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ANATOMICAL DISTRIBUTION AND SPATIO-TEMPORAL VARIATION IN PARALYTIC SHELLFISH TOXIN COMPOSITION IN TWO BIVALVE SPECIES FROM THE GULF OF MAINE

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ABSTRACT Marine bivalve molluscs accumulate paralytic shellfish poisoning (PSP) toxins through filter-feeding on blooms of toxic dinoflagellates, specifically, *Alexandrium* spp. on the Atlantic coast of North America. To determine the seasonal variation in PSP toxin composition in various anatomical compartments, inshore and offshore populations of the sea scallop *Placopecten magellanicus* and the surfclam *Spisula solidissima*, two bivalve species noted for prolonged toxin retention, were sampled periodically over two consecutive years in the Gulf of Maine. Individuals were dissected into tissue fractions for the determination of toxin composition (molar% and nmol g^{-1}) by high-performance liquid chromatography with fluorescence detection (HPLC-FD). The individual tissues included digestive gland, adductor muscle, gill and mantle, plus siphon and foot for clams and gonads for scallops. The calculated toxicity (μgSTXeq 100 g^{-1} shellfish tissue) confirmed the distributional trend of parallel mouse bioassays performed upon the tissues, but did not match quantitatively the bioassay results over a seasonal time scale. Partitioning of PSP toxin components among various organs was markedly different for the two bivalve species. For both sea scallops and surfclams, substantial differences in the relative amounts of PSP toxins among tissue compartments and seasonal variation were more evident than were differences between geographical populations of the same species. Analysis of PSP toxin profiles from a representative isolate of *Alexandrium tamarense* from the Gulf of Maine supported previous findings that the toxin composition in bivalves may differ considerably from that of toxigenic dinoflagellates. A pronounced seasonal toxin shift from the less potent N-sulfocarbamoyl toxins (C1/C2), which dominate in the dinoflagellate, to higher toxicity carbamate derivatives (e.g., GTXs, NEO, and STX) was found in both bivalve species. Relative to sea scallops, surfclams have a much higher capacity for *in vivo* PSP toxin conversion to decarbamoyl analogues. Metabolic and physico-chemical mechanisms which may be involved in PSP toxin transformation are compared among bivalve species.

KEY WORDS: *Placopecten, Spisula*, PSP toxins, biotransformation, saxitoxin

INTRODUCTION

The neurotoxins associated with paralytic shellfish poisoning (PSP) are among the most potent phycotoxins (toxins of algal origin) found in the marine environment. The accumulation of PSP toxins in suspension-feeding bivalves harvested in coastal zones constitutes a major public health risk to human consumers and has severely restricted the exploitation and development of both natural shellfish resources and aquaculture production. Although acute cases of PSP are relatively rare in advanced industrialized countries, due to the implementation of shellfish toxin monitoring programs and strict inspection procedures (Shumway et al. 1988, Cembella and Todd 1993), there have been few achievements in mitigating techniques to minimize toxin accumulation or to enhance detoxification of contaminated shellfish to levels below the acceptable regulatory limit (80 μgSTXeq [saxitoxin equivalents] $100g^{-1}$) adopted by many countries.

The shellfish toxicity associated with PSP has been a recurrent problem in the Gulf of Maine for several decades. As a result, a comprehensive shellfish toxicity monitoring program based upon the mouse bioassay procedure (AOAC 1984) has been in effect for nearshore waters of the coast of Maine since the 1970s (Shumway et al. 1988). A recent dramatic increase in PSP toxicity in key shellfish species from both the Canadian and American sectors of Georges Bank since 1989 (Watson-Wright et al. 1989, Shumway et al. 1993, White et al. 1993a), has seriously threatened the economic viability of the offshore 'roe-on' fishery for sea scallops, *Placopecten magellanicus* and the harvest of whole surfclams, *Spisula solidissima*.

The present review incorporates recent evidence of PSP toxin accumulation and biotransformation in commercially-important natural populations of sea scallops and surfclams from the Gulf of Maine. The distribution of PSP toxins in inshore and offshore sea scallops in Maine coastal waters was compared with surfclams from an inshore site and populations from Georges Bank (40–43°N 66–70°W). Where relevant, comparative data on anatomical distribution and spatio-temporal variation in PSP toxin composition in other bivalve molluscs have been considered. Expressly excluded are detailed discussions of PSP toxin uptake kinetics, detoxification rates, resistance to deleterious effects of accumulated toxin, and other species-specific physiological responses to toxin exposure.

PSP Toxins in Toxigenic Dinoflagellates

The PSP toxins comprise a suite of at least 18 naturally-occurring neurotoxic tetrahydropurine derivatives produced among several species of free-living planktonic marine dinoflagellates, including *Alexandrium* spp., *Pyrodinium bahamense* var. *compressum*, and *Gymnodinium catenatum* (Hall and Reichardt 1984, Taylor 1984, Oshima et al. 1990) (Fig. 1). These PSP toxin derivatives can be classified according to their chemical structure and specific potency in mammals (as sodium channel blocking agents); the carbamate toxins (GTX1-GTX4, NEO, STX) are the

R1	R2	R3	Carbamate Toxins	N-Sulfocarbamoyl Toxins	Decarbamoyl Toxins
H	H	H	STX	B1	dc-STX
OH	H	H	NEO	B2	dc-NEO
OH	H	OSO_3^-	GTX 1	C3	dc-GTX 1
H	H	OSO_3^-	GTX 2	C1	dc-GTX 2
H	OSO_3^-	H	GTX 3	C2	dc-GTX 3
OH	OSO_3^-	H	GTX 4	C4	dc-GTX 4

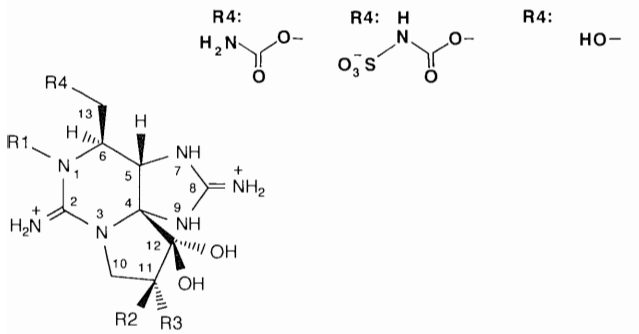

Figure 1. Structures of PSP toxins found in toxigenic dinoflagellates and shellfish, which include carbamate, N-sulfocarbamoyl, and decarbamoyl derivatives. Saxitoxin = STX; neosaxitoxin = NEO; gonyautoxins 1,2,3,4 = GTX1,2,3,4; dc- = decarbamoyl analogues.

most potent, whereas the N-sulfocarbamoyl derivatives (B1, B2, C1–C4) have a much lower specific toxicity. The decarbamoyl (dc-) analogues, of intermediate toxicity, are generally less abundant in toxigenic dinoflagellates, particularly *Alexandrium* spp., but they may be important toxin components in certain bivalve species (Sullivan et al. 1983).

No natural toxigenic dinoflagellate population or individual isolate in culture has been found to contain all naturally occurring PSP toxin analogues, and non-toxic variants of indistinguishable morphotypes are often found. In toxigenic varieties, a rather complex spectrum of PSP toxin derivatives may be produced at physiological equilibrium during exponential growth phase. In the absence of environmental stress, the toxin profile of *Alexandrium* cells is considered to be characteristic of the strain (Cembella et al. 1987, Anderson 1990). This conservative toxin profile (presumably fixed genetically) has been employed with some success to define geographical populations (Cembella et al. 1987, Oshima et al. 1989).

Alexandrium spp. Associated with PSP Toxicity in the Gulf of Maine

At the generic level, the organism(s) responsible for PSP toxicity in shellfish along the Atlantic coast of North America is no longer a matter of dispute. The taxonomic history of marine dinoflagellates implicated in PSP toxicity in this region is, however, convoluted and requires further clarification. The species causing PSP toxicity in the lower estuary and Gulf of St. Lawrence and in the Bay of Fundy was identified originally as *Gonyaulax tamarensis* (Needler et al. 1949), according to a description of this species from the type locality near Plymouth, UK. Later attempts to differentiate populations from eastern North America into varieties *sensu* Braarud (1945) (i.e., var. *excavata* versus var. *tamarensis*) proved ultimately to be unconvincing. After these varieties were elevated to species, subsequent alteration of the descriptions to include characters such as toxicity, bioluminescence and the presence of a ventral pore were used to redefine the species (Loeblich and Loeblich 1975). Thus, the New England red-tide species was assigned to *G. excavata*. With the eventual recognition that these toxigenic species were not "true" *Gonyaulax* (reviewed by Taylor 1984), several alternative generic solutions (*Protogonyaulax, Gessnerium,* or *Alexandrium*) were proposed. Regardless of the respective merits of these morphotaxonomic treatments, now accompanied by biochemical and molecular genetic data (Scholin et al. 1993), it is clear the PSP toxicity in eastern North American waters can be attributed to species referable to the genus *Alexandrium* (Halim) emend Balech (1990), principally *A. excavatum, A. tamarense,* and *A. fundyense*.

Blooms of *Alexandrium* spp. associated with PSP toxicity are recurrent events along the coast of eastern North America, generally following the annual vernal warming (Hurst and Yentsch 1981), but populations tend to be sub-surface and visible water discolourations ("red-tides") are rarely (if ever) observed. Bloom initiation, development and dispersion appear to be largely driven by hydrodynamic factors involved in tidal mixing, upwelling, density stratification and longshore currents arising from geostrophic flow (Franks and Anderson 1992). In the Gulf of Maine, the respective contribution to PSP toxicity in shellfish attributable to localized blooms versus longshore transport of toxic vegetative cells remains to be established.

In any case, even cryptic *Alexandrium* blooms are capable of causing high toxicity levels in inter-tidal and neritic populations of bivalve shellfish, including clams, mussels, oysters, and scallops, in Maine coastal waters (Shumway et al. 1988). In a comprehensive review of PSP toxicity in scallops, Shumway and Cembella (1993) cited levels as high as 150,000 μgSTXeq 100 g^{-1} in scallop digestive glands from the Bay of Fundy in eastern Canada, where *A. fundyense* is considered to be the source of the toxicity. The New England coastline is subject to periods of intense annual PSP toxicity in shoreline molluscs, with a gradual diminution in maximum toxicity, frequency and duration in toxic events towards the south. In the aftermath of the catastrophic meterological events associated with Hurricane Carrie in 1972, apparently resulting in a major bloom dispersion, PSP toxicity has become endemic in Massachusetts, albeit at a generally reduced intensity since the original episode. There is some evidence that the net toxicity per cell in *Alexandrium* populations tends to decline from north to south along a latitudinal gradient, resulting from a shift in the toxin composition and a decrease in the amount of PSP toxin per cell (Maranda et al. 1985).

The exact identity and population dynamics of the organism responsible for this offshore toxicity on Georges Bank are currently unknown. A recent net sample from Georges Bank yielded cells of *Alexandrium tamarense* (Shumway et al. 1993), a plausible candidate species as the cause of PSP toxicity in this region. However, the presence of *Alexandrium* spp. in the gut contents of surf clams from Georges Bank was not confirmed.

Net Accumulation of PSP Toxicity

Time-series data from shellfish toxin monitoring programs based upon the AOAC (1984) mouse bioassay have indicated both geographical and seasonal variation in net PSP toxicity among diverse bivalve species (reviewed by Quayle 1969, Prakash et al. 1971, Shumway et al. 1988, 1993, Shumway and Cembella 1993). Specifically, in sea scallops from the Gulf of Maine, wide seasonal fluctuations in toxicity have been reported (Bourne 1965, Jamieson and Chandler 1983, Watson-Wright et al. 1989, Gillis et al. 1991),

occasionally with the appearance of fall and winter maxima. All bivalve species known to accumulate PSP toxins exhibit marked differences in the distribution of toxicity among the various organs (Prakash et al. 1971, Blogoslawski and Stewart 1978, Maruyama et al. 1983). As PSP toxins are released after digestion of toxic cells in the viscera, the digestive system is invariably found to contain the highest toxicity levels immediately following exposure to toxic algal blooms. However, the kinetics of PSP toxin elimination and the sequestration of toxin in other organs follow a characteristic pattern in each bivalve species (reviewed by Shumway and Cembella 1993 for scallops). Both sea scallops (Medcof et al. 1947, Prakash et al. 1971, Jamieson and Chandler, 1983) and surfclams (White et al. 1993a, Shumway et al. 1993) are capable of prolonged retention of PSP toxins, thus they are suitable candidate species for comparative studies of long term changes in PSP toxicity and toxin composition.

Seasonal partitioning of PSP toxicity in various anatomical compartments was compared for individual tissues of adult surfclams from an inshore site at Head Beach, ME and offshore stations on Georges Banks (1990–91), and for sea scallops from inshore (20 m depth) and offshore (180 m depth) stations in the Gulf of Maine near Boothbay Harbor (1988–89) (Fig. 2). Tissues selected for both species included adductor muscle, mantle (rims), digestive gland (viscera) and gill. For scallops, gonads were dissected and analyzed separately; the prominent foot and the distal extension of the mantle (siphon) were analyzed as separate tissues for surfclams. Toxicity was determined by the mouse bioassay (AOAC 1984). The tissues of randomly selected individuals of surfclams (n = 6) and sea scallops (n = 8) were pooled for homogenization in 0.1 M HCl, followed by heating at 100°C (5 min), pH adjustment to 3.5–3.7, and centrifugation to clarify the supernatant. After intraperitoneal injection of 1 mL of tissue extract into adult white mice (n = 3), toxicity was determined by interpolation of mouse death time within 15 min from the calibrated dose response table prepared by injection of purified STX.

For comparison with the mouse bioassay, two alternative methods of high-performance liquid chromatography with fluorescence detection (HPLC-FD) (Sullivan and Wekell 1986, Oshima et al. 1989) were applied to toxin extracts of tissues from the same sites in the Gulf of Maine. The analytical methods were optimized to preserve the native toxin composition in the tissues by extraction in 0.1 M acetic acid without heating (Bricelj et al. 1990, 1991, Cembella et al. 1993). Net toxicity (in μgSTXeq 100 g^{-1}) was calculated from toxin concentrations (in μmol l^{-1}) measured by HPLC, based upon specific toxicity values (in μgSTXeq μmol $^{-1}$) (Fig. 3) determined empirically from mouse bioassay calibration data using purified toxins (Sullivan et al. 1985, Oshima 1992). For scallop tissues, the 11-hydroxysulfate toxins GTX1 and GTX4 were combined for data analysis due to inconsistent epimerization.

The calculated toxicity results determined by HPLC generally reflected the toxicity trend of the corresponding mouse bioassays for populations of both bivalve species, although the bioassay values were usually substantially higher (Fig. 4). For both species, the summer toxicity peaks in digestive glands, which are inferred to indicate the occurrence of toxic blooms, were more pronounced in the bioassay results than for the HPLC-FD data. The mouse bioassays indicated as much as three-fold higher toxicity in surfclam digestive glands than the HPLC method and differences for inshore sea scallops were often even more dramatic. Since previous validations of the HPLC-FD method (Sullivan et al. 1985, Sullivan and Wekell 1986, Martin et al. 1990) have shown good correlations ($r^2 \geq 0.9$) with the mouse bioassay when performed on extracts prepared according to the AOAC (1984) protocol, it is likely that the discrepancy was due primarily to differences in sample preparation.

In the AOAC (1984) bioassay procedure, extraction of toxins with hot 0.1 M HCl tends to increase net toxicity (known as Proctor enhancement) due to indeterminate hydrolysis of low potency N-sulfocarbamoyl toxins (C1–C4) to their GTX analogues (Fig. 1); some degradation of the low toxicity components B1 and B2 to the non-sulfated carbamate toxins STX and NEO, respectively, can also occur, resulting in increased toxicity (Hall and Reichardt 1984, Boyer et al. 1986) (Fig. 3). A chemically-induced shift in the ratios of α-:β-epimers of the C-11 sulfated derivatives (GTX2/GTX3; GTX1/GTX4) is also expected, although this would have little effect on net toxicity. The efforts to avoid artifactual toxin conversion in the extraction procedure for HPLC-FD

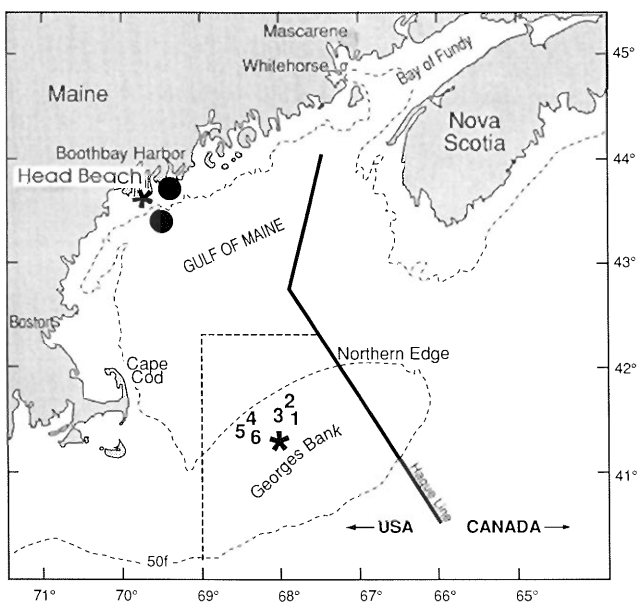

Figure 2. Map of primary sampling sites for sea scallops ● and surfclams * in the Gulf of Maine.

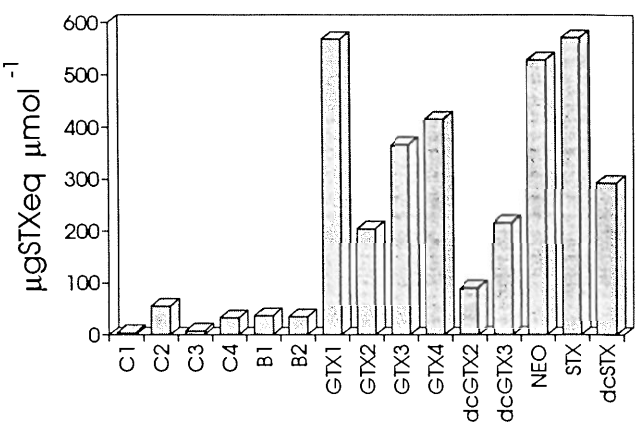

Figure 3. PSP toxin conversion factors for the calculation of specific toxicity (μgSTXeq μmol^{-1}) based upon values determined empirically by mouse bioassays (mouse units [M.U.] μmol^{-1}) (Oshima 1992), assuming 1 M.U. = 0.23 μgSTXeq. The factor for B2 was calculated from a value given by Sullivan et al. (1985) in M.U. μmol^{-1}.

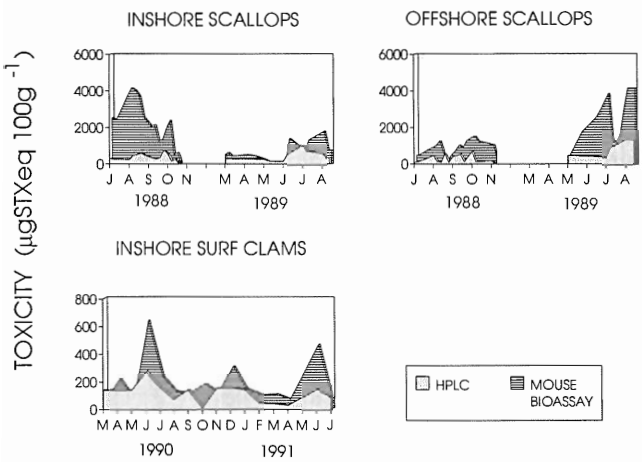

Figure 4A,B. Comparison of mean seasonal variation (n = 8) in toxicity (µgSTXeq 100 g^{-1}) in digestive glands of offshore and inshore scallops (A) and inshore surf clams from Head Beach (B), determined by AOAC mouse bioassay and calculated from HPLC-FD chromatograms using toxin specific conversion factors (µgSTXeq µmol^{-1}).

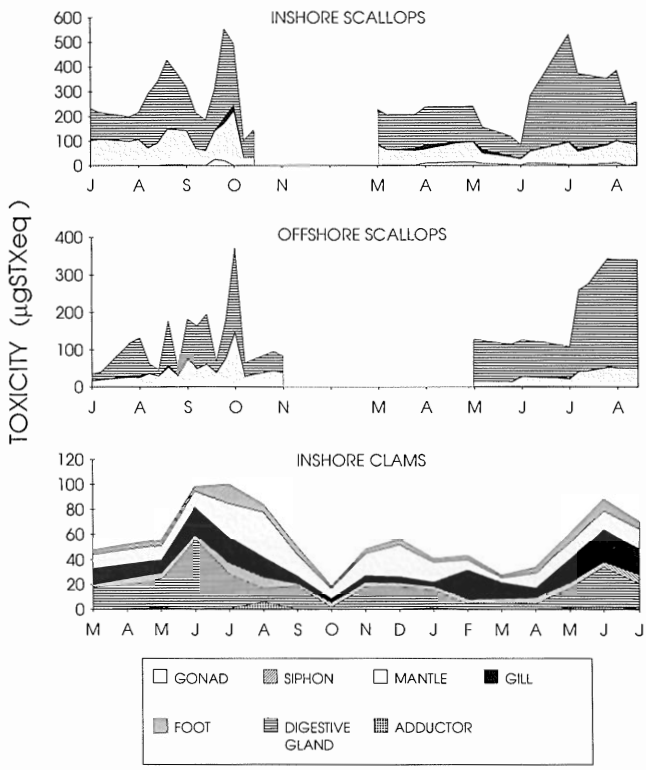

Figure 5. Seasonal variation in mean toxin burden (µgSTXeq per individual) in tissues of sea scallops (1988–89) and surfclams (1990–91) from the Gulf of Maine, calculated from HPLC-FD values.

analysis thus yield values representing "actual" toxicity rather than an approximation of "potential" toxicity. This explanation for the difference in net toxicity as determined by these alternative methods was supported further by the fact that the greatest discrepancies were almost invariably found during summer toxicity peaks when the relative contribution of the N-sulfocarbamoyl toxins (particularly C1/C2) to the total toxin body burden was at maximum, apparently indicating the recent ingestion of dinoflagellates rich in these derivatives.

On a weight-normalized basis (µgSTXeq 100 g^{-1}), toxicity in digestive glands from sea scallops was often much higher than in surfclams from the inshore site, according to the mouse bioassay data (Fig. 4). However, it is unwise to attribute much validity to this comparison, as the sampling dates did not overlap and the inshore sites for each species were in close proximity but not identical. Both analytical techniques revealed a winter peak in toxicity in inshore surfclams during November to February, which is difficult to explain in terms of conventional toxic bloom dynamics. Unfortunately, no samples of sea scallops were available during the winter from the Gulf of Maine to prove whether or not toxicity was present throughout the winter months, when toxic *Alexandrium* blooms are not expected to occur. Nevertheless, the substantial (although declining) body burden of toxicity in both scallop populations in the late fall, prior to the suspension of sampling for the winter, and elevated toxicity (>200 µgSTXeq 100 g^{-1}) found in early spring when sampling was resumed (Fig. 5), offers circumstantial evidence that considerable toxicity persisted in digestive gland and mantle tissue during the winter.

This is consistent with mouse bioassay data acquired from 1985–87 for combined fractions (digestive gland, mantle, and gill) of sea scallops from Gulf of Maine sites (Shumway et al. 1988). Whereas high toxicity levels were maintained throughout the year in the offshore zone, the peak toxicity in inshore scallops occurred during early summer, with persistent toxicity extending into the fall and winter. Moreover, peak toxicity was reported previously to occur in scallops from the Bay of Fundy during fall and winter, in the apparent absence of toxic blooms (Bourne 1965, Jamieson and Chandler 1983).

In the present study, the general pattern of toxicity among various tissues, as determined by the HPLC-FD method, essentially substantiated that found previously for natural populations of both sea scallops (reviewed by Shumway et al. 1988 and Shumway and Cembella 1993) and surfclams (Shumway et al. 1993) using the AOAC mouse bioassay. For sea scallops, the typical rank order of toxicity burden (µgSTXeq) throughout the year was as follows: digestive gland > mantle ≫ gill > gonad ≫ adductor muscle (Fig. 5), although mantles were briefly more toxic on a weight-normalized basis (µgSTXeq 100 g^{-1}) than digestive glands during the post-bloom period in the fall. This toxicity hierarchy was supported by the corresponding mouse bioassay data for individual tissues (not shown), albeit that toxicity values for gills, gonads, and adductor muscles remained consistently below the bioassay detection limit (<58 µgSTXeq 100 g^{-1}) throughout the two-year sampling period, except for a brief toxicity peak (maximum: 426 µgSTXeq 100 g^{-1}) in gonads from the offshore population in the summer of 1989.

For sea scallop populations, rapid increases in toxicity burden in digestive glands were usually accompanied by concomitant, but less dramatic, increases in toxicity in other organs, particularly in mantles (Fig. 5). The prominent rise in toxicity in digestive glands in the summer of 1989 was delayed by a month in the offshore zone, relative to the inshore population. The scallop populations exhibited a peak in toxicity of similar magnitude in all tissues during the fall of 1988.

During summer toxicity peaks, the distribution of PSP toxins among various organs was strikingly similar for inshore and offshore scallops; in excess of 95% of the total toxin load (nmol per individual organ) was partitioned into the digestive gland plus mantle tissues (Fig. 6). As expected, the relative contribution of

basis (%molar), except during summer toxicity peaks, when the toxin profile became more complex. The N-sulfocarbamoyl toxins were prevalent for short periods during toxicity peaks in digestive gland, gill, foot and siphon tissue, whereas they were barely registered in mantle and adductor muscles. During the summer toxicity maximum, the ratio of β-to α-epimers of the C-11 OSO_3^- derivatives (GTXs) rose in all tissues, except in the viscera, where there was strong evidence of epimerization (Fig. 12B).

The relative distribution of N-1 hydroxy derivatives also exhibited some seasonal variation among surf clam tissues; there was a prominent maximum in these toxins which corresponded temporally to the initial toxicity peak in early summer in all tissues (Fig. 13B). As overall toxin levels decreased following the winter toxicity peak (Fig. 5), the ratio of N-1 hydroxy toxins to total components also declined (Fig. 13B).

The origin of the winter toxicity maximum from November to January in digestive glands and mantles of surf clams (cryptic late-season bloom? sinking of senescent fall bloom? toxic benthic cysts?) is difficult to explain by invoking arguments based upon the toxin spectrum. Substantial amounts of dcSTX were accumulated in the fall, especially in gills, mantles and siphons, and these high relative levels were maintained throughout the winter and subsequent spring. Biotransformation alone cannot account for the winter toxicity increase; the relatively high levels of STX and dcSTX in the most toxic tissues (digestive gland, mantle, gills) indicated that substantial toxin catabolism had already occurred prior to the toxicity peak. The lack of significant N-sulfocarbamoyl toxins during the winter also suggests that "new" toxin was not introduced from cryptic winter blooms. Nevertheless, the shift towards an increase in the β-:α-epimeric ratios of the C-11 OSO_3^--toxins and the relative increase in N-1 hydroxy toxins observed during early winter would support the proposed scenario that there was a exogenous toxin source at this time.

With reference to previous studies on other clam species, the fact that relative and absolute amounts of STX retained in the siphon were not dramatically elevated in surfclams was rather surprising. Early work on the PSP toxin content of butter clams from Alaska (reviewed by Schantz 1984) tended to emphasize this organ as the major repository for STX. Although this is now seen as an oversimplification, given that the first efforts at toxin fractionation tended to regard STX as the sole PSP toxin component, subsequent work has not contradicted this observation. According to Sullivan's (1982) studies on natural butter clam populations from Puget Sound, WA and from controlled feeding trials, STX and dcSTX were retained primarily in the siphons. This was confirmed subsequently by Beitler and Liston (1990) who also found STX accumulation mainly in the siphon.

In sea scallops, the most important contributors to total toxin content in digestive glands were toxins GTX2 and C1/C2 throughout most of the year. The epimeric ratio of GTX2:GTX3 in sea scallops approximated 3:1 and did not exhibit much seasonal variation, especially in digestive glands and mantles (Fig. 12a). The N-1 hydroxy carbamate toxins (GT1/GTX4, NEO) represented >30% of the molar toxin composition in the Gulf of Maine dinoflagellate, yet these components were relatively less abundant in scallop populations (Fig. 13a). Apart from the occasional appearance of GTX1/GTX4, there was little variation in relative toxin composition in digestive glands from either scallop population within a given year.

The proportion of N-sulfocarbamoyl derivatives C1–C4 in scallop digestive glands was higher in 1989 than in the preceding year, but was less persistently elevated in the inshore population. The higher C1/C2 content and N-sulfocarbamoyl:carbamate ratio in digestive glands from the early spring of 1989, relative to the previous autumn, may indicate recent exposure to a toxic bloom. Unlike surfclams, where this ratio could be used to identify peaks in the occurrence of summer dinoflagellate blooms, the corresponding seasonal trend in the proportions of carbamate:N-sulfocarbamoyl toxins in scallop digestive glands was less clearly defined.

The typical toxin profile of scallop mantles was similar to that of the digestive gland, with the carbamate epimers GTX2 and GTX3 as the dominant toxins in both populations. There was a lesser contribution by toxins C1/C2 than in the digestive tissues, particularly during toxicity peaks. Trace quantities of N-sulfocar-

TABLE 1.

Mean coefficient of variation* (S.D./$\bar{X}$ as %) of weight-normalized toxicity (μgSTXeq $100g^{-1}$) for tissues of individual sea scallops (n = 8) and surfclams (n = 6) from populations in the Gulf of Maine.

	C1-C4	GTX1/GTX4	GTX2	GTX3	NEO	dcSTX	STX	Total
INSHORE SCALLOPS								
Digestive gland	37.6	73.1	52.6	57.3	63.6		73.9	53.0
Gill	27.2	111.2	90.2	98.6	43.7		111.6	58.6
Mantle	33.3	72.8	43.5	41.4	59.6		47.6	40.3
Gonad	67.1	27.6	138.4	146.8	77.0		47.9	122.0
OFFSHORE SCALLOPS								
Digestive gland	41.1	81.1	42.4	43.8	56.4		36.9	42.2
Gill	24.4	84.7	67.7	76.7	52.6		123.6	74.2
Mantle	32.9	73.4	49.7	50.5	50.8		56.7	45.7
Gonad	63.0	50.4	102.3	100.9	57.9		61.0	103.5
SURF CLAMS (Head Beach)								
Digestive gland	13.5	48.4	64.5	71.2	71.6	55.8	72.5	54.0
Gill	8.4	52.4	67.0	80.1	124.1	45.2	65.5	58.9
Mantle	20.1	99.7	50.9	63.9	51.1	46.6	62.8	54.8
Siphon	19.8	67.6	50.7	77.6	75.9	48.5	74.3	62.3
Foot	4.9	85.5	66.8	89.2	58.6	16.7	79.0	72.1
Adductor	5.9	57.5	65.4	84.3	14.8	9.4	102.2	93.6

* Number of observations averaged: inshore scallops (n = 26); offshore scallops (n = 17); inshore surfclams (n = 15).

bamoyl toxins C3 and C4 were detected in both mantle and digestive glands during the summer. Virtually all toxin was present as carbamate derivatives in mantles in both scallop populations during 1988, and the N-sulfocarbamoyl:carbamate toxin ratio was consistently lower than in other tissues. That the N-sulfocarbamoyl fraction was not present in the mantle in strict equilibrium with the digestive gland suggests that these toxins are preferentially eliminated from mantles or are transformed in the digestive gland prior to export to the mantles.

Among all scallop tissues, the toxin profile in gills was the most erratic on a seasonal basis, and some geographical variation between inshore and offshore population was evident. During toxicity peaks, the principal toxin analogue in gills was NEO, although GTX2 and C1/C2 sometimes co-dominated. Averaged seasonally on a relative molar basis, toxins C1 + C2 were the most significant toxins in the inshore scallop population, usually comprising half of the total toxin content (nmol g^{-1}). This was not the case for the offshore stocks, where NEO and GTX2 usually tended to dominate. Trace concentrations of C3 and C4 were found, particularly in association with high levels of toxin C2 occurring at maximum toxicity.

The PSP toxin profile in scallop gonads (when toxin was present) fluctuated seasonally and was dominated by C1/C2, GTX2 and GTX3, with the gonyautoxin components accounting for most of the toxicity. The N-sulfocarbamoyl toxin content in inshore scallop gonads was unusual in the summer in that significant amounts of toxin C4 were accumulated. The clearest evidence for biotransformation in scallop tissues from the Gulf of Maine was found in offshore gonads in 1989. Dominance of the C-toxin fraction in the spring shifted to a large relative increase in GTX2/GTX3 and a decrease in NEO accompanied by the appearance of STX in summer. This pattern is consistent with the loss of the N-21 sulfocarbamoyl moiety and reductive loss of the N-1 hydroxyl group. Since STX appeared only rarely in gonads, during summer when abundance in digestive glands was maximal, it is likely that transfer efficiency of this toxin analogue from surrounding tissues is rather low. There was no apparent systematic seasonal trend in the ratio of carbamate:N-sulfocarbamoyl toxins in gonads, yet shifts in this ratio closely corresponded to those occurring in digestive glands. The gonadal toxin composition was very similar to that of associated digestive glands in offshore scallops, particularly in 1988. In contrast, in inshore scallop gonads, toxins C1/C2 typically comprised a greater fraction of the toxin components than in either digestive glands or offshore scallop gonads. In gonads from the inshore population, there were wide fluctuations in the GTX2:GTX3 ratio (range: >9:1 to <1:9) in both years which did not appear to be linked temporally with toxic dinoflagellate blooms in an obvious manner. This discrepancy may be accounted for by the effects of gonadal maturation on toxin dynamics, as inshore scallop gonads are expected to be more active reproductively than their offshore counterparts (Barber et al. 1988).

As for surfclams, the adductor muscles of sea scallops were distinguished from other organs by the low relative abundance of N-sulfocarbamoyl toxins. When toxins were present in scallop adductor muscles, GTX2 and GTX3 were usually found, although occasionally trace levels of GTX1/GTX4 or STX were identified. As the HPLC-FD detection limits for GTX2/GTX3 are much lower than for STX and N-1 hydroxy derivatives, the spectrum of toxins reported here for weakly toxic scallop adductor muscles may be somewhat biased towards the highly fluorescent derivatives produced from toxins GTX2/GTX3. That STX was not relatively abundant in adductor muscles could also indicate that this derivative is not readily transferred due to its high binding affinity for the viscera.

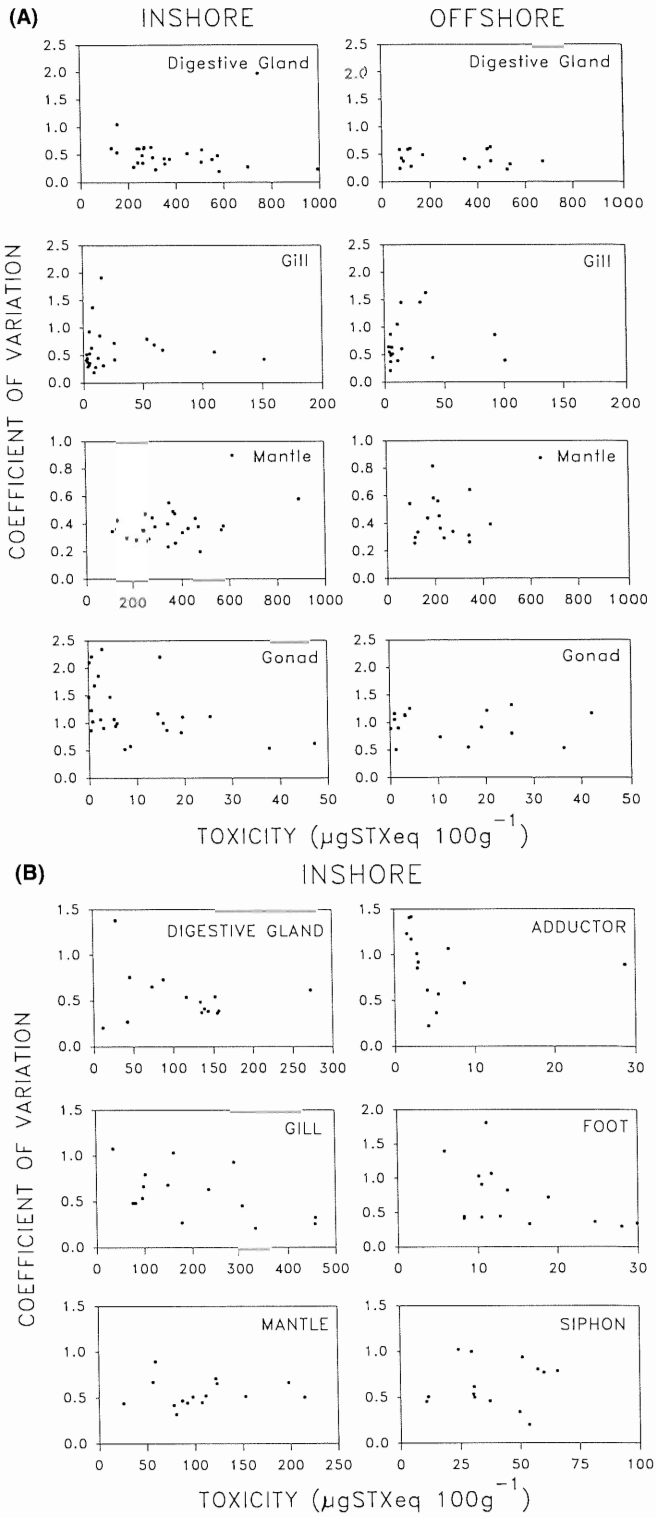

Figure 14A,B. Mean toxicity (μgSTXeq 100 g^{-}) as determined by HPLC-FD versus the coefficient of variation (C.V. = S.D./$\bar{X}$) in different organs of inshore and offshore scallops (A) and surfclams (B) from the Gulf of Maine.

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