clams (Table 2). Only the butter clams were found to contain PSP toxins, averaging 73 μg of STX equiv./100 g of tissue and ranging from 45 to 94 μg of STX equiv./100 g. PSP was not detected in either soft shell and little neck clam samples.

TABLE 1.

PSP toxicity in moonsnails from Double Bluff and Agate Passage collected in September 1994.

Sample	Toxicity (μg STX equiv./100g tissue)
Agate Passage	
940901-1-viscera	171
940901-2-viscera	164
940901-3-viscera	114
940901-4-viscera	155
940901-5-viscera	116
940901-6-viscera	213
940901-7-viscera	80
Viscera average	**145**
940901-6-foot	ND*
Double Bluff	
940902-1-viscera	ND
940902-2-viscera	95
940902-3-viscera	ND
940902-4-foot	ND
940902-4-viscera	ND
940902-5-foot	ND
940902-5-viscera	DNQ**
940902-6-foot	ND
940902-6-viscera	54

* ND, not detected, treated as 0 μg of STX equiv./100g of tissue for calculations.

** DNQ, toxicity detected, not quantified. No deaths, but animals were distressed; response was not characteristic of PSP.

October 1994 Sampling

Washington DOH monitoring indicated elevated concentrations of PSP in Mystery Bay in October. During sample collection, moonsnails were not present in this bay; therefore, other shellfish

TABLE 2.

Other shellfish collected at Agate Passage in September 1994.

Sample*	Toxicity (μg of STX equiv./100g of tissue)
Butter clams	
940901-bc-1	45
940901-bc-2	90
940901-bc-3	86
940901-bc-4	94
940901-bc-5	75
940901-bc-6	46
Softshell clams	
940901-ss-1	ND
940901-ss-2	ND
Pacific littleneck clams	
940901-ln-1	ND
940901-ln-2	ND

* Abbreviations: bc, butter clams (*S. gigantus*); ss, soft shell clams (*M. arenaria*); ln, Pacific littlenecks (*P. stamina*).

species were collected: the predatory dogwinkle (*N. lamellosa*), an omnivorous snail (*S. dira*), Pacific oysters, and two species of mussels. Because of their size, the Pacific oysters and the horse mussel (*M. modiolus*) were analyzed individually, whereas the meats from the blue mussel samples and dogwinkles were composited. Blue mussels were highly toxic (652 μg of STX equiv./100 g of tissue); all of the other species collected also contained PSP toxins (Table 3), although at very reduced levels. With the exception of the mussels, all of the other samples were below the health guideline of 80 μg of STX equiv./100 g. Nevertheless, the dogwinkle sample contained the second highest levels of PSP (72 μg of STX equiv./100 g). Because mussels are known to both accumulate and depurate PSP toxins rapidly, such high levels of PSP in mussels indicated a probable bloom of PSP-producing phytoplankton.

April 1995 Sampling

Approximately 8 months after the initial moonsnail collection (September 1994), another survey was made in the same area (Agate Passage), on April 13, 1995. PSP levels were expected to be low during this period in Puget Sound. In addition to moonsnails, samples of butter clams, littleneck clams, and two red rock crab (*C. productus*) were collected and analyzed for PSP. PSP levels are reported in Table 4. As expected, the early spring collection produced moonsnail and butter clam samples with relatively low PSP levels. All moonsnail viscera examined contained PSP activity, ranging from 43 to 53 μg of STX equiv./100 g. However, only four of six butter clam samples were found to contain PSP activity. In contrast, the September 1994 sampling found all of the butter clam samples to be toxic. Toxin levels in clams collected in the spring 1995 survey ranged from 40 to 77 μg of STX equiv./100 g. Littleneck clams and red rock crab hepatopancreas were not toxic.

Domoic Acid Analyses

All samples subjected to PSP analysis were also analyzed for domoic acid. No domoic acid was detected in any sample. At the time and places of collection, no blooms of *Pseudonitzschia* sp. were observed or reported.

Mineral Composition

Mineral analyses of the moonsnail viscera homogenates were undertaken to gain a better understanding of the degassing observed during the acidification step of the PSP assay. Mineral composition is reported on both a dry and a wet weight basis in Table 5. Marked differences were noted in the average moisture content of the moonsnail samples collected in September 1994 (84.5% moisture) and April 1995 (74.2% moisture), which, in turn, was apparently reflected in the mineral composition of these samples (Table 5). Ash content, expressed on a wet weight basis, was significantly different between the 1994 (1.4%) and the 1995 (2.7%) samples; however, on a dry weight basis, no significant differences were observed between the samples (9.4% for 1994 and 10.5% for 1995). On both dry and wet weight bases, sodium was present at the highest level, as expected. Magnesium was present at the second highest level in both the 1994 and the 1995 samples. On a wet weight basis, the macromineral levels of sodium and potassium varied considerably between the 1994 and the 1995 samples, with 1995/1994 ratios of 1.86 and 2.20, respectively. However, when expressed on a dry weight basis, these sodium and potassium ratios were less dramatic, 1.07 and 1.29, respectively. Magnesium concentrations, on a dry weight basis, were similar in both the 1994 and the 1995 samples. However, on a wet weight basis, the September 1994 samples were almost one-half of the April 1995 level. On a dry weight basis, calcium levels were similar in both samples, 3,444 and 3,564, respectively. However, on a wet weight basis, the 1995 samples were 1.8 times higher than the 1994 samples. The concentrations of the trace minerals (chromium, copper, manganese, and zinc) were similar between the 1994 and the 1995 samples on a wet weight basis. As expected for shellfish, zinc levels were high in both 1994 and 1995, 102 and 116 ppm on a wet weight basis, respectively.

TABLE 3.

PSP assay of whole-body shellfish samples collected October 28, 1994, at Mystery Bay, WA.

Sample	Toxicity (μg of STX equiv./100g of tissue)
941028-dogwinkle (*N. lamellosa*)	72
941028-blue mussel (*M. edulis*)	652
941028-1-Pacific oyster (*Crassostrea virginicus*)	41
941028-2-Pacific oyster (*C. virginicus*)	44
941028-3-Pacific oyster (*C. virginicus*)	48
941028-4-Pacific oyster (*C. virginicus*)	47
941028-1-horse mussel (*M. modiolus*)	45
941028-2-horse mussel (*M. modiolus*)	51
941028-1-snail (*S. dira*)	50

TABLE 4.

PSP levels in moonsnails, littleneck clams, and red rock crab collected on April 13, 1995, at Agate Passage.

Sample*	Tissue Analyzed	Toxicity (μg of STX equiv./100g of tissue)
Moonsnails (*P. lewissi*)		
950413-msv-1	Viscera	45
950413-msm-1	Foot	0
950413-msv-2	Viscera	49
950413-msm-2	Foot	0
950413-msv-3	Viscera	54
950413-msm-3	Foot	0
950413-msv-4	Viscera	49
950413-msm-4	Foot	0
950413-msv-5	Viscera	44
950413-msm-5	Foot	0
950413-msv-6	Viscera	47
950413-msm-6	Foot	0
Butter clams (*S. gigantus*)		
950413-bc-1	Whole body	47
950413-bc-2	Whole body	76
950413-bc-3	Whole body	40
950413-bc-4	Whole body	44
950413-bc-5	Whole body	0
950413-bc-6	Whole body	0
Pacific littleneck (*P. stamina*)		
950413-ln-1	Whole body	0
Red rock crab (*C. productus*)		
950413-rch-1	Whole body	0

* Abbreviations: msv, moonsnail viscera; msm, moonsnail foot tissue; bc, butter clam; ln, littleneck.

TABLE 5.

Elemental composition, presented on both a dry and a wet weight basis, of moonsnail viscera collected in September 1994 (940901) and April 1995 (950413).

Element	950413 Average (ppm) Dry Wt. (N = 6)	940901 Average (ppm) Dry Wt. (N = 6)	Ratio 950413/940901	950413 Average ppm Wet Wt.	940901 Average ppm Wet Wt.	Ratio 950413/940901
Ca	3,564	3,444	1.0	930	522	1.8
Cr	1.8	2.8	0.62	0.44	0.43	1.1
Cu	35	56	0.64	9.0	8.5	1.1
Fe	323	316	1.0	82	48	1.7
K	7,270	5,618	1.29	1,880	854	2.2
Mg	15,801	14,231	1.11	4,113	2,205	1.9
Mn	23	33	0.68	5.8	5.2	1.1
Na	18,330	17,104	1.1	4,598	2,474	1.9
P	6,482	5,828	1.1	1,682	885	1.9
Sr	37	35	1.0	9.4	5.3	1.8
Zn	458	606	0.76	116	102	1.1

DISCUSSION

Both the moonsnail, *P. lewissi*, and the frilled dogwinkle, *N. lamellosa*, are predatory marine carnivores (Keep 1911, Ricketts and Calvin 1968, Barth and Brushears 1982) and attack their prey using a special boring device, a radula (Russel 1993, Carriker 1961). Both species are commonly found from British Columbia to at least southern California, with *Nucella* preferring rocky beaches, whereas moonsnails prefer sandy bottoms (Ricketts and Calvin 1968, Rice 1968). The favored prey for these species includes mussels and clams (Ricketts and Calvin 1968), but *N. lamellosa* also feeds on barnacles (*Balanus* sp.) (Emlen 1966). Mussels and clams are phytoplankton feeders and have the highest risk of containing marine biotoxins, e.g., domoic acid (from the consumption of *Pseudonitzschia* spp.) or PSP (from consuming *Alexandrium* spp.). Boring into their prey, these carnivores consume the viscera, which potentially carry the highest levels of toxins. Because these animals live in a zone that frequently experiences blooms of *Alexandrium* capable of producing PSP, and prey on shellfish that are phytoplanktonic feeders, it is not surprising that they can and do accumulate PSP toxins.

Until the mid- to late 1970s, PSP was not commonly found within the Puget Sound basin. Before then, monitoring and closures were common along the Strait of Juan de Fuca, on beaches west of the city of Port Angeles (Fig. 1). However, in 1977, we and others measured very high levels (>14,000 μg of STX equiv./100 g) of PSP toxins in mussels from Puget Sound (J. Wekell, unpublished data, 1977). Since that time, PSP has become a constant risk throughout the basin. Predatory snails and other potential human food items besides bivalve shellfish have not been routinely monitored for the presence of PSP or other marine toxins.

In the Puget Sound basin, PSP levels in shellfish generally begin to rise in May and continue through the summer and early fall months, although high levels of PSP have been noted as late as November and December. Because of their rapacious nature, it is not surprising that PSP levels in moonsnails and other predatory gastropods follow a pattern similar to that observed in other shellfish in Puget Sound. Butter clams are known to accumulate and retain PSP toxins consistently for long periods along the coasts of Oregon, Washington, British Columbia, and Alaska. Although the levels of PSP in predatory snails found in Puget Sound appear to be low, our September 1994 survey found levels well in excess of the regulatory closure limit of 80 μg of STX equiv./100 g; this indicates that the consumption of these shellfish does represent a potentially serious human health risk.

Unlike data reported by Shumway (1995) and White et al. (1993a and 1993b) for *E. heros* (=*Polinices*) taken from Georges Bank, we found PSP activity restricted to the viscera of the Puget Sound moonsnail (*P. lewissi*) in both samplings (fall of 1994 and spring of 1995). In the Georges Bank survey, *Euspira* PSP levels were considerably higher (>2,500 μg of STX equiv./100 g of tissue) than the toxicities observed in our studies (i.e., <250 μg of STX equiv./100 g of tissue). In addition, the sampling in this survey was considerably smaller than the sampling conducted in the Georges Bank survey; however, the variation of PSP levels from the September sampling at Agate Passage is similar to that reported by White et al. (1993b) for moonsnails taken from Georges Bank. Nagashima et al. (1995) reported that the trumpet shell (*Charonia lampas*) collected from the Galician Coast in Spain contained PSP toxins in the digestive gland in a range similar to those we found in Puget Sound snails. Interestingly, the toxin suite found in the Galician shells was composed of mainly the decarbamoyl derivatives of STX (dcSTX), gonyautoxin2 (dcGTX2), and gonyautoxin3 (dcGTX3), with only minor amounts of GTX2 and GTX3.

In order to obtain some understanding about the variation at each collection site, individual shellfish were analyzed. With these constraints, modifications to the AOAC method were necessary because of the nature of the tissues and the amounts available for analysis. Samples of snails from the Double Bluff area were used to test and develop modifications to the PSP assay procedure, i.e., tissue sampling and handling. Moonsnail viscera presented a problem in adjusting the pH. Because viscera consisted of soft tissue, homogenizing with an equal volume of water was unnecessary. For the viscera analysis, 1 part homogenized viscera was mixed with 1 part 0.1N HCl, but in order to achieve the desired pH 4 with some 1994 samples, considerably more acid was required because a higher than expected initial pH (pH 7.6–8.6) was observed. In order to achieve the desired pH, as required in the AOAC procedure, it was necessary to add a more concentrated acid, i.e., 5 N HCl, to the mixtures (10 mL), requiring 20–25 drops or about 1 mL. Because this amount increased the volume nearly 10%, adjustment to the final calculations was applied. It was noted that the

addition of this acid caused an apparent release of gas, and care had to be taken during the heating step to ensure that material was not lost as the result of frothing and leakage from the tubes. The use of polyethylene centrifuge tubes with fitted screw-cap lids helped reduce these losses and reduce the chances of breakage. With the exception of only one sample, moonsnail viscera from samples collected in April 1995 had normal expected pH values (i.e., pH 6–7) that did not produce gas when acidified. Nevertheless, sample 950413-msv-3 had an initial pH of 8.1 and produced negligible amounts of gas when acidified. It is presumed that this gas is carbon dioxide, because it was both odorless and colorless. Because it is released on acidification, it is probably present as carbonate ion.

The high pH encountered suggests that carbonate would be associated with some typical biological alkaline cation, such as calcium, sodium, or magnesium. Accordingly, we expected higher concentrations of these cations in the viscera, which included the digestive gland/gonadal tissues. Calcium was considered to be the likely cation because of its high concentration in seawater and its involvement in shell synthesis. However, the mineral analyses indicated the opposite of what was expected. That is, the calcium, magnesium, and sodium concentrations on a wet weight basis were approximately one-half of the element concentrations seen in 1995, in which little or no frothing occurred. Thus, mineral analyses alone are not sufficient to explain the presence of apparent excess carbonate ion.

The assessment of health risks posed by the consumption of predatory shellfish and the development of programs for their management will be difficult to implement because our current knowledge, based on this work and other studies in the Puget Sound basin, is limited. To develop an acceptable risk model and program, more information and data concerning the distribution and quantity of these shellfish (seasonally and geographically) will be required. In addition, information about the toxins will be required, for example, levels in specific tissues, total body burden, and seasonal variability. The implementation of a management program will also require detailed information about consumers and their use of these nongame marine invertebrates (NGMI). For example, Carney and Kvitek (1991) found in their survey that over 50% of the collectors harvesting NGMI were Asian, Korean, or Filipino. The usage and consumption rates of NGMI among these ethnic groups are unknown but are suspected to be much higher than that reported for the whole population (6.5 g/day) (Connie Nakano, Project Coordinator, Asian and Pacific Islander Seafood Consumption Study, Seattle, WA, personal communication, 1995).

CONCLUSIONS

The results from this survey indicate that Puget Sound predatory marine snails accumulate PSP toxins to levels above the regulatory level (80 μg of STX equiv./100 g). Further, to increase the likelihood of detection, the viscera of the moonsnails appears to be the tissue of choice in analyzing these animals for PSP toxins. Modification of the sample preparation method may be required. Other parts of the moonsnail either do not accumulate the PSP toxins or accumulate it at levels that are below the current AOAC method's detection limit. In addition, these survey data indicate that other predatory and omnivorous snails accumulate PSP toxins. If the recreational or subsistence collection of these species continues or grows, some form of monitoring may be required for the protection of public health. The management of these health risks will require more information on the seasonality, distribution, uptake, and depuration of toxicity in these molluscan species within the Puget Sound basin. Perhaps most important, however, will be more detailed knowledge of the patterns of human collection, preparation, and consumption of these shellfish.

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