ANATOMICAL DISTRIBUTION OF PARALYTIC SHELLFISH TOXINS IN SOFT-SHELL CLAMS

JENNIFER L. MARTIN,* ALAN W. WHITE,** AND JOHN J. SULLIVAN***
*Department of Fisheries and Oceans, Aquaculture and Invertebrate Fisheries, Biological Station, St. Andrews, New Brunswick E0G 2X0 Canada; **Sea Grant Program, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543 U.S.A.; ***U.S. Food and Drug Administration, Seafood Products Research Center, Seattle, Washington 98174 U.S.A.

ABSTRACT

Soft-shell clams, *Mya arenaria*, were collected from two sites in the southwestern Bay of Fundy in January, April, May, July, September and November, 1986. The sites were Crow Harbour, where clams exceed the quarantine toxin level year-round, and Lepreau Harbour, where clams exceed the quarantine level only during the annual, summer *Alexandrium* bloom. Clams were dissected into digestive gland, gonad, gills, muscles (siphon, foot, pallial muscle and adductor muscle), and "remainder" which consisted of a small amount of material near the digestive gland and included the kidney, heart and brown gland. The concentration of total toxins (µg STX equiv./100 g) in the "remainder" was up to 10 times greater than in the other tissues throughout the year except in July. During the *Alexandrium* bloom in July, the digestive gland also contained high toxin concentrations; much lower levels occurred in the gills and gonads. In the other months, the gills contained about the same toxin concentrations as the digestive gland.

The patterns of abundance of the individual toxins in the various anatomical parts were similar within and between collection sites. In July, the sequence of abundance of the individual toxins in the clams was GTX I, GTX IV and GTX III, with STX usually the lowest of the six or seven detectable toxins. The sequence in July plankton dominated by *Alexandrium* was GTX IV, GTX III, NEO, and GTX I. Clam samples from the other months showed a striking difference from the July pattern; STX was nearly always present at the highest level, often followed by NEO, and GTX IV was always the lowest. Results indicate that soft-shell clams contain high toxin concentrations in parts other than the digestive gland. Further, results suggest that interconversion or selective retention of the toxins occurs within these animals.

INTRODUCTION

Blooms of *Alexandrium fundyense* are an annual summer occurrence in the Bay of Fundy and are responsible for soft-shell clams, *Mya arenaria*, accumulating paralytic shellfish toxins while filter feeding which result in the closure of most areas to harvesting. Shellfish toxicities for the southern Bay of Fundy behave as a unit and follow the *Alexandrium* bloom closely due to the tremendous turbulence and mixing within the system. Since 1980, several soft-shell clam sites have remained closed year round due to low, but unacceptable levels of PSP toxins. Although prior reports suggested the use of ozone for detoxifying clams [1], our efforts to do so from a permanently closed area such as this, with toxins stored for long periods, proved unsuccessful [2]. This study was undertaken to determine whether clams from an area where natural detoxification does not occur behave in a similar manner to those from an area where detoxification occurs, and to determine how the toxins are transformed or retained.

Published 1990 by Elsevier Science Publishing Co., Inc.
Toxic Marine Phytoplankton
Edna Granéli et al., Editors

MATERIALS AND METHODS

During January, April, May, July, September and November of 1986, soft-shell clams, *Mya arenaria*, were collected from Crow Harbour and Lepreau Harbour, New Brunswick. Clams were kept moist by covering with seaweed during transport (1-2 hours) to the laboratory and refrigerated at 2°C overnight. Since preliminary tests showed a decreased range in shellfish toxicity in clams of uniform size, only those with shell lengths from 4.0 to 6.0 cm were used for these experiments. For each site and each sampling, 20 clams were analyzed for whole tissue concentrations, and 55 clams for tissue and organ concentrations (digestive glands, gonads (including visceral mass), gills (plus mantle), muscles (pallial and adductor muscles plus siphon and foot) and remainder (heart, kidney and brown or Keber's gland) (Fig. 1)). The whole clams or parts were processed for "6-mouse" bioassays [3] and subsamples were removed from each to be centrifuged. The supernatant was passed through YM-10 ultrafiltration membranes, frozen and shipped to Seattle, Washington for HPLC analysis [4].

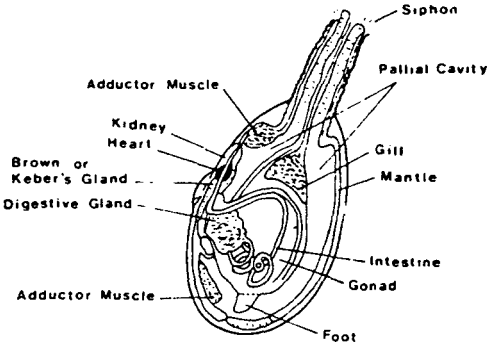


FIG. 1. Internal anatomy of the soft-shell clam.

To determine the occurrence of the PSP toxins in nature, plankton was collected with a 20-µm mesh net of 0.5 m diameter during the bloom of *A. fundyense* from three locations in southwestern Bay of Fundy: Station Prince 5 (44°57'N, 66°51'W), near the Wolves Islands (45°00'50"N, 66°47'00"W), and northeast of Grand Manan Island (44°51'97"N, 66°41'65"W) (Fig. 2). Net contents were kept on ice during the 2- to 3-hour return trip to the laboratory where the plankton was processed for toxin concentration and cell counts were determined according to the method described by White [5].

RESULTS AND DISCUSSION

Comparison between the mouse bioassay and the HPLC technique showed a good correlation between the two methods. The HPLC results in most cases were higher than those for the mouse bioassay, except at toxicities less than 30 µg STX equiv./100 g and toxicities greater than 1000 µg STX equiv./100 g where the HPLC values tended to slightly underestimate the total toxicity. Figure 3 shows the only exception to the latter from all our results - the total toxicity at Crow Harbour determined by HPLC during the *Alexandrium* bloom (July) was 78% greater than that determined by mouse bioassay. Sullivan et al. [6] observed similar results when comparing the two methods using a variety of species.

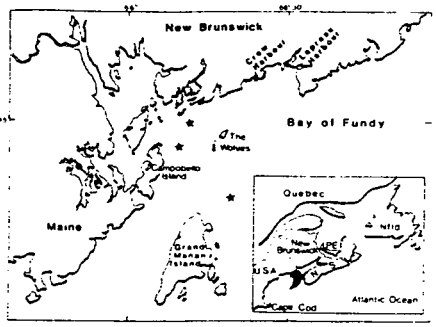


FIG. 2. Map of sampling area.

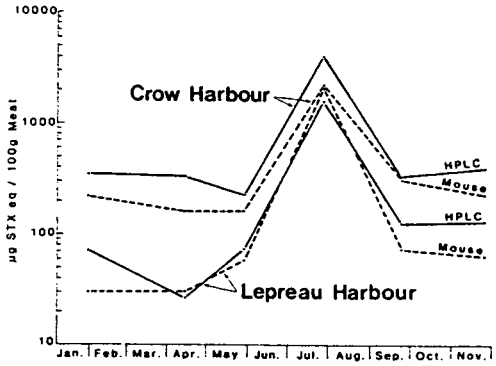


FIG. 3. Variation in PSP toxin levels in whole soft-shell clam extracts.

In general, the toxicities from whole tissue extracts show similar patterns for clams from Crow Harbour and Lepreau Harbour throughout the year (Fig. 3). Total toxin levels for Crow Harbour were from 160-310 (mouse bioassay) and 229-397 (HPLC) µg STX equiv./100 g during non-bloom periods (September-May), with a high in July of 2260 (mouse) and 4039 (HPLC) µg STX equiv./100 g meat, causing this particular area to remain closed to harvesting throughout the year. Similarly, at Lepreau Harbour, shellfish toxicities peaked in July with toxicities of 2103 (mouse) or 1602 (HPLC) µg STX equiv./100 g meat but dropped to acceptable levels by mouse bioassay (<30-75 µg STX equiv./100 g) to permit harvesting between September and May. Total toxicities for the individual organs from Crow Harbour and Lepreau Harbour also showed similar patterns. The remainders contained the most toxins throughout the year, except at Lepreau Harbour in July, and were followed by digestive glands, gills or gonads and muscles (Fig. 4). These results differ from those of Medcof et al. where toxins tended to migrate to the gills in the off-season [7]. Toxin levels increased to maximum levels for all tissues in July, one week after concentrations of *Alexandrium* reached 9.3 x 10^4 cells/L northeast of Grand Manan (Fig. 2). Earlier studies in the Bay of Fundy indicate highest concentrations of *A. fundyense* cells tend to be located offshore (northeast of Grand Manan) [8] from where they are dispersed and result in inshore

shellfish toxicities. Thus, the rise in toxicity closely follows the bloom. PSP toxins were found above the level of 30 µg STX equiv./100 g in all tissues throughout the year from Crow Harbour, while the gills, muscles and gonad from Lepreau Harbour were less during April and May, resulting in a lower overall toxicity from these clams (Fig. 4).

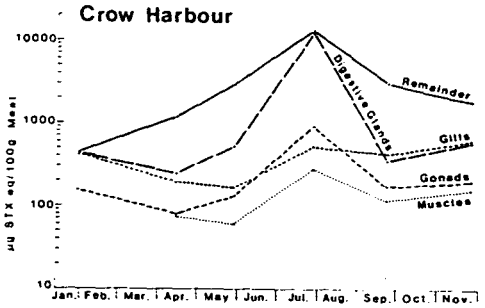
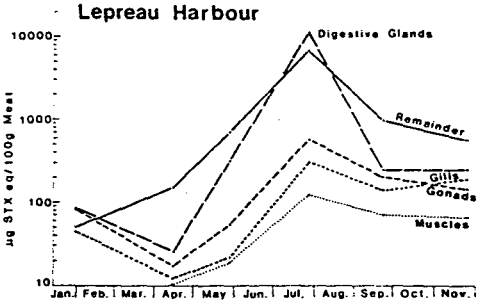


FIG. 4. Total toxin content for gills, gonads, digestive glands, muscles and remainder.



Amount of toxins in each tissue can be calculated as a percentage of the total toxins. Highest percentages were concentrated in the remainder throughout the year in Crow Harbour clams and during September and November in Lepreau Harbour clams. July was the exception for both sites when most of the toxins were located in the digestive glands (Fig. 5). This agrees with Medcof et al.'s [6] work in 1945 on toxin distribution in *Mya* from the Bay of Fundy where they also found the digestive glands to be the most toxic when the shellfish are acquiring the toxins from their food. A rise in percentage for digestive gland toxin content in May coincides with the annual early *Alexandrium* "mini" bloom that is the precursor to the July bloom (Martin, unpublished). All other tissues (gonads, gills, muscles) retained consistently low percentages of total toxins throughout the year. In July, clam toxicity values reached levels of 4039 and 1602 (HPLC) µg STX equiv./100 g at Lepreau Harbour and Crow Harbour, respectively, with clams at Lepreau Harbour able to eliminate the toxins sufficiently to allow harvesting, whereas those from Crow Harbour retained 65-70% of toxins [

the remainder, and resulting in the prohibit of harvesting throughout the year. Although high percentages of toxins were retained in the remainder, when toxins from this source were combined with the rest of the clam during homogenization, the overall toxin value ranged from 160-397 µg STX equiv./100 g. These results suggest either that there may be strain variation or difference in selective retention in clams between the two areas where high percentages of toxins are stored in the kidney or brown gland of Crow Harbour clams during the non-bloom periods or the clams from Crow Harbour contained 2.5 times the levels of toxins from Lepreau Harbour (by HPLC analysis) in July and were unable to detoxify sufficiently during the non-bloom months to permit harvesting.

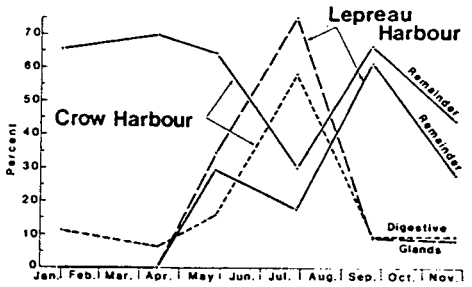


FIG. 5. Percent total body burden of toxins in digestive glands and remainder

The toxin content of *A. fundyense* cells collected from Prince 5, The Wolves and Northeast Grand Manan (Fig. 2) ranged from 7.37×10^{-5} to 8.87×10^{-5} µg STX equiv./cell. Although most analyses pool GTX I and GTX II with their respective isomers, GTX IV and III, due to difficulties in establishing equilibrium ratios, the toxin data from this experiment have been left in the raw form and the toxins treated individually. Eight of the PSP toxins were detected by HPLC analysis from the plankton in the following order of abundance: GTX (gonyautoxin) IV, GTX III, NEO (neosaxitoxin), GTX I, STX (saxitoxin), GTX II and C1/C2. Results indicate that transformation of toxins from the swimming cells occurs fairly rapidly upon ingestion by the clams. Soft-shell clams sampled in July during the bloom showed highest concentrations of GTX I, GTX IV and GTX III (with STX, GTX II, NEO, C1/C2 present at lower levels) in all tissues from Lepreau Harbour. Similar profiles were observed in the remainder, muscles and digestive glands from Crow Harbour whereas dominant toxins from the other tissues were: gills - GTX I and IV followed by STX; gonads - GTX I, GTX III and NEO; and whole clams - GTX IV, GTX III and NEO. The toxin composition through the rest of the year did not alter as markedly. STX was the main component for Crow Harbour clams from all extractions whereas at Lepreau Harbour this applied only for the remainder and muscles. In gills, gonads, digestive gland and whole clams from Lepreau Harbour, STX, NEO and GTX I or III tended to interchange as the most abundant.

Previous studies also show STX to be the major component in toxins in the butter clam (*Saxidomus giganteus*) [9] and scallops (*Placopectin magellanicus*) [10] and suggest that toxins may be enzymatically (metabolically) converted to STX or selectively retained as STX. The ability of Lepreau Harbour clams to convert major toxins from STX to NEO or GTX I or III and lower toxin levels to acceptable levels for harvesting may be either due to a variation in strains or the presence of different

enzymes creating different toxin transformation than those from Crow Harbour. Kotaki et al. have demonstrated the ability of bacteria to transform GTX I, II or III to STX [11]. This further suggests the possibility that Crow Harbour and Lepreau Harbour may possess different bacteria - either within the clams or in the environment.

Results of this investigation indicate that although highest concentrations of PSP toxins occur in the digestive gland of the soft-shell clam during the bloom, the toxins tend to migrate to the remainder during the off-season. Included in the remainder were the heart, kidney and brown or Keber's gland. Although the exact function of the brown gland is uncertain, it is thought to function as a part of the excretory system [12]. Results suggest the PSP toxins are transferred within a short time from the digestive gland to the excretory system and can be retained within this system for an extended period.

This study suggests the possibility that the toxins may be either metabolically transformed by either bacteria, clams or the cells themselves or chemically altered. This may relate to variation in the ability of clams to detoxify from different areas and the storage of high concentrations or selective retention of STX in the remainder of the Crow Harbour clam during non-bloom periods.

ACKNOWLEDGMENTS

We thank B. Best, S. Bellis, F. Cunningham and B. McMullon for preparation and drafting of the manuscript. Support for A.W. White was in part by NOAA National Sea Grant College Program Office, Department of Commerce, under Grant No. NA86-D-SG090, WHOI Sea Grant Project No. M/0-2.

REFERENCES

1. W.J. Blogoslawski, M.E. Stewart, J.W. Hurst and F.G. Kern, Toxicon 17, 650-654 (1979).
2. A.W. White, J.L. Martin, M. LeGresley and W.J. Blogoslawski in: Toxic Dinoflagellates, D.M. Anderson, A.W. White and D.G. Baden, eds. (Elsevier, New York, 1985) pp. 473-478.
3. Official Methods of Analysis, 14th Edition, Procedure 18.086.092 (Association of Official Analytical Chemists, Washington, D.C. 1984).
4. J.J. Sullivan and M.M. Wekell in: Seafood Quality Determination, D.E. Kramer and J. Liston, eds., Sea Grant College Program, Anchorage, AK, pp. 357-371 (1988).
5. A.W. White, Toxicon 24, 605-610 (1986).
6 J.J. Sullivan, M.G. Simon, W.T. Iwaoka, J. Food Sci. 48, 1312-1314 (1983).
7. J.C. Medcof, A.H. Leim, A.B. Needler, A.W.H. Needler, J. Gibbard and J. Naubert, Bull. Fish. Res. Board Can. LXXV, 1-32 (1947).
8. J.L. Martin and A.W. White, Can. J. Fish. Aquat. Sci. 45, 1968-1975 (1988).
9. J.J. Sullivan, Paralytic shellfish poisoning Analytical and biochemical investigations, Ph.D. Thesis, University of Washington, 232 pp. (1982).
10. Y. Shimizu and M. Yoshioka, Science 212, 547-549 (1981).
11. Y. Kotaki, Y. Oshima and T. Yasumoto in: Toxic Dinoflagellates, D.M. Anderson, A.W. White and D.G. Baden, eds. (Elsevier, New York, 1985) pp. 287-292.
12. A.W. Martin and F.M. Harrison in: Physiology of Mollusca, Vol. II, K.M. Wilbur and C.M. Yonge, eds., pp. 353-386 (1966).