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Seasonal Resistance of the Shore Crab, *Hemigrapsus oregonesis,* to Saxitoxin Injections

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The accumulation of paralytic shellfish poisons (PSP) by filter feeding molluscan shellfish ingesting toxic dinoflagellates represents a constant source of concern to the shellfish industry and public health officials alike. Since the original finding by Sommer et al. (1937), that outbreaks of paralytic shellfish poisons along the California coast were attributed to the presence of marine dinoflagellates of the genus Gonyaulax catenella, numerous reports of PSP have been registered along the British Columbia coastline with the dinoflagellates G. acatenella and G. catenella (Prakash and Taylor 1966). In all cases, saxitoxin (STX), a potent neurotoxin has been isolated along with other less toxic paralytic poisons (Schantz et al. 1966; Sullivan et al. 1985).

Although the retention of PSP in bivalve molluscs is well documented, a variety of other marine animals have also been identified to contain PSP. In studies with the crab, Foxall et al. (1979) reported PSP in Cancer irroratus fed toxic shellfish, and Jonas-Davis and Liston (1985) have correlated the accumulation of PSP in crabs with the occurrence of toxic dinoflagellate blooms in the Northwestern U.S. Recently, Yasumura et al. (1986) identified a number of PSP in numerous crab species responsible for human intoxication. In addition, resistibility of xanthid crabs against PSP and tetrodotoxin has been of interest (Koyama et al. 1983). The present study was initiated to determine if the small shore crab Hemigrapsus oregonesis also developed a resistance to PSP, and if so, whether this resistance could be associated with the accumulation of PSP and the appearance of red blooms at specific locations on the British Columbia coast.

MATERIALS AND METHODS

Neurotoxin standards, Saxitoxin (100 ug/mL in 20% ethanol; 95% purity; Food and Drug Administration, Division of Microbiology Cincinnati, OH 45202) and Tetrodotoxin (citrate free, Sigma Chemicals, St. Louis, MO) were used in this study.

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Male and female shore crabs (1.5 - 3.5 g) Hemigrapsus oregonesis were collected at low tide from 3 different coastal locations: (see Fig 1) Towers Beach (Vancouver, 1985 to 1986), Okeover Arm (Powell River, 1986) and Porpoise Bay (Seshelt, 1986). The last two locations were known to be contaminated with toxic dinoflagellate blooms at time of collection. In addition, clams and mussels were collected at Okeover Arm for PSP determinations. A total of ten crabs were tested for sensitivity to saxitoxin, (SAX; dose range: 1×10^{-3} ug to 1×10^{-2} ug) and tetrodotoxin (TTX; dose range: 5×10^{-3} ug to 5×10^{-2} ug), within 24 hours of collection. Toxins were injected in separate crabs at the Milne-Edwards opening using a 25 G 7/8 needle and a uniform volume (50 uL) of injectate. Crab death times were recorded when tactile stimulation of both eyes and legs elicited no response.

The standard mouse lethality bioassay (AOAC 1980) was used for the detection of PSP in shellfish. Body tissues were blended to homogeneity and a 100 g sample added to 100 ml of 0.1 N HCl. This mixture was boiled for 5 min. and then cooled to room temperature. The pH was adjusted to 2.0 to 4.0 and the sample was then diluted to 200 ml. This mixture was allowed to settle until the supernatant was translucent. 1 ml of this extract was then injected intraperitoneally into 3 mice (Swiss, female) weighing 19 to 22 g. The time of inoculation was noted and time of death recorded to the nearest second. Toxicity of the extracts was calculated after reference to Sommer's Table.

Samples of naturally incurred toxic shellfish meats; clams (Tapes japonica), and mussels (Mytilus edulis) as well as the small shore crab (Hemigrapsus oregonesis) were obtained during a toxic bloom of G. acatenella at Okeover Arm on the B.C. coast. All samples were homogenized and stored at -80°C until analysis. One half of the sample was used in the normal mouse bioassay and the remainder was used to quantitate a fluorescent derivative of STX (2 amino-8,9-dihydro-4-hydroxy-methyl pyrimide (2,1-6 purin-7 (1H)-one)) in the samples according to the method of Bates and Rapoport (1975). Briefly, samples of toxic shellfish homogenate were boiled with 0.5 M TCA and adjusted to pH 5 with NaOH. After centrifugation (12,000xg, 10 minutes), the supernatant was chromatographed on ion exchange columns (0.8 cm id x 5 cm) containing equilibrated Bio Rex 70 (50-100 mesh) resin (Bio-Rad, Rich. Calif.). The samples were washed through the column with 0.2 M sodium acetate buffer (pH 5.0) and distilled water prior to being eluted with 0.25 M sulfuric acid. The toxic fraction was oxidized with alkaline hydrogen peroxide and acidified to pH 5 with glacial acetic acid. Fluorescent intensities were monitored (Shimadzu, RF0450 Spectrofluorophotometer, Kyoto, Japan) with an excitation wavelength of 330 nm and an emission wavelength of 380 nm. STX content of samples was determined from a STX standard curve.

RESULTS AND DISCUSSION

The standard curves depicting both saxitoxin and tetrodotoxin

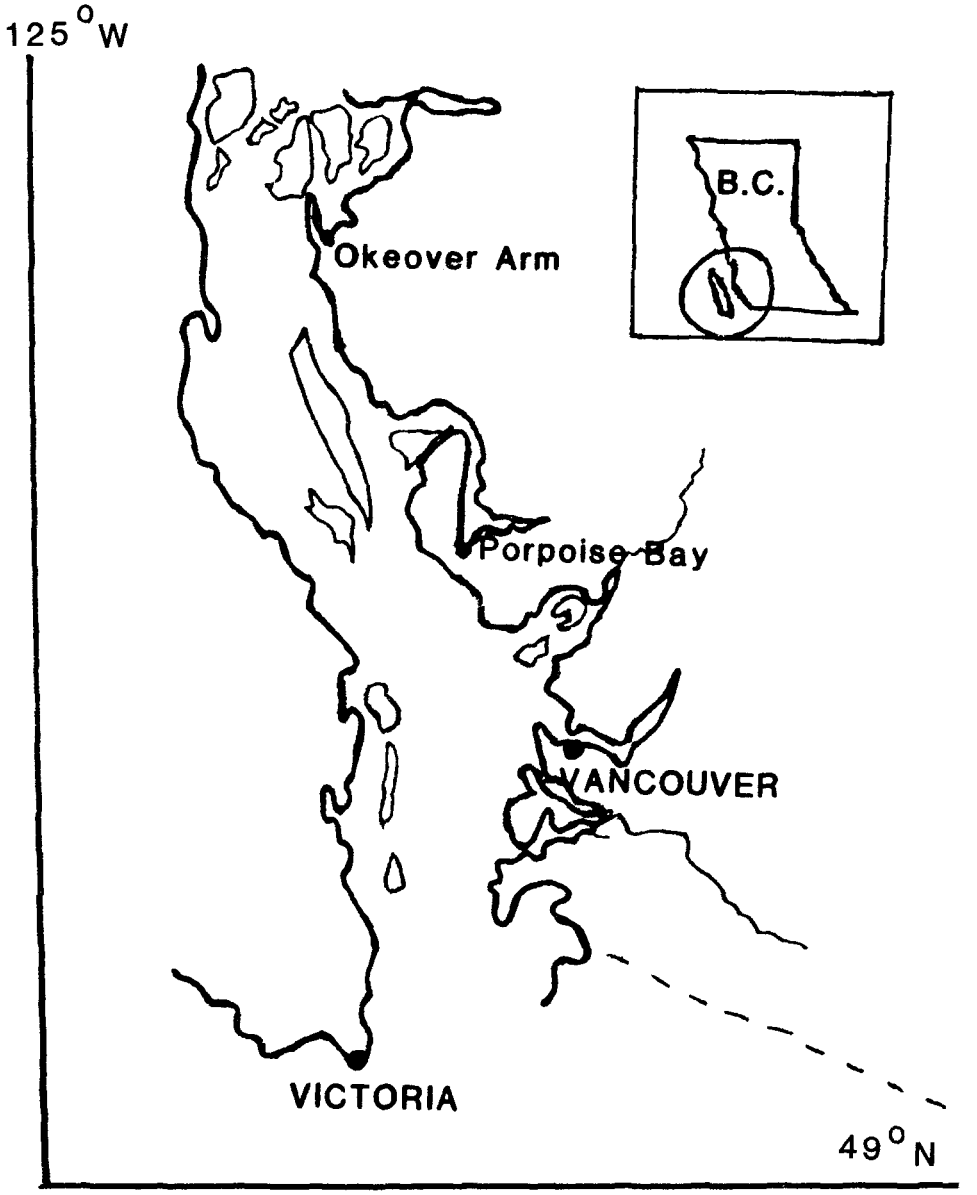

Figure 1. Resistance in small crabs from various BC locations contaminated with red tides.

dosages and associated death times in sensitive shore crabs, obtained from Tower Beach, are presented in Figure 2 (insert). Crab death times for saxitoxin ranged from 0.75 to 9.39 minutes over a dose range of 1×10^{-2} ug to 1×10^{-3} ug, and was expressed as $y = 17.80 + 4.04x$ ($r^2 = 0.79$; $n = 20$) where y = crab death time and x = dose of saxitoxin. Similarly, death times for tetrodotoxin ranged from 2.86 to 8.71 minutes for dosages of 5×10^{-2} ug to 5×10^{-3} ug and this response was described by $y = 4.55 + 2.46 \times$ ($r^2 = 0.62$; $n = 20$). From these results, a standard dose of 5×10^{-2} ug was chosen for both saxitoxin and tetrodotoxin, respectively.

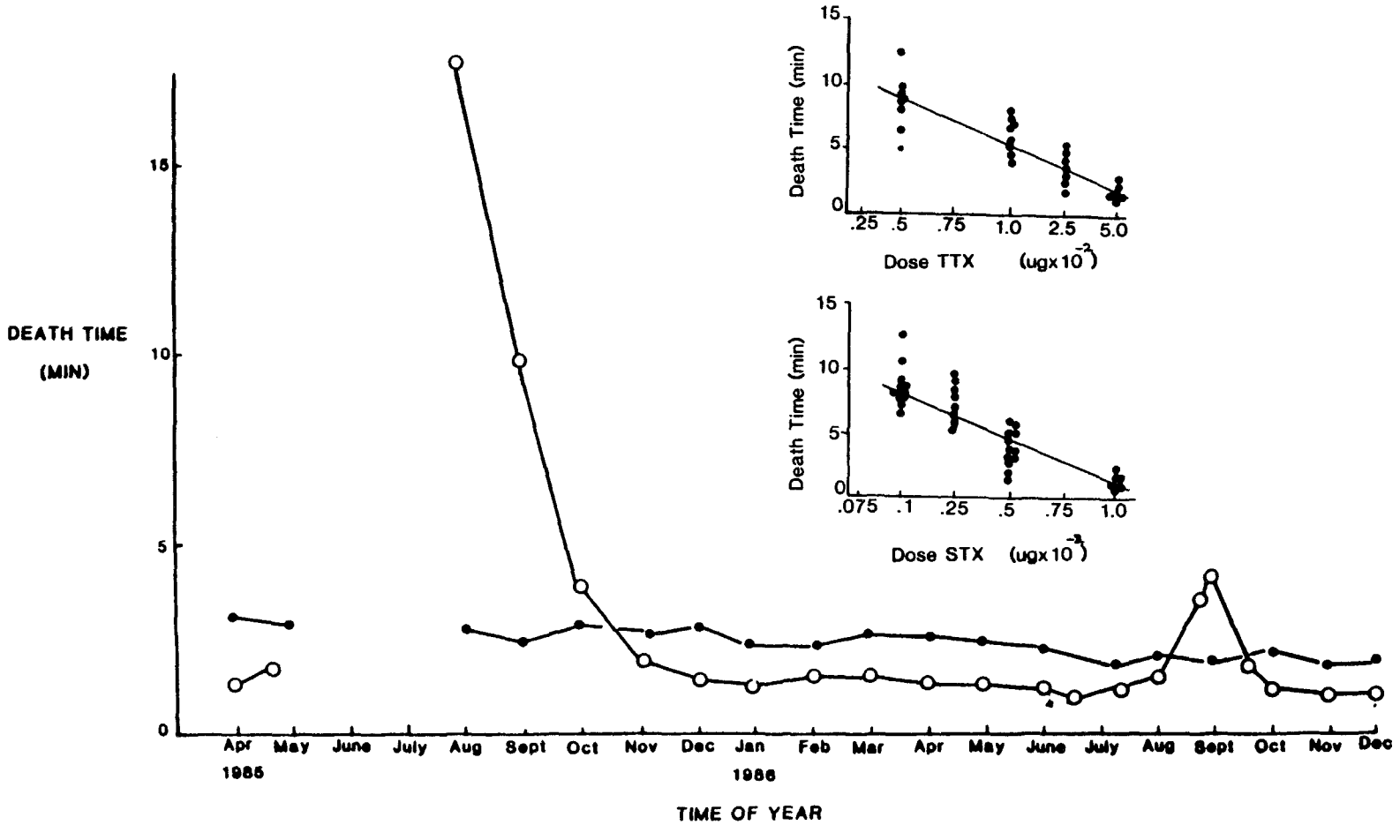

Figure 2 - Seasonal pattern of sensitivity to saxitoxin (STX, 0.05 ug ○ ○) and tetrodotoxin (TTX, 0.05 ug ● ●) in the small shore crab Hemigrapsus oregonesis. Insert represents the standard curve for STX and TTX lethality in the shore crab. Break in curve indicates no samples available.

The 21-month seasonal patterns of crab sensitivity to fixed dosages of saxitoxin and tetrodotoxin are also presented in Figure 2. Mean crab death times due to tetrodotoxin injections remained relatively constant (2 to 4 minutes) over the entire 21-month period, demonstrating no seasonal change in crab sensitivity to this toxin. Conversely, a wide seasonal fluctuation was observed in mean death times of crabs injected with saxitoxin. Although the average death time for a single dose of saxitoxin was approximately 1 minute in sensitive crabs during the spring of 1985, a marked increase in death times approaching 30 minutes was observed in crabs collected during August 1985. This apparent resistance to saxitoxin injections was found to gradually disappear and eventually returned to sensitive levels (1 minute) in November 1985. Crab sensitivity to saxitoxin injections persisted throughout the winter and fall until the following summer (July 1986), when crabs began to again show an increased resistance to saxitoxin injections. Peak resistance in crab death times was observed in August 1986 and reached a level which was substantiallysmaller than the previous year (August 1985). Nevertheless, the same temporal pattern of relative resistance to saxitoxin injections was observed and crabs were found to again lose their resistance to saxitoxin by October 1986. This observation that the small shore crab, Hemigrapsus oregonesis, exhibits resistance to saxitoxin injections is supported by previous research on the xanthid crabs, Atergates floridus (Koyama et al., 1983) and Zosimus aeneus (Noguchi et al., 1985). These workers showed crab resistance to both extracted paralytic shellfish toxins and tetrodotoxin throughout the year, whereas this present study showed a seasonal resistance. It is noteworthy that the resistant shore crabs in this study remained relatively sensitive to tetrodotoxin despite reduced sensitivity to saxitoxin. This finding suggests that the mechanism in which crabs develop resistance to PSP could be associated with the presence of the dominant toxin in the crabs' environment. While PSP and tetrodotoxin have been found in a marine macro-alga inhabiting Japanese waters all year round (Kotaki et al., 1983), dinoflagellates elaborating PSP in the Pacific Northwest show a seasonal pattern of toxicity (Gaines and Taylor, 1985). Moreover, tetrodotoxin does not occur naturally in southern B.C. and therefore shore crabs are not likely to come into contact with this toxin.

The possibility that the resistance of shore crabs to saxitoxin administration in late summer was attributed to the presence of dinoflagellate blooms at Towers Beach could not be confirmed because of the lack of PSP testing in that area. Therefore, small shore crabs were also collected at two locations on the B.C. coastline, namely Okeover Arm and Porpoise Bay (Fig. 2) during registered red tide blooms. Similar extended death times of crabs following saxitoxin injections were observed in crabs collected from Okeover Arm (30 minutes) and Porpoise Bay (10 minutes). These crab responses corresponded to PSP contamination levels of 14,000 ug PSP and 1,700 ug PSP per 100 g shellfish for Okeover Arm and Porpoise Bay, respectively.

Table 1. Saxitoxin content and lethality of shellfish and crab extracts at Okeover Arm

Source[1]	Fluorometric Determination (ug STX/100 g)	Mouse Bioassay (ug PSP/100 g)
Mussel[2]		
Meat	65.33± 4.81	
Total	80.03± 4.81	196.0
Clam		
Total	65.33± 11.39	87.0
Crab[3]		
H. oregonesis(R)	21.5± 2.35	32.0
H. oregonesis(S)	0.0	0.0

1 Mussel, clam and resistant crabs [H. oregonesis (R)] were collected during a red tide bloom at Okeover Arm on the British Columbia Coast.

2 Total Activity is equivalent to mussel meat and juice obtained after thawing of samples.

3 H. oregonesis (R) = resistant crab, collected during a bloom; H. oregonesis (S) = sensitive crab, collected after bloom had subsided.

The concentration of saxitoxin and its associated lethality, as measured by both fluorometric and the standard mouse bioassay procedures in shellfish and resistant crab extracts obtained during a toxic bloom of G. acatenella at Okeover Arm is presented in Table 1. Both fluorometric analysis and lethality after extraction were observed to be greatest in the toxic shellfish samples compared to the resistant crab. In all cases, PSP values obtained by fluorometric determinations were lower than the bioassay results, suggesting that other paralytic shellfish toxins in addition to saxitoxin, were present in the samples. No trace of saxitoxin was detected in crabs that were collected after the bloom had subsided from the area. Similarly, additional crabs collected at this time were found to be sensitive to saxitoxin injections. In the mouse bioassay, lethality was observed only after injections of crab extracts obtained from resistant crabs and not sensitive ones (Table 1). These results confirm previous findings in other crab species which have been shown to accumulate PSP when fed toxic shellfish (Foxall et al., 1979) or when in the presence of toxic marine organisms (Kotaki et al., 1983; Jonas-Davies and Liston, 1985) and extend the present knowledge of a definite relationship between crab resistibility to PSP and accumulation of shellfish toxins.

In summary, the small shore crab, Hemigrapsus oregonesis, was found to exhibit remarkable seasonal resistance to saxitoxin administration, which was attributed to the presence of red tide blooms and the accumulation of PSP in crab visceral tissue. No seasonal resistance to tetrodotoxin was observed in additional crabs collected simultaneously. This finding strongly points to the specificity of the PSP induced resistance. Aside from the possible protective behavioral responses which may account for developed resistance to PSP in some marine species (Dupuy and Sparks, 1967), very little is known about the mechanism involved in PSP induced resistance. Further studies are now in progress to understand the possible detoxification mechanism(s) responsible for the seasonal resistibility of the small shore crab to saxitoxin. Moreover, the usefulness of this small crab in monitoring regional red tide blooms has not yet been examined.

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