

PARALYTIC SHELLFISH TOXINS IN GEODUCK CLAMS (*PANOPE ABRUPTA*): VARIABILITY, ANATOMICAL DISTRIBUTION, AND COMPARISON OF TWO TOXIN DETECTION METHODS

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ABSTRACT The geoduck clam, *Panope abrupta*, is a valuable economic resource in Washington State. Prior to the mid 1970s, the levels of paralytic shellfish poisoning (PSP) toxins in Washington State geoducks were not considered by the Washington State Department of Health (WDOH) to be a risk to public health because the viscera were presumed to be discarded. Recent monitoring information indicates that geoducks accumulate high levels of toxins, primarily in the viscera. The purposes of this study were to determine: (1) the seasonal concentration of paralytic shellfish toxins in geoduck clams at two sites and at two depths within each site; (2) the variability of PSP toxin levels among individual clams within each site; (3) the anatomical distribution of toxins; and (4) the correlation between two methods for estimating PSP toxins. From the summer of 1997 through the winter of 1998, 12–24 geoducks were collected biweekly from a shallow (7 m) and a deep (17 m) location in each of two tracts in Puget Sound, Washington: Quartermaster Harbor (QH) and Agate Pass (AP). Geoducks, dissected into siphon, mantle, and visceral portions, were assayed separately using the mouse bioassay (MBA), while only the visceral portions were assayed using the receptor-binding assay (RBA). Results indicated that toxin variability between individual clams was high in the shallow areas, with coefficients of variation (CVs) ranging from 20–98%, and lower in the deep areas (CV = 18–62%). In QH, only geoducks from the shallow water had toxin levels greater than the regulatory level of 80 µg saxitoxinequivalents (STX eq) · 100 g shellfish tissue⁻¹, while all geoducks from AP contained toxin above the regulatory level, with clams from shallow water considerably more toxic than those from deep water. Anatomically, the highest concentrations of PSP toxins were localized in the viscera of geoducks. There was a significant positive correlation between toxin levels measured by the MBA compared to values obtained using the RBA ($r^2 = 0.83$). The large differences in toxicity between geoducks sampled at different depths and harvest tracts indicate that careful management plans must be designed in order to ensure public health.

INTRODUCTION

General Background

Toxins that cause paralytic shellfish poisoning (PSP) are accumulated by filter-feeding bivalve mollusks when they ingest toxic dinoflagellates from the genus *Alexandrium*. These algae produce the toxins naturally, and the PSP syndrome results from the human consumption of toxic bivalves. Large-scale problems with PSP stem from the extreme difficulty in predicting the timing and extent of dinoflagellate blooms, in turn making it difficult to monitor toxicity in shellfish efficiently (Boesch et al. 1996, Horner et al. 1997). Difficulties also arise because each species of shellfish is unique in the kinetics of uptake and elimination of toxins. In addition, shellfish toxicities do not always coincide with observed toxic algal blooms (Cembella and Shumway 1993, Bricelj and Shumway 1998).

The geoduck clam, *Panope abrupta*, is a valuable economic resource in Washington State, with revenues ranging from \$5–7 million annually (Washington State Department of Natural Resources (WDNR), unpublished data, 1997). Recently, the demand from newly developed large markets both domestically and overseas (e.g., Hong Kong, Japan, and Singapore) has sent the price of geoducks up from \$1.50 per pound in the late 1980s to a current average price range of \$12–14 per pound. Public demand is for

whole, live geoducks; the market for shucked or frozen product is very small. While geoducks are one of many species of bivalves known to filter and accumulate toxic dinoflagellates, few data exist that describe PSP toxins in this organism (Shumway 1990; Bricelj and Shumway 1998). The risk of PSP to consumers is therefore increased, which may lead to devaluation of the geoduck as a food item for human consumption if toxic product reaches the market.

Washington's Geoduck Fishery

Prior to the mid 1970s, PSP toxin levels in Washington State geoducks were not considered by the Washington State Department of Health (WDOH) to be a risk to public health because the geoduck viscera were presumed to be discarded. However, we now know that the viscera are consumed by some members of tribal and immigrant communities, who use them in soup (K. Chew, University of Washington pers. comm., 1996, M. Antee, WDOH pers. comm., 1997). In addition, toxic algal blooms are extending into previously benign areas of central and southern Puget Sound (Nishitani and Chew 1988, F. Cox, WDOH pers. comm., 1997), which is leading to unprecedented high levels of PSP toxicity in geoducks and toxicity that lasts well into the winter months, resulting in thousands of dollars of an unharvestable resource. The recent increased demand for geoduck meat is resulting in new tribal and state commercial tracts being opened in some areas of

central and northern Puget Sound where PSP is known to occur (F. Cox, WDOH pers. comm., 1997).

Little information exists regarding PSP toxicity in geoducks. However, recent monitoring programs indicate considerable inter- and intrapopulation variability (F. Cox pers. comm., 1997).

An understanding of the reasons for toxin variability is crucial in designing a regional monitoring and sampling program. The current method used by the WDOH in monitoring and testing for PSP in the geoduck does not account for individual variability in the clams because composite viscera from three clams are tested for toxicity as one sample. In the absence of variability and anatomical distribution information, it is difficult to assess the effectiveness of the current Washington State geoduck monitoring program in protecting public health. This study describes toxin variability in geoducks in relation to water depth and geographical location, thereby providing basic information that can be integrated into future monitoring efforts by the WDOH.

MATERIALS AND METHODS

Sampling

Quartermaster Harbor

Quartermaster Harbor (QH), located between the southern tips of Vashon and Maury Islands (Fig. 1), is currently a prohibited harvest area due to consistent levels of PSP toxicity $> 80 \mu\text{g}$ of saxitoxin equivalents (STXeq)/100 g of tissue (all toxicities are given in micrograms of STXeq/100 g of shellfish tissue) (Nishitani and Chew 1984) and pollution problems resulting from failing septic systems (Washington Department of Fish and Wildlife 1997). There are two tracts in QH, and tract number 10300 was randomly chosen as the study site (Washington Department of Fish and Wildlife 1997).

A shallow and a deep sampling location within this tract were randomly selected. The depth of the shallow location averaged 7

m, adjusted to mean lower low water, and the deep location averaged 17m (mean lower low water). A diver collected 6–14 geoducks within a circular area approximately 27 m in diameter from both depth locations, at 2-wk intervals from June through October 1997.

Agate Pass

Tract number 0700 in Agate Pass (AP), located north of Arrow Point on the west side of Bainbridge Island (Fig. 1), is currently a WDOH-approved harvest tract (Washington Department of Fish and Wildlife 1997). A shallow and a deep sampling location were randomly selected in the same manner as in QH. Divers collected geoducks at 2-wk intervals from August 1997 through January 1998. All of the geoducks from deep water consistently came from the same sampling location. In the shallow zone, however, the lack of sufficient numbers of geoducks necessitated a constant lateral shift in collection sites, but all of the shallow sites were within an approximately 300-m section along the shoreline.

Laboratory Determinations

Geoducks were dissected, and toxicities of the siphon, mantle, and visceral portions of individual geoducks were determined by mouse bioassay (MBA) (Association of Official Analytical Chemists 1965). All of the visceral tissue, except the gills, was combined and tested. The gills were saved for future testing, time and funding permitting. Additionally, the visceral portions were tested using the receptor-binding assay (RBA) (Davio and Fontelo 1983, Doucette et al. 1997, Trainer and Poli, 2000). In this assay, nerve terminal membrane from the rat brain, containing sodium channel receptors (STX binding sites) is used to test for the presence of STXeq in a sample. Toxin in the sample displaces radioactively labeled STX from its specific receptor sites, thereby reducing the level of radioactivity in the shellfish sample. Geoduck samples analyzed using this method had toxin levels ranging from 40–1,800 μg (determined by MBA). No samples below the detection limit of the MBA were used.

RESULTS

Anatomical Distribution

The actual toxin levels (given in micrograms of STXeq per 100 g of shellfish tissue) in each of the dissected tissues (siphon, mantle, and viscera) from all clams collected from QH and AP ($n = 361$), are shown in Figure 2. In QH samples, detectable levels of toxins were found in the mantle portion of three individual clams, but the values were well below the fishery closure level (80 μg) at 46, 47, and 51 μg (Fig. 2B). In AP samples, detectable levels of toxin were found in the mantle portion of seven individual clams and in the siphon portion of nine individual clams (Fig. 2B), however, the values were again well below the fishery closure level. At no time during the study period did the siphon portion from any geoduck show detectable levels of toxicity. All toxicities above the fishery closure level were in the visceral portion only.

Quartermaster Harbor

Shallow Water

Toxin levels above the fishery closure level were detected on all eight sampling dates from June through October, except July 27

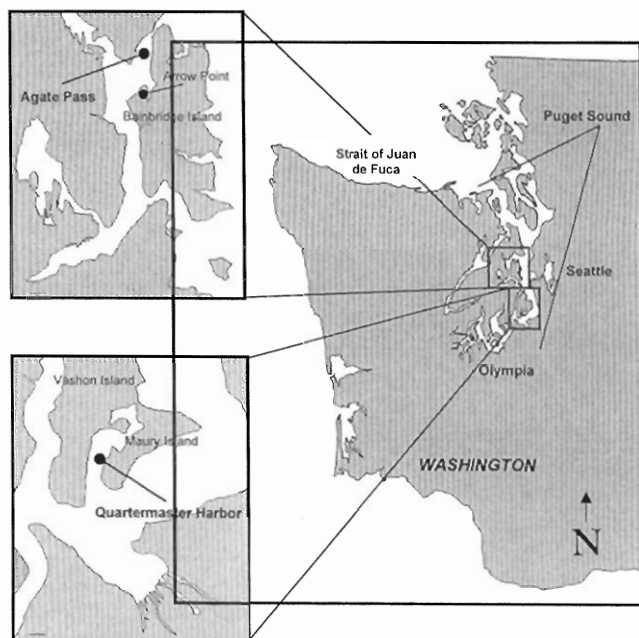


Figure 1. Map of coastal Washington and Puget Sound showing the study collection sites at AP and QH.

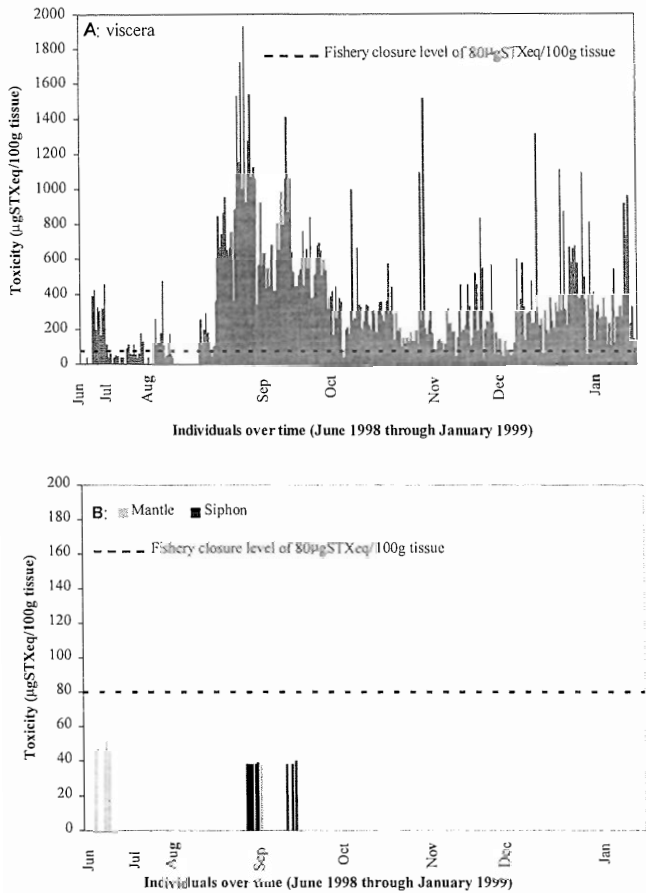


Figure 2. Toxicity levels in each of the dissected tissues (A = viscera; B = mantle and siphon). Each bar represents an individual geoduck. All geoducks collected from QH and AP during the study are included ($n = 361$) and are shown in chronological order of collection. The mantle portions of 10 geoducks and the siphon portion of 9 geoducks had detectable levels of toxin but were still below the fishery closure level.

(Fig. 3A). When toxicity was above closure levels, there was a large variation in toxin levels among individual clams. On July 27, variability was low and toxicity levels ranged from 0–61 μg . The largest variation occurred on October 5, with toxin levels ranging from 38–998 μg (Table 1).

Deep Water

In QH deep water, toxicity was consistently below the closure level, and values were considerably lower than those observed in the shallow location (Fig. 3B). Toxin levels ranged from nondetectable to 38 μg on all collection dates except October 20, when toxin levels ranged from 0–67 μg . Variability between individuals was low on all sampling dates.

AP

Shallow Water

Toxicities were consistently above the fishery closure level on all 12 collection dates from August through January with the exception of three individual clams, one each on November 12 and 25, and January 6 (Fig. 4A). There was a large variation in toxin levels among individuals on all sampling dates.

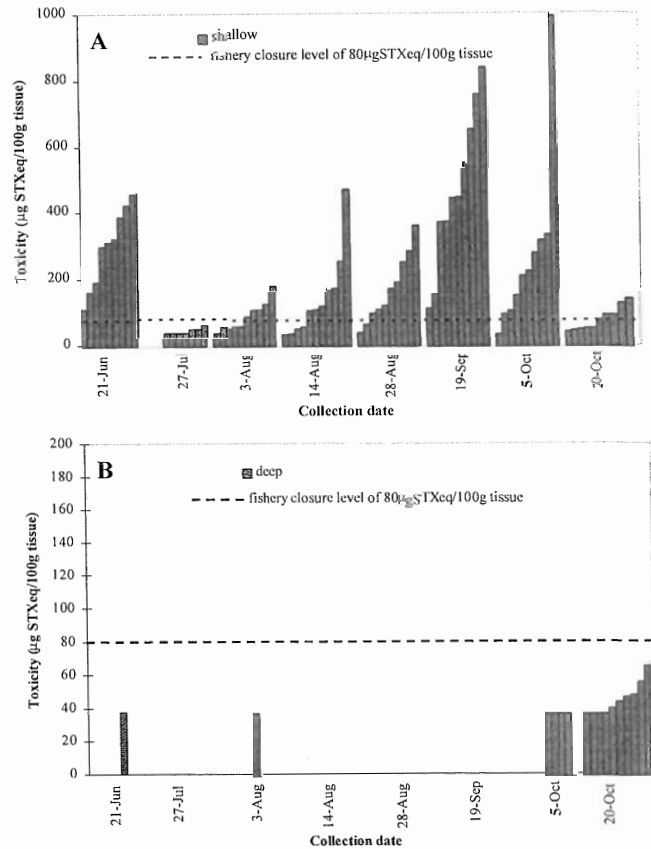


Figure 3. Toxicity of the viscera in geoducks from the QH collection areas taken from June through October 1997. Each bar represents an individual geoduck. The dashed line indicates the regulatory closure level of 80 $\mu\text{g STXeq}/100\text{ g}$ of shellfish tissue. A = shallow; B = deep.

Deep Water

Toxicities were consistently above the fishery closure level of 80 μg on most of the 11 collection dates, except December 10 when 50% were below and 50% were above the closure level of 80 μg (Fig. 4B). There was a large variation in toxin levels among individuals on all sampling dates. The largest variation occurred on January 20 with toxicities ranging from 107–545 μg .

Comparison of PSP Detection Methods

Correlations between the MBA and the RBA methods are shown in Figure 5. Figure 5A illustrates the relationship between all samples tested, which ranged from 60–1,700 μg (by MBA). A comparison of the two methods showed a significant positive correlation ($r^2 = 0.83$). Figure 5B illustrates the relationship between samples with toxicities < 85 μg (by MBA) and demonstrated a significant positive correlation between the two methods ($r^2 = 0.55$).

DISCUSSION

Variability

The high degree of toxin variability observed among individual geoducks (Figs. 3, 4) is not surprising and has been seen in many other shellfish species. For example, Atlantic surfclams (*Spisula solidissima*) taken off the coast of Maine showed an average coefficient of variation (CV) of 48.6%, and ocean quahogs (*Arctica islandica*) showed a mean CV of 56% (White et al. 1993). Soft-

TABLE 1.

Summary of the variation in levels of PSP toxins among individual geoducks collected from QH and AP during each 1-d collection period, with clams separated by depth.

Area	Sampling Date	n	Geoducks with >80 µg of Toxin	Range ^a	Mean ± SD ^a	Pooled SD	CV (%)	Mean CV (%)	
QH shallow	June 21	9	9	113–460	298.8 ± 119		40		
	July 27	10 ^c	0	0–61	53 ± 7		13		
	August 3	11	6	39–179	95 ± 43		44		
	August 14	11	7	38–475	158 ± 130		82		
	August 28	10	8	44–365	173 ± 103		60		
	September 19	10	10	116–845	474 ± 237		50		
	October 5	10	9	38–998	305 ± 274		90		
	October 20	10	5	46–146	81 ± 36	153	44	53	
QH deep	June 21	6	0	0–38	N/A ^b		N/A ^b		
	August 3	6	0	0–38	N/A ^b		N/A ^b		
	August 14	14	0	0	N/A ^b		N/A ^b		
	October 5	10	0	0–38	N/A ^b		N/A ^b		
	October 20	11 ^d	0	0–67	51 ± 9	N/A	18	N/A	
AP shallow	August 19	12	12	892–1,937	1,272 ± 335		27		
	September 2	10	10	530–1,413	885 ± 258		29		
	September 17	9	9	290–692	476 ± 160		34		
	October 7	9	9	203–666	334 ± 140		42		
	October 14	10	10	102–577	289 ± 143		49		
	October 28	5	5	172–1,521	649 ± 621		96		
	November 12	6	5	49–318	202 ± 88		44		
	November 25	10	9	38–835	398 ± 219		55		
	December 9	14	14	138–1,314	408 ± 292		72		
	December 23	13	13	224–1,113	606 ± 288		48		
	January 6	11	10	61–813	346 ± 195		57		
	January 20	10	10	98–966	431 ± 318	271	74	52	
	AP deep	August 19	10	10	359–958	717 ± 164		23	
		September 2	11	11	342–930	546 ± 166		30	
September 17		12	12	212–544	409 ± 107		26		
October 7		11	9	38–441	247 ± 115		46		
October 14		11	11	183–357	271 ± 61		23		
October 28		10	9	116–195	144 ± 29		20		
November 12		10	10	81–304	151 ± 74		49		
November 25		10	10	106–521	278 ± 150		54		
December 9		12	6	47–183	92 ± 44		48		
December 23		10	9	65–386	252 ± 94		37		
January 20		10	10	107–545	233 ± 144	115	62	38	

^a Values given as micrograms of STXeq/100 g of shellfish tissue.

^b N/A = not applicable. These values were below detection level and could not be determined.

^c Only three geoducks had toxicities >38 µg. These values were used to calculate mean, SD, and CV.

^d Only six geoducks had toxicities >38 µg. These values were used to calculate mean, SD, and CV.

shell clams (*Mya arenaria*) from the Bay of Fundy showed an average CV of 49% (Medcof et al. 1947). Prior to the present study, the only variability information available for geoducks was from an unpublished study in Alaska, where the mean CV for 10 sets of geoducks was 41% (Ketchikan Public Health Laboratories, unpublished data, 1981).

Some variability in PSP toxin levels among individual geoducks can be accounted for by the variability ($\pm 20\%$) in the MBA test (McFarren 1962). The mean CVs for each set of geoducks (defined by collection area and depth) were close to or greater than twice that in the MBA (38%, 52%, and 53%). However, within sets of geoducks, the CV reached 96% (Table 1), indicating that there was considerable variability between individual geoducks that was not due to an error in the MBA.

Many factors have been suggested to account for variations between individual shellfish, including differences in feeding

rates, availability of food due to vertical and horizontal depth gradients, reproductive condition, individual sensitivity to PSP toxins, and variation in body mass (Prakash and Medcof 1962, Nishitani and Chew 1984, Bricelj et al. 1991, Bricelj and Laby 1996, Mackenzie et al. 1996). Much of the variation between individual geoducks within one depth may be attributable to differences in feeding rates (D. Williams, WDNR pers. comm., 1997). At any given time, geoducks are expected to have a 70% "show factor," meaning that only 70% of the population will have their siphons protruding out of the sand but will not necessarily be feeding. This show factor varies with the time of year and could be attributed to changes in water temperature or localized disturbances (e.g., the presence of divers, crabs, siphon-nipping fish, or marine mammals), causing the geoducks to retract their siphons.

The availability of food, often directly related to the behavior of algal cells, is very likely to be the reason for the high degree of

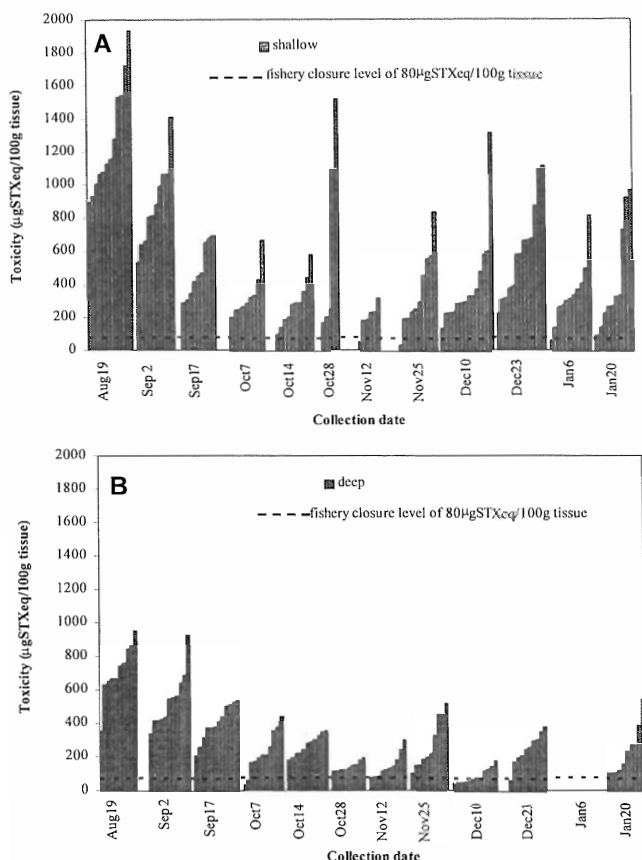


Figure 4. Toxicity of the viscera in geoducks from the AP collection areas taken from August 1997 through January 1998. Each bar represents an individual geoduck. The dashed line indicates the regulatory closure level of 80 µg STXeq/100 g of shellfish tissue. A = shallow; B = deep.

variability between depths (shallow and deep). The toxic *Alexandrium* cells have been found to undergo diel vertical migrations, reaching a maximum depth of 8 m (Nishitani and Chew 1984). The depth of the dinoflagellates also depends on currents and winds, which mix them deeper into the water column. This may explain why the geoducks from the deep water of QH, a shallow, quiet bay without strong currents or vertical mixing, were never over the toxicity closure level, while the ones from the shallow water exhibited high toxin levels (Fig. 3). It is likely that the geoducks from the shallow water were exposed to the toxic dinoflagellates more frequently than the ones from the deep water, thus increasing their overall toxicity. Because AP experiences mixing due to strong and variable currents, with speeds ranging from 0.3–6.6 knots (U.S. Department of Commerce 1973), cells are mixed to greater depth within the water column, making them available for uptake by the geoducks in deeper areas. However, it is likely that the geoducks from the shallow water were exposed to toxic cells more frequently, accounting for their higher overall toxicity.

The difference between collection depths has some implications for the geoduck industry. Currently, harvesting for the market and collection for PSP monitoring occurs primarily in the shallower depths of a harvest tract (D. Winfrey, Puyallup Tribe pers. comm., 1997, D. Williams, WDNR pers. comm., 1998). Geoducks are easier to find, and the divers can collect more clams in a shorter amount of time. In order to accommodate the toxicity differences,

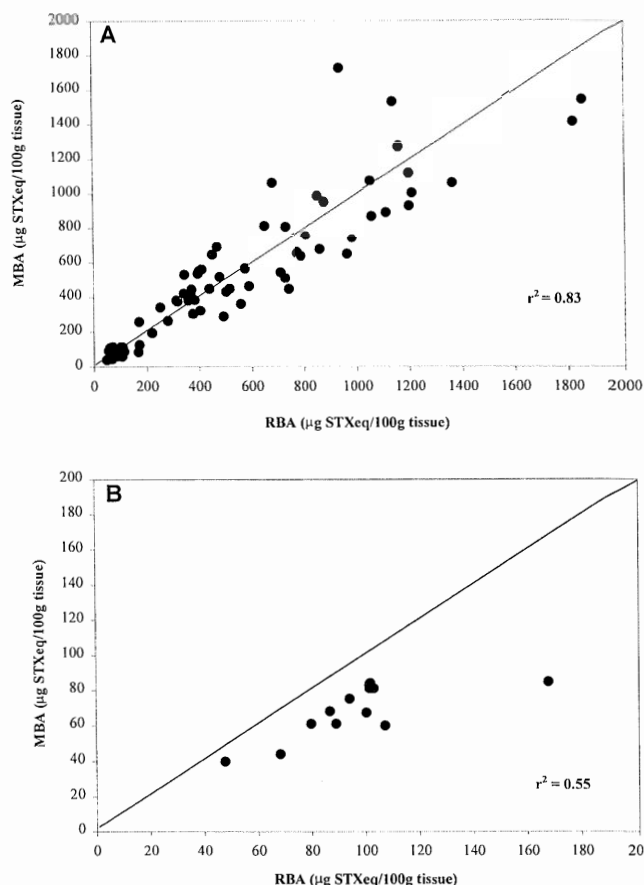


Figure 5. Correlation between the MBA and the RBA. A = all samples analyzed ($n = 73$); B = samples with toxin levels between 32 and 85 µg (by MBA, $n = 12$). The diagonal line represents perfect correlation ($r^2 = 1$). There was a significant correlation between the two methods: $r^2 = 0.83$ for all samples and $r^2 = 0.55$ for samples < 85 µg.

harvests could be limited to certain depths during periods of high toxicity (typically in the summer through early winter months). For example, as toxicity levels increase, harvest depths could also be required to increase.

The large difference in overall toxicities between AP and QH is difficult to explain (Figs. 3, 4). It is not unreasonable to suspect that QH would have higher toxicities since it is a “breeding bay” for *A. catenella* (Nishitani and Chew 1984). Breeding bays are defined as certain shallow, protected bays in which strong thermal stratification occurs relatively frequently. In these bays, dense populations of *A. catenella* can develop and become available to the shellfish. However, the AP study area had toxicity levels up to five times (Table 1) those seen in the QH study area, even though AP is an area of strong currents and very little thermal stratification. One explanation for the higher toxicities in AP is the possibility of dinoflagellate cysts in this area, which can be more toxic than motile cells (Dale et al. 1978). Since *A. catenella* forms dormant cysts, it is possible that the geoduck harvesters or the strong currents in AP are stirring up the toxic dormant cysts in the sediments, making them available for uptake by the geoduck and, thereby, accounting for the higher overall toxicities in that area. In addition, QH is a closed harvest area with no diver activity and slow currents, further supporting the explanation for lower overall toxicities.

An additional explanation for higher toxicities in AP could be that motile toxic cells are potentially being exported from a nearby breeding bay. Puget Sound, a fjord with a long, deep main channel, has numerous relatively shallow and often poorly flushed bays, where blooms of *A. catenella* could potentially originate (Horner et al. 1997). The toxic cells, if exported horizontally during periods of reduced turbulence, could cause toxicity levels in shellfish in a nearby area to be several times greater than in the breeding bay itself (Nishitani and Chew 1984).

Individual shellfish within the same sampling population are known to exhibit differential sensitivities to PSP toxins (Bricelj and Laby 1996). This has not been studied in geoducks. Variations in body mass also may have effects on individual toxin accumulation rates. Smaller individual clams can reach equal or higher toxicities than larger individuals collected from the same location (Medcof et al. 1947, Aalvik and Framstad 1981). In this study, there was no relationship between geoduck weight and toxicity level on any date or in any collection area.

Anatomical Distribution

In this study, all PSP toxin levels above closure level were concentrated in the visceral ball in geoducks from all collection sites (Fig. 2). Only the siphon and mantle portions contained an amount of toxin that was below regulatory levels, and, therefore, these were the only portions that would be considered safe to consume during periods of PSP intoxication. This raises the important question of: "How do we protect the public health from the dangers of consuming toxic geoduck viscera?"

The current program used by the WDOH in monitoring for PSP in geoducks could be modified to better protect public health and will be discussed in the next section. Second, geoducks could be shucked and eviscerated prior to being sent to the market. However, 80% of the current market, both domestic and overseas, is for whole, live geoduck, where consumers often pay \$12 or more per pound. There is not a large demand for processed geoduck meat (J. Lo, Evergreen International Food Stuff, pers. comm., 1999; L. Elliott, E.C. Phillips and Son, pers. comm., 1999). Typical prices for shucked body meat range from \$3–5 per pound, and for neck meat, from \$12–24 per pound. These prices depend mainly on the economy and on the availability of whole, live product. In addition, the volume is so small that even the high prices for neck meat do not make up for the overall value of live product. A third solution to the question of how to protect public health is to increase public awareness and education on the dangers of consuming toxic geoduck viscera.

PSP Monitoring

The large difference in toxicity levels between depths and between tracts has implications for the industry and the WDOH. Each harvest area will have to be treated separately when determining sample size and PSP monitoring effort. Perhaps a larger number of samples could be taken in the shallow areas, since most harvest activity occurs in those zones. Because sites vary widely in wind patterns, bathymetry, tidal currents, and turbulence, the extent to which toxicity differences will actually occur may also be expected to vary considerably and can best be tested on a site-by-site basis. It must be noted that the information gained from this study is only applicable to the specific study sites. However, generalizations, such as high variability between individual geoducks, can be made to other populations.

Assay Comparison

In this study, the RBA overestimated MBA results by an average of 22.8%. Doucette et al. (1997) found that the RBA agreed very closely with MBA results from one laboratory but tended to overestimate those originating from a second source. The reasons for overestimation are unclear. Differences are expected, given that the RBA is performed on a static system and the MBA is performed on a dynamic system (live mice). Resulting toxicities can be affected by metabolic changes in the mice. Other work has shown that the MBA is known to underestimate actual toxicity by as much as 60% at lower toxicity levels (McFarren 1957, Park et al. 1986). Therefore, the lower levels of toxicity obtained by MBA in this study could have been underestimated by as much as 60%, accounting for most of the overestimation by the RBA. Variability at low toxicity levels in the MBA is affected by many factors, including salt content during sample preparation, pH, and storage (McFarren 1957, Park et al. 1986). Last, after initial sample preparation, some degradation of the low-toxicity compounds B1 and B2 (N-sulfocarbamoyl toxins) to the nonsulfated carbamate toxins, STX and neosaxitoxin, could have occurred, resulting in increased toxicity by the RBA (Cembella et al. 1993).

The overestimation of toxicity levels by the RBA has implications for the industry and the WDOH if this were chosen as the approved method of toxin detection. At very low levels of toxicity (near the regulatory level of 80 µg), the geoduck fishery would be closed to harvest more often. However, the RBA could prove to be a useful tool in prescreening shellfish for PSP toxins. It also may have applications as a diagnostic tool in suspected cases of STX poisoning in humans and marine animals. Overall, the two methods were in very good agreement, as confirmed by a significant correlation coefficient ($r^2 = 0.83$ for all samples, $r^2 = 0.55$ for samples < 85 µg). The assay warrants consideration as a rapid, reliable, and cost-effective alternative to the MBA.

CONCLUSIONS

1. Geoducks collected at shallow depths in both tracts were more variable in levels of toxicity and were more toxic than geoducks from the deeper waters.
2. Toxicity levels in the shallow AP area were about two times those in the shallow QH area. Toxicities in the deep AP area were about five times those in the deep QH area. In the deep AP area, toxicity levels were almost always well above the closure level, while those in the deep QH area were always below closure level.
3. Results indicate substantial variability in toxicity levels among individual geoducks within a small population. It appears that the overall variability among geoducks in both shallow areas can be generally characterized as having a CV of about 53%, and in the AP deep area having a CV of 38%. In the QH deep area, the CV could not be measured because of an insufficient number of geoducks with detectable levels of toxicity.
4. All toxin levels recorded above the regulatory closure level (80 µg STXeq/100 g of tissue) were in the viscera only.
5. At low levels of toxicity (< 85 µg STXeq/100 g of tissue), the RBA overestimated the MBA. However, most of the overestimation can be accounted for by the inherent variability in the MBA and its tendency to underestimate low levels of toxicity by as much as 60%. Overall, the two

methods had a high degree of correlation ($r^2 = 0.83$ for all samples, $r^2 = 0.55$ for samples $< 85 \mu\text{g STXeq}/100 \text{ g}$ of tissue).

The results of this research have implications for the geoduck industry and public health agencies. The following recommendations can be implemented to improve geoduck sampling and analysis.

1. Due to the toxicity differences in harvest depth, the collection of geoducks during the PSP season could be limited to the deeper areas of a harvest tract to avoid fishery closures.
2. Farmers interested in culturing subtidal geoducks should consider doing so in deeper areas to avoid the high toxicities found in the shallow areas.
3. From a risk-management standpoint, a larger number of samples collected from shallow areas would have to be analyzed to reduce the risk of PSP intoxication in consumers.
4. The toxicity difference between tracts implies that the physical aspects of each tract may have to be considered when sampling and monitoring for PSP in geoducks.
5. Geoducks should be tested for PSP on an individual basis

rather than as a composite of three samples, to account for the high degree of individual variability seen in this study.

6. The viscera could be immediately removed and discarded prior to consumption of the siphon and mantle portions, which have been shown to be safe to consume even during times when viscera are highly toxic.

ACKNOWLEDGMENTS

We are grateful to Dr. K. Chew at the University of Washington, and F. Cox, L. Hanson, M. Antee, J. Tebaldi, M. Guichard, J. Jernigan, G. Hilton, M. Panoke, and D. Nguyen, at the Washington State Department of Health (WDOH) for their help and support in this research. Thanks also go to S. Jennison, J. Markert, M. Chevalier, and D. Williams at the WDNR, and to D. Winfrey with the Puyallup Tribe for all of their support in this research. Thanks go to J. Wekell and B. Conrad for statistical advice, and to R. Horner who offered critical comments on the manuscript. Geoducks, divers, boat operators, and boat time were provided by the WDNR and the Puyallup Tribe. Funding for this research was provided by the Washington Sea Grant Program, WDNR, WDOH, the Tulalip Tribe, and the University of Washington School of Fisheries.

LITERATURE CITED

- Aalvik, B. & K. Framstad. 1981. Assay and detoxification experiments with mytilotoxin in mussels (*Mytilus edulis*) from Nordasstraumen, western Norway, 1979 and 1980. *Sarsia*. 66:143–146.
- Association of Official Analytical Chemists. 1965. Paralytic shellfish poisoning biological method. *In: Official Methods of Analysis of the AOAC*, 10th ed. Association of Official Analytical Chemists, Arlington, VA. pp 282–284.
- Boesch, D., D. Anderson, R. Horner, S. Shumway, P. Tester & T. Whitledge. 1996. Harmful algal blooms in coastal waters: options for prevention, control and mitigation. *In: National Oceanic and Atmospheric Administration Coastal Ocean Decision Analysis Series*, No. 10. National Oceanic and Atmospheric Administration Coastal Ocean Office, Silver Spring, MD. 46 pp.
- Bricelj, V., J. Lee & A. Cembella. 1991. Influence of dinoflagellate cell toxicity on uptake and loss of paralytic shellfish toxins in the northern quahog, *Mercenaria mercenaria*. *Mar. Ecol. Prog. Ser.* 74:33–46.
- Bricelj, V. & D. Laby. 1996. Differential sensitivity and PSP toxin accumulation in two clam species, *Spisula solidissima* and *Mya arenaria* (abstract) *J. Shellfish Res.* 15:502.
- Bricelj, V. & S. Shumway. 1998. Paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics and biotransformation. *Rev/ Fisheries Sci.* 6:315–383.
- Davio, S. & P. Fontelo. 1983. A competitive displacement assay to detect saxitoxin and tetrodotoxin. *Analyt. Biochem.* 141:199–204.
- Dale, B., J. Hurst & C. Yentsch. 1978. Toxicity in resting cysts of the red tide dinoflagellate *Gonyaulax excavata* from deeper water coastal sediments. *Science*. 201:1223–1225.
- Doucette, G., M. Logan, J. Ramsdell & F. Van Dolah. 1997. Development and preliminary validation of a microtiter plate based receptor-binding assay for paralytic shellfish poisoning toxins. *Toxicon*. 35:625–636.
- Homer, R., D. Garrison & F. G. Plumley. 1997. Harmful algal blooms and red tide problems on the U.S. west coast. *Limnol. Oceanogr.* 42:1076–1088.
- Hwang, D., S. Lu, T. Noguchi, K. Hashimoto, I. Liao & S. Jeng. 1990. Seasonal variations of paralytic toxins in purple clam, *Soletellina diplos*. *J. Fish. Soc. Taiwan*. 17:305–311.
- Mackenzie, L., D. White & J. Adamson. 1996. Temporal variation and tissue localization of paralytic shellfish toxins in the New Zealand tuatua (surfclam), *Paphies subtriangulata*. *J. Shellfish Res.* 15:735–740.
- McFarren, E. 1957. Chemical determination of paralytic shellfish poison in clams. *In: Conference on Shellfish Toxicology*. United States Public Health Service. Washington, DC. pp.77–95.
- McFarren, E. 1962. Present status of the paralytic shellfish poison problem. Proceedings of the Shellfish Sanitation Workshop, November 1961, at Washington, D.C. United States Department of Health, Education and Welfare, Public Health Service, Washington, D.C. pp 275–277.
- Medcof, J., A. Leim, A.B. Needler, A.W. Needler, J. Gibbard & J. Naubert. 1947. Paralytic shellfish poisoning on the Canadian Atlantic coast. *Bull. Fish. Res. Bd. Can.* 75:1–32.
- Nishitani, L. & K. Chew. 1984. Recent developments in paralytic shellfish poisoning research. *Aquaculture*. 39:317–329.
- Nishitani, L. & K. Chew. 1988. PSP toxins in the Pacific coast states: monitoring programs and effects on bivalve industries. *J. Shellfish Res.* 7:653–669.
- Organization for the Prohibition of Chemical Weapons (OPCW). Background Paper On Saxitoxin Transfers. EC-VIII/TS.3, Executive Council, January 28, 1998.
- Park, D., W. Adams, S. Graham & R. Jackson. 1986. Variability of mouse bioassay for determination of paralytic shellfish poisoning toxins. *J. Assoc. Offic. Analyt. Chemists*. 69:547–550.
- Prakash, A. & J. Medcof. 1962. Hydrographic and meteorological factors affecting shellfish toxicity at Head Harbor, New Brunswick. *J. Fish. Res. Bd. Can.* 19:101–112.
- Prakash, A., J. Medcof & A. Tennant. 1971. Paralytic shellfish poisoning in eastern Canada, Bulletin No. 177. Fisheries Research Board of Canada. 87 pp.
- Shumway, S. E. 1990. A review of the effects of algal blooms on shellfish aquaculture. *J. World Aquaculture Soc.* 21:65–104.
- Trainer, V. & M. Poli. 2000. Assays for dinoflagellate toxins, specifically brevetoxin, ciguatoxin and saxitoxin. *In: Rochat, H. and M.-F. Martin-Eauclaire (eds.)*. Animal Toxins. Facts and Protocols. Birkhauser, Berlin. pp 1–19.
- U.S. Department of Commerce, National Oceanic and Atmospheric Administration. 1973. Tidal current charts, Puget Sound southern part. 3rd ed. National Ocean Survey, Rockville, MD.
- White, A., S. Shumway & J. Nassif. 1993. Variation in levels of paralytic shellfish toxins among individual shellfish. *In: T. Smayda and Y. Shimizu (eds.)*. Toxic Phytoplankton Blooms in the Sea. Elsevier, Amsterdam, the Netherlands. pp 441–446.

