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ANATOMICAL AND SPATIO-TEMPORAL VARIATION IN PSP TOXIN COMPOSITION IN NATURAL POPULATIONS OF THE SURFCLAM *SPISULA SOLIDISSIMA* IN THE GULF OF MAINE

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ABSTRACT

The surfclam, *Spisula solidissima*, a bivalve mollusc which retains PSP toxins for extended periods, was sampled over two consecutive years from populations in the Gulf of Maine. To determine the seasonal and geographical variation in toxin composition, adult individuals (n=8) were dissected into six tissue fractions for toxin analysis by high-performance liquid chromatography (HPLC). The net toxicity calculated from HPLC data confirmed the general pattern of toxicity among the tissues, as determined by parallel AOAC mouse bioassays. There were substantial differences in the relative amounts of PSP toxins among various tissue compartments, with the following typical trend in net toxicity as a portion of total body toxin burden: digestive gland > mantle = gill > siphon = foot > adductor muscle. Seasonal variations were more pronounced than were geographical differences between the two populations. Metabolic toxin conversion was indicated by the elevated STX levels, the *de novo* appearance of decarbamoyl STX, the rapid epimerization of GTX derivatives, and the sharp decline in N-sulfocarbamoyl toxin concentration in all surfclam tissues following the mid-summer toxic dinoflagellate bloom(s).

INTRODUCTION

Blooms of the toxic dinoflagellate *Alexandrium* are a recurrent cause of toxicity associated with paralytic shellfish poisoning (PSP) in the Gulf of Maine (Cembella *et al.*, 1994). The surfclam *Spisula solidissima* is known for prolonged PSP toxin retention (>1 yr); this species can accumulate high toxin levels which persist throughout the winter in the Gulf of Maine (Shumway *et al.*, 1994). Recently, high PSP toxicity in commercially harvested shellfish from offshore zones on Georges Banks (White *et al.*, 1993) has led to intensive efforts to characterize the persistence and extent of this toxicity.

Filter-feeding bivalve molluscs vary widely in their capacity to ingest toxigenic dinoflagellates and to sequester PSP toxins in various anatomical

compartments (Lassus *et al.*, 1989; Bricelj *et al.*, 1991; Bricelj and Cembella, this volume). Certain shellfish are also capable of profoundly modifying the composition of ingested toxins via metabolic transformations (Sullivan *et al.*, 1983), thereby affecting net toxicity. High-performance liquid chromatography with fluorescence detection (HPLC-FD) was used to determine: 1) the seasonal persistence of PSP toxins over two successive years; 2) the anatomical distribution of toxins; and 3) progressive shifts in the toxin profile indicative of biotransformation or selective retention, in natural surfclam populations from the Gulf of Maine.

MATERIALS AND METHODS

Adult surfclams were collected in 1990-91 by digging from an inshore site at Head Beach, ME and by hydraulic dredge from offshore stations on Georges Bank in the Gulf of Maine. Individual tissues (digestive gland, gill, mantle, siphon, foot, adductor muscle) were dissected from randomly selected specimens (n=8), then lyophilized and prepared for PSP toxin analysis by homogenization in 0.1 M acetic acid (Bricelj *et al.*, 1991). After high-speed centrifugation, and ultrafiltration of the supernatant (10 000 MW cut-off), toxin composition was determined by two reverse-phase HPLC techniques (Sullivan and Wekell, 1987; Oshima *et al.*, 1989). The method of Oshima *et al.* (1989) was used to resolve the N-sulfocarbamoyl and decarbamoyl derivatives. Conversion factors for calculating specific toxicity (in μgSTXeq $100g^{-1}$) from toxin concentrations (in μmol l^{-1}) were provided from empirical mouse bioassay data (Y. Oshima, pers. comm.). The HPLC toxicity data were compared with those of mouse bioassays (AOAC, 1984) performed on individual surfclam tissues from the same sites.

RESULTS

The seasonal pattern of total PSP toxin concentration (nmol g^{-1}) in surfclams at the inshore site indicated a biphasic peak in digestive glands in the spring to fall of 1990, followed by high levels maintained throughout the winter, and a subsequent rise in the spring and early summer of 1991 (data not shown). This general distribution was also reflected in the other tissues, although the tendency for bimodal toxin peaks in the summer was less pronounced. Except for the gills, toxin concentration maxima in other anatomical compartments occurred several weeks after the initial toxin rise in the viscera, when toxin concentration was declining in digestive tissues. The precipitous decline in total toxin concentration in the viscera to levels <0.5 nmol g^{-1} in the fall was accompanied by toxin levels in mantle and gill tissues which exceeded those in the viscera for several weeks. The mouse bioassay results paralleled the toxicity values calculated from HPLC data, including the mid-winter toxicity rise in digestive gland and mantle tissues. The toxicity derived by HPLC analysis, however, was as much as three-fold lower than that determined by mouse bioassay, particularly during toxicity peaks.

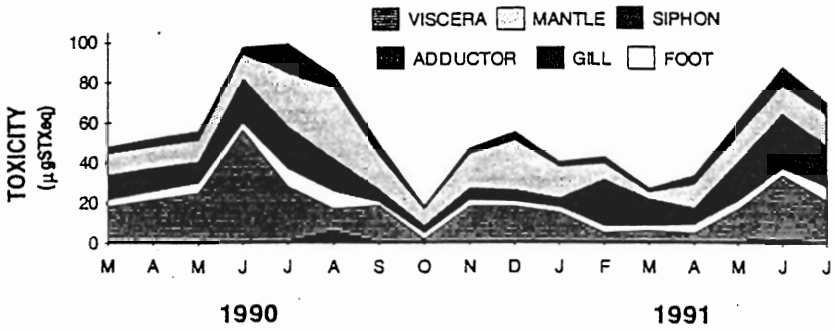

Fig. 1 Anatomical distribution of PSP toxicity (µgSTXeq individual^{-1}) in adult surfclam tissues from Head Beach (1990-91) in the Gulf of Maine.

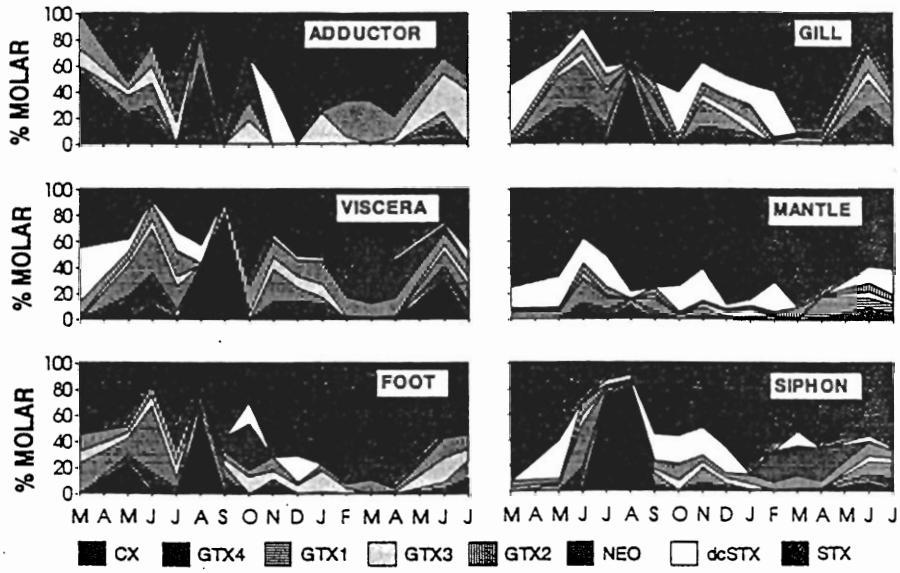

Fig. 2 Seasonal variation in mean relative composition (%molar) in inshore surfclams from Head Beach (1990-91).

On the basis of total body toxin burden (µgSTXeq per individual surfclam) (Fig.1), the viscera constituted the most significant toxic component (>50% at the toxicity peak) during the late spring to fall period, whereas mantle and gill tissues were approximately equal sub-dominant constituents. The foot and siphon contained substantially less total toxicity than these tissues. Toxin burden in adductor muscles was a significant portion of total body burden (up to 5%) only

when total toxicity was declining from the summer maximum. During the early winter of 1990, however, toxin burden in mantles surpassed that in the viscera; later in the winter and in early spring of 1991, the gills became the most toxic organ.

The PSP toxins found in surfclams included carbamate (GTX1-GTX4, NEO, STX), N-sulfocarbamoyl (Cx=C1/C2), and decarbamoyl (dcSTX) derivatives (Fig.2). On a relative basis (%molar), STX was usually the dominant toxin in most tissues, except during summer toxicity peaks. 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 mantles and adductor muscles. A shift in the ratios of C-11 OSO_3^- toxins from ß- to α-epimers was evident during the summer toxicity maximum in all tissues, except in the viscera. 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. The relative distribution of N-1 hydroxy derivatives also exhibited some seasonal variation among the tissues; there was a prominent maximum in these toxins which corresponded temporally to the initial toxicity peak in early summer in all tissues.

DISCUSSION

Maximal PSP toxicity in surfclam viscera coincides with the seasonal occurrence of *Alexandrium* blooms along the coast of the Gulf of Maine. Experiments involving juvenile surfclams fed toxic *Alexandrium* cells (Bricelj and Cembella, this volume) revealed that PSP toxins are readily accumulated, with no obvious indications of feeding inhibition even at high toxin burden ($>10^4$ µgSTXeq $100g^{-1}$ of digestive tissue). Prolonged toxin retention by surfclams results in high toxicity throughout the winter in the Gulf of Maine. The magnitude of the PSP toxin peak varies from year to year, as a direct result of ephemeral toxic blooms, at inshore stations (Shumway *et al.*, 1994) and on Georges Bank (White *et al.*, 1993). The slow detoxification rate in this species may result in cumulative PSP toxin sequestration from one year to the next, particularly in the offshore populations.

The peak in winter toxicity, remains, nevertheless, to be explained. The toxin profile in surfclam tissues throughout the winter (rich in STX and dcSTX) indicated that substantial toxin catabolism had already occurred, rather than suggesting the introduction of "new" toxin from cryptic winter blooms. Yet the winter toxicity peak cannot be explained solely by toxin bioconversion to more potent derivatives, since the HPLC results showed a clear rise in both PSP toxin concentration and total body toxicity burden during this period.

The substantial discrepancies between the mouse bioassay and HPLC data, particularly during summer toxicity maxima, can be attributed mostly to recent ingestion of toxic dinoflagellates rich in low potency N-sulfocarbamoyl toxins. In the HPLC procedure, actual toxin composition is used to calculate toxicity,

whereas the hot acid extraction employed in the mouse bioassay converts many of these low toxicity components to higher toxicity carbamate analogues, hence increasing net toxicity.

Post-digestion toxin metabolism may increase net toxicity substantially, through conversion of N-sulfocarbamoyl toxins to carbamate derivatives (e.g., GTX1-4, NEO, and STX). The toxin composition of ingested dinoflagellates is altered more profoundly by surfclams than by other Atlantic coast shellfish species, including sea scallops (Cembella *et al.*, 1994), quahogs (Bricelj *et al.*, 1991), and blue mussels (Chebib *et al.*, 1993). Particularly noteworthy was the *de novo* formation of substantial quantities of dcSTX in surfclams; decarbamoyl derivatives are comparatively rare in dinoflagellates from the Atlantic coast. In fact, this is the first report of the seasonal accumulation of decarbamoyl toxin in natural bivalve mollusc populations from the northwest Atlantic. In the surfclam digestive gland, biotransformation is mediated enzymatically through the activity of a "carbamoylase" (A. Cembella, N. Ross, and V.M. Bricelj, unpubl. obs.), as suggested by Sullivan *et al.* (1983) for the little-neck clam from the Pacific coast.

Although the specific toxin profile of the dinoflagellate responsible for the PSP toxicity in surfclams from the Gulf of Maine remains unknown, inferences may be made based upon cultured isolates from this region. The apparent conversion of C-11 ß-GTX epimers, which tend to dominate in *A lexandrium* isolates from the east coast (A. Cembella, unpubl. obs.), to α-epimers, was substantiated in surfclams. Comparison of PSP toxin profiles from representative *A. tamarense* isolates from the Gulf of Maine (Cembella *et al.*, 1994) showed that surfclams were richer in carbamate derivatives, particularly STX, than dinoflagellates and contained relatively less N-sulfocarbamoyl toxins. Thus, the dominance of ß-GTX epimers in the viscera during the summer peak of toxicity apparently reflects recent ingestion of toxic dinoflagellates, followed by transfer of epimerized toxin to adjacent tissues. This hypothesis is supported by evidence of high relative levels of N-sulfocarbamoyl toxins (characteristic of *A lexandrium* blooms) found in the viscera and siphon during the summer toxin maximum.

In summary, the elevated STX levels, the *de novo* appearance of dcSTX, the rapid epimerization of GTX derivatives, and the sharp decline in C-toxin concentration found in all surfclam tissues following the mid-summer toxic dinoflagellate bloom(s) provided strong evidence of metabolic toxin conversion. Analysis of toxin profiles proved useful in hindcasting toxic blooms, particularly when spikes in N-sulfocarbamoyl toxins typical of recent exposure to toxic dinoflagellates can be identified. However, toxin biotransformation processes occur on a time scale much shorter than the sampling intervals often selected for shellfish toxin monitoring programs. In addition, seasonal variations and differences in the relative amounts of PSP toxins among various tissue compartments are more significant than geographical differences between populations. Species-specific differences in toxin retention and biotransformation must be recognized in the development of effective toxin monitoring strategies and could be exploited as site selection criteria for shellfish aquaculture.

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