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Paralytic Shellfish Poison in Various Bivalves, Port Moresby, 1973[1]

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ABSTRACT: Toxicity studies of various bivalves at Port Moresby were carried out by mouse bioassay. *Crassostrea echinata* was found to lose paralytic shellfish poison after 3 weeks in a closed seawater system, although toxin is retained for a much longer period *in vivo*. Four bivalve species tested were as toxic at 10 meters depth as they were at 2 meters. Toxin was not distributed evenly through the tissues of bivalves investigated.

POISONOUS BIVALVE SHELLFISH have been reported from Papua New Guinea coincidently with the appearance of red tides or blooms of the dinoflagellate *Pyrodinium bahamense* (Maclean, 1973). After the blooms had disappeared, shellfish toxicity was found to decrease at different rates in different intertidal bivalve species (Worth, Maclean, and Price 1975), although sampling at that time was insufficient to indicate the rate of toxin loss. The symptoms produced by both shellfish tissues and *Pyrodinium* samples were those of paralytic shellfish poisoning.

In this study, the longevity of toxin in *Crassostrea echinata* at Port Moresby, Papua New Guinea, was determined. Bioassays were made also of species from below the intertidal zone and of dissected bivalves to ascertain toxin distribution in the various tissues.

I would like to thank Dr G. K. Worth, Department of Agriculture, Stock and Fisheries, Papua New Guinea, who carried out the bioassays.

METHODS

The mouse bioassay method was used (Horwitz 1970: 305). However, because of a continuing shortage of suitable mice, only a relative scale of toxicity can be given. If the mice died in 3 minutes or less after being injected with tissue extract, shellfish were termed highly toxic (+++); from 3 to 7 minutes, toxic (++); from 7 to 20 minutes, mildly toxic (+); and longer than 20 minutes, nontoxic (< 0). This scale is based on that of Worth, Maclean, and Price (1975), which was formulated according to the relative severity of human symptoms during an outbreak of poisoning in Papua New Guinea.

RESULTS

Rate of Toxin Loss

The rate of toxin loss from *Crassostrea echinata* was determined from specimens that had been collected from Port Moresby Harbor on 15 February 1973 and introduced into a closed (*Pyrodinium*-free) seawater system. Samples for bioassay were taken over the next 3 weeks.

Results are shown in Figure 1. Further details are included in Table 1. The initially toxic oysters reached the minimum recognized level of mild toxicity at the end of 3 weeks, after which they would be considered nontoxic (< +, Table 1).

Depth of Penetration of Toxin

Previously, shellfish samples for toxin bioassay were taken from the intertidal and immediate sublittoral zones (Worth, Maclean, and Price 1975). Because the *Pyrodinium* red tide band occurs on occasions down to at least 9 meters (unpublished data), I wanted to learn whether deeper living bivalves were also affected.

On 20 March 1973, during the red tide season, specimens of various species were collected at depths of 2 and 10 meters in Port Moresby Harbor. Ten meters represents the lower edge of most of the reef area in the harbor, below which the substrate is sand.

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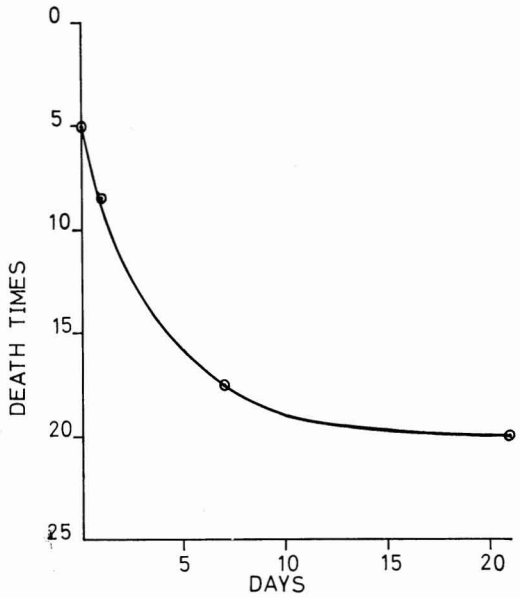

FIGURE 1. Breakdown of toxin in *Crassostrea echinata* at intervals after removal from the sea, shown as mean mouse death times in minutes.

Bioassay results are shown in Table 2. Species assayed at both depths, *Spondylus* sp., *Chama* sp., *Ostrea trapezina* Lamarck, and *Barbatia parvivillosa* Iredale, were all highly toxic. Specimens from 2 meters were only slightly more toxic than were those from 10 meters. Three more species of bivalves were obtained on 10 April 1973 from 10 meters only: *Pinna* sp., *Pterocarpa* sp., and *Pycnodonte hyotis* (Linnaeus). Bioassay results are included in Table 2. *Pinna* was highly toxic, *Pycnodonte* was toxic, and *Pterocarpa* was mildly toxic.

Distribution of Toxin in Shellfish Tissues

The toxin is localized in particular organs in some bivalve species. Prakash, Medcof, and Tennant (1971) observed that the digestive gland is the main poison site for most of the bivalve species that they investigated. Seasonal changes in the organ of highest toxin concentration may also occur (Halstead 1965), although such changes may be due to differential rate of loss of toxin in different tissues.

To investigate the distribution in shellfish tissues of *Pyrodinium* toxin, I dissected several

TABLE 1

TOXICITY OF *Crassostrea echinata* SAMPLES IN A CLOSED SEAWATER SYSTEM

DATE	NO. OF MICE	NO. OF DEATHS	MEAN DEATH TIME (MINUTES)	TOXICITY
5 Feb 1973	5	5	5	++
6 Feb 1973	5	5	8.5	+
12 Feb 1973	5	5	17.5	+
26 Feb 1973	6	3	20	<+

NOTE: ++, toxic; +, mildly toxic. See text.

specimens each of the edible oyster *Crassostrea echinata* from Bootless Bay near Port Moresby, 12 February 1973, and the pearl oyster *Pinctada maxima* from the pearl farm in Port Moresby Harbor, 8 March 1973 (these oysters had been brought to the farm from Western Australia approximately 2 years previously) and bioassayed the organs for toxicity.

Bioassay results are shown in Table 3.

The organs dissected are not all the same for the two species.

In the case of *C. echinata*, all parts were toxic to some extent. Gonad, palps, and possibly digestive glands (the latter were difficult to separate from gonads) were toxic, whereas mantle, gills, and muscle were mildly toxic. For *P. maxima*, the gonad-digestive gland fraction and heart were toxic; foot, mantle, and gills were mildly toxic; and the muscle was nontoxic.

DISCUSSION

The results show that bivalve shellfish are poisonous to some extent during *Pyrodinium* red tides in Port Moresby. The shellfish are toxic throughout the depth range of native divers seeking them for food.

Toxin is not distributed evenly through the tissues of the species investigated. This has proven fortunate in the case of pearl oyster (*P. maxima*) flesh, which is eaten extensively by pearl farm workers during harvesting (there was a minor harvest in March 1973 when these samples were taken). Only the muscle is eaten

TABLE 2

TOXICITY OF VARIOUS BIVALVES AT DIFFERENT DEPTHS

SPECIES AND DATE COLLECTED	DEPTH (METERS)	NO. MICE	NO. DEATHS	MEAN DEATH TIME (MINUTES)	TOXICITY
20 March 1973					
Chama sp.	2	2	2	2.5	+ + +
Chama sp.	10	4	4	3.0	+ + +
Spondylus sp.	2	4	4	1.25	+ + +
Spondylus sp.	10	4	4	1.5	+ + +
Barbatia parvivillosa Iredale	2	4	4	2.5	+ + +
Barbatia parvivillosa Iredale	10	4	4	3.5	+ +
Ostrea trapezina Lamarck	2	4	4	3	+ + +
Ostrea trapezina Lamarck	10	4	4	3	+ + +
10 April 1973					
Pinna sp.	10	3	3	2.5	+ + +
Pterocarpa sp.	10	3	3	7.75	+
Pycnodonte hyotis (Linnaeus)	10	3	3	4.5	+ +

NOTE: + + +, highly toxic; + +, toxic; +, mildly toxic. See text.

TABLE 3

RELATIVE TOXICITY OF SHELLFISH TISSUES, PORT MORESBY AREA

SPECIES AND ORGAN ASSAYED	NO. OF MICE	NO. OF DEATHS	MEAN DEATH TIME (MINUTES)	TOXICITY
Crassostrea echinata (Quoy & Gaimard)*				
Gonad and Digestive Gland	4	4	4	+ +
Gonads	3	3	4.5	+ +
Palps	3	3	5	+ +
Mantle	6	6	9	+
Gills	6	6	10	+
Muscle	6	5	11	+
Pinctada maxima (Jameson)†				
Gonad and Digestive Gland	6	6	2.5	+ + +
Heart (mainly ventricle)	3	3	5.5	+ +
Foot	5	5	10	+
Mantle	6	6	15.5	+
Gills	6	6	18	+
Muscle	6	0	–	< +

NOTE: + + +, highly toxic; + +, toxic; +, mildly toxic. See text.
* Collected 12 February 1973.
† Collected 8 March 1973.

by these workers, and, as noted, this is the only toxin-free tissue.

The relatively high toxicity of gonads in the edible oyster also has a bearing on consumption. These organs form a significant proportion of the flesh, and their removal would make the oysters unattractive, such that they could not be trimmed for marketing in the red-tide season.

The rate of toxin loss in *C. echinata* is comparable to that of *C. gigas* after the dinoflagellate *Gonyaulax acatenella* has disappeared from the plankton (Quayle 1969). The rate is also comparable to that of *C. virginica*, which loses *Gymnodinium* toxin in 2 to 6 weeks (Morton and Burklew 1969). However, *C. virginica* eliminates all poison from *Gonyaulax tamarensis* as soon as the latter disappears from the plankton (Prakash, Medcof, and Tennant 1971).

Worth, Maclean, and Price (1975) found that *C. echinata* was still highly toxic 15 weeks after *Pyrodinium bahamense* had disappeared from the plankton in Port Moresby Harbor. At that time, specimens were collected from the sea at intervals after the end of the red tide season. The present findings, using specimens in a closed system, suggest the continued presence of toxin in seawater after the *Pyrodinium* blooms have ended.

Morton and Burklew (1969) found a secondary rise in toxicity of *Crassostrea virginica* after *Gymnodinium* had disappeared from the plankton. They thought that this may have been due to assimilation of toxin from lysed cells, since laboratory experiments have shown that the oysters are capable of assimilating some free toxin. The longevity of dinoflagellate toxin in seawater has not been determined, but it is possible that the accumulated poison from generations of organisms in blooms might survive weeks or months in concentrations sufficient to cause or enhance shellfish toxicity. Spiegelstein, Paster, and Abbott (1973), for instance, found that the neurotoxin from *Gymnodinium* is stable in distilled or saline water for several months.

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