Fisheries Research Board of Canada



# paralytic shellfish poisoning in eastern Canada

A. Prakash ⋆ J.C. Medcof ⋆ A.D. Tennant



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# PARALYTIC SHELLFISH POISONING IN EASTERN CANADA

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BULLETIN 177

# paralytic shellfish poisoning in eastern Canada

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# Preface

The interest and involvement of the Fisheries Research Board of Canada in the paralytic shellfish poison problem are of long standing. The first major report on this subject in Canada appeared in 1947 as Fisheries Research Board of Canada Bulletin 75. That bulletin was prepared by Dr J. C. Medcof and several other scientists and dealt mainly with the problem in the Bay of Fundy. In the two decades since, there has been a growing awareness of the importance of the problem on both our coasts and of the need for more information on it. Expanded research effort in Canada and abroad and experience in developing and implementing control programs on the Canadian Atlantic and Pacific coasts have greatly increased our understanding of the many aspects of the illness. The problem in British Columbia has been recently reviewed by Dr D. B. Quayle of the Board's Biological Station at Nanaimo, B.C., in Fisheries Research Board of Canada Bulletin 168.

The present book is a composite effort by three authors, each a specialist in his own field, and brings together all pertinent information to date on paralytic shellfish poisonings in eastern Canada. Dr A. D. Tennant contributed the section on measuring shellfish toxicity, Dr J. C. Medcof the sections on features of poisoning, toxicity of shellfish, and control programs, and a major part of effects of processing on toxicity, and Dr A. Prakash the sections on the causative organism, mechanisms of poison accumulation and elimination, the toxin, and detoxification of shellfish. Dr Prakash also organized and coordinated the various sections and compiled the extensive bibliography.

J. C. STEVENSON
*Editor and*
*Director of Scientific Information*

*Ottawa, 1971*

# Abstract

Outbreaks of paralytic shellfish poisoning (PSP) from consumption of toxic shellfish are common in eastern Canada and have involved over 200 reported illnesses and 23 deaths since 1880. The middle and lower reaches of the Bay of Fundy and the lower estuary of the St. Lawrence River are the affected areas. Poisonings occur mainly during summer (June–September) and are associated with abundance of the planktonic marine dinoflagellate *Gonyaulax tamarensis*. Features of poisonings, including symptoms, levels of human sensitivity to shellfish poison, treatment, and prevention, are described.

Measurements of shellfish toxicity using mouse bioassays show that toxicity varies with species of shellfish, area, and position in the intertidal zone. Shellfish species also differ in anatomical distribution of the poison. Although 14 species of shellfish have been shown to become toxic, soft-shell clams and blue mussels account for about 90% of the poisonings. Blue mussels accumulate the largest amounts of toxin in the shortest time. Possible mechanisms of toxin accumulation in molluscan shellfish, both filter feeding and nonfilter feeding, are discussed.

The causative organism, *G. tamarensis*, synthesizes a potent endotoxin; its toxigenic and growth characteristics and environmental factors influencing its seasonal abundance are discussed. The toxins extracted from shellfish and from *G. tamarensis* cultures are identical in their pharmacological characteristics and there is little doubt that they are structurally similar. Methods for measuring shellfish toxicity are described and factors affecting accuracy of mouse bioassays are reviewed.

Home cooking and commercial canning each reduce shellfish toxicity by 70–90%, and storage of toxic canned shellfish further reduces their toxicity. Toxin in live shellfish can be reduced or eliminated if they are transplanted to nontoxic areas or subjected to sudden changes in salinity and temperature.

A control program to reduce risks of poisoning without jeopardizing the shellfish fishery has been instituted. This program involves classification of shellfish-producing areas according to patterns and levels of toxin accumulation; year-round monitoring of toxicity in seriously affected areas; closures and patrolling of areas when toxin scores reach hazardous levels; and warning the public of dangers. The effectiveness of control schemes and the problems in implementing them are discussed.

# Introduction

This is an up-to-date review of most aspects of paralytic shellfish poisoning (PSP) in eastern Canada. The aim of the bulletin is to reduce the hazard of poisoning to residents of and visitors to shore communities. It is intended for use by physicians, scientists, fisheries administrators, public health officers, fishermen, processors of fisheries products, and the general public.

Paralytic shellfish poisoning in humans and animals has been recorded from various parts of the world for about three centuries. Early records are mostly from Europe and North America and are not detailed. Later records cover a much wider geographical area and include information on species of shellfish involved, epidemiology, sources and characterization of the poison, pathology, and control methods.

Much of this advanced knowledge and the early history is reviewed by Halstead (1965). He lists over 900 cases of PSP with over 200 human fatalities from various parts of the world between 1689 and 1962. According to our estimate, worldwide incidence of PSP now totals 1600 cases. Most cases have occurred in the subtropical and temperate zones (between 30 and 60° N lat). The most seriously affected area is the Pacific coast of North America from central California to the Aleutian Islands (Fig. 1). Present-day understanding of PSP problems stems largely from investigations carried out in that area (Sommer and Meyer 1937; Sommer et al. 1937; McFarren et al. 1960; Schantz and Magnusson 1964; Quayle 1969).

The Atlantic coast of North America is fourth in importance among areas of the world where PSP is a public health hazard (Fig. 1). The only regions on this vast coastline that have been affected are the Bay of Fundy and the estuary of the St. Lawrence (Fig. 2). The PSP problem in eastern Canada has been studied for over 25 years and there are few places in the world with such detailed records. Much of the early information on the problem of PSP in eastern Canada is contained in a bulletin (Medcof et al. 1947) dealing mainly with PSP in the Bay of Fundy. Since 1947 much additional information has been assembled.

Shellfish do not produce the paralytic poison; they acquire it by feeding on certain species of marine dinoflagellates, which are one-celled planktonic organisms. Bivalve shellfish, such as mussels and clams, strain these from the water, digest them, and accumulate their poison. The shellfish do not seem to be harmed by the poison, but become toxic to humans and other animals that feed on them. The poison is 50 times as strong as the paralytic poison curare, and as little as 0.5 mg of purified poison can be fatal to man.

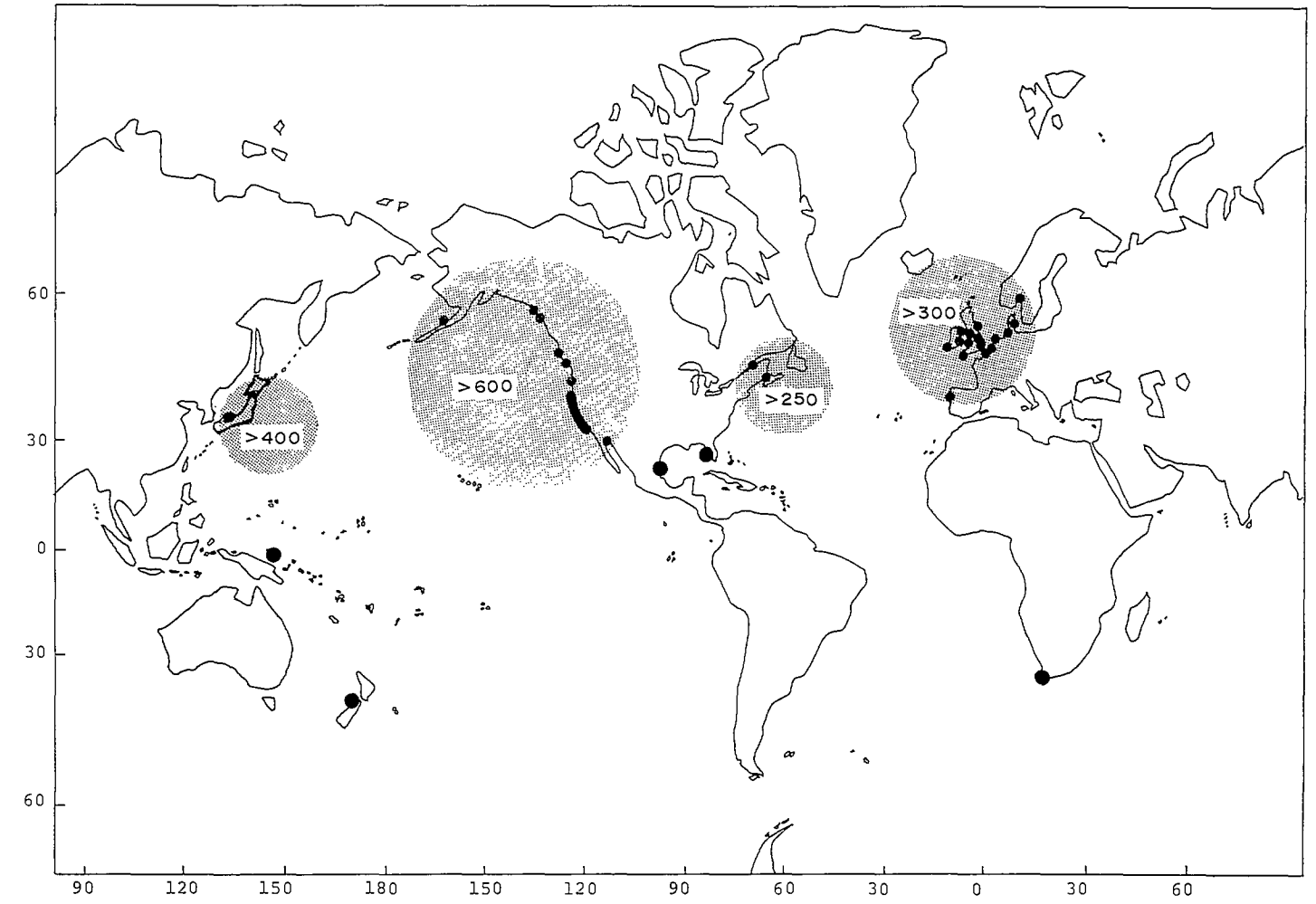


FIG. 1. World distribution of paralytic shellfish poisoning (PSP), 1689–1970 (modified after Halstead 1965). Numerals in hatched areas indicate approximate total numbers of human poisonings reported from the four major areas affected; solid circles, individual outbreaks.

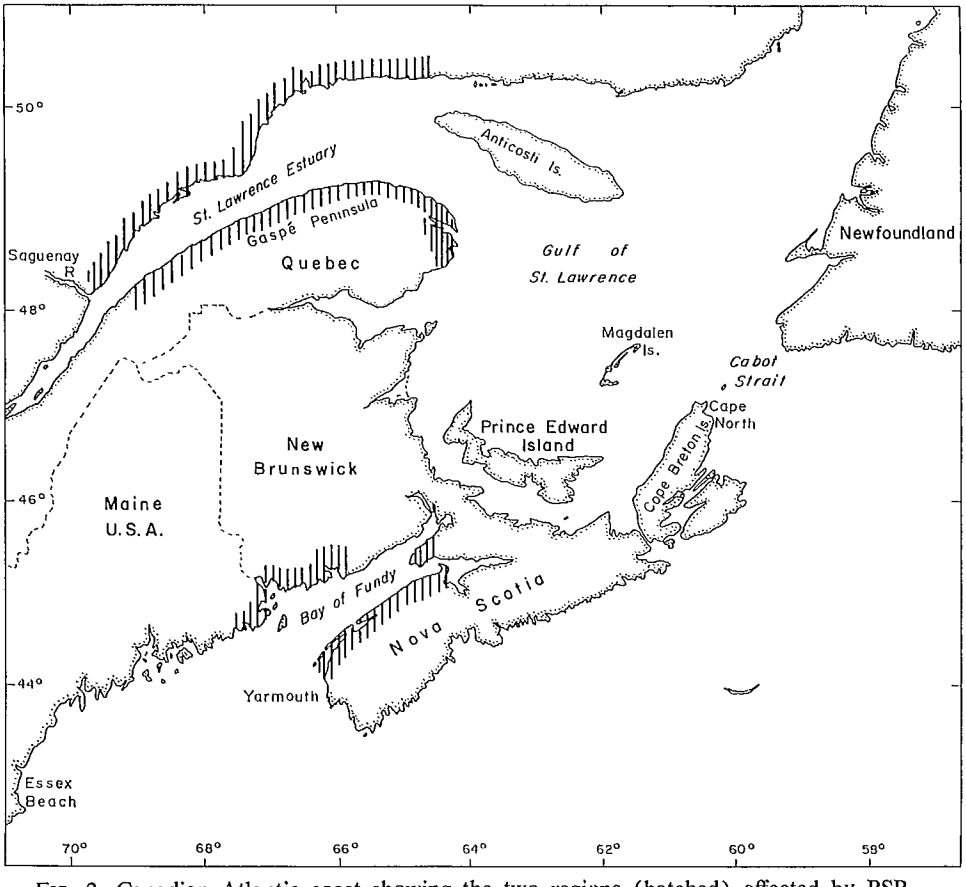


FIG. 2. Canadian Atlantic coast showing the two regions (hatched) affected by PSP.

The amount of poison present in shellfish is measured by preparing extracts of their meats, injecting them into mice, determining the time it takes injected mice to die, and then calculating the poison content on the basis of known standards. The results (scores) are expressed in micrograms (μg) of poison present in 100 g of shellfish meat. Scores are often stated numerically with or without "μg" as a suffix. The lowest concentration of poison that can be detected by the standard mouse test now used in Canada is 44 μg/100 of shellfish meat (before May 1966, it was 32 μg/100 g). Thus, when injection of a shellfish extract has no effect on mice, its score is now reported as <44 (before May 1966, it would have been <32). For practical purposes such scores are regarded as equivalent to zero.

Contrary to popular belief and early claims (Von Haranghy 1942), it is impossible to distinguish between toxic and nontoxic shellfish by sight, taste, or smell.

The first report of PSP in Canada is found in the diary of Mr Menzies, naturalist and surgeon to Captain George Vancouver's expedition to the Pacific

TABLE 1. Confirmed cases of paralytic shellfish poisoning in the Bay of Fundy region, 1889–1969 (illnesses include number of nonfatal and fatal poisonings).

| Year | Month | Source of shellfish | Shellfish eaten | Illnesses | Fatalities | Reference |
|---|---|---|---|---|---|---|
| Prior to 1889 | ? | The Wolves, N.B. | Red mussels | 1 | 1 | Ganong (1889) |
| 1934 | Apr. | Digby, N.S. | Scallop roes | 1 | 0 | Medcof and Gibbons (MS 1948) |
| 1936 | July or Aug. | Pocologan, N.B. | Red mussels | 1 | 0 | " |
| 1936 | July | Centreville, N.S. | Red and blue mussels | 2 | 1 | Murphy (1936); Gibbard et al. (1939) |
| 1936 | " | East Ferry, N.S. | Red and blue mussels | 3 | 1 | " |
| 1936 | " | Digby Co., N.S. | ? | $(35 \pm 5)^a$ | 0 | Medcof and Gibbons (MS 1948) |
| 1936 | " | Freeport, N.S. | Blue mussels | 3 | 0 | " |
| 1938 | ? | Meteghan, N.S. | ? | 2 | 0 | " |
| 1945 | Aug. | Lepreau Basin, N.B. | Clams | 18 | 0 | Medcof et al. (1947) |
| 1945 | " | Pocologan, N.B. | " | 1 | 0 | " |
| 1945 | " | New River Beach, N.B. | " | 2 | 0 | " |
| 1945 | " | Thorne Cove, N.S. | " | 5 | 0 | " |
| 1947 | July | Lepreau Basin, N.B. | " | 1 | 0 | Medcof (MS 1949) |
| 1957 | June | Lepreau Basin, N.B. | " | 23 | 0 | Bond and Medcof (1958) |
| 1957 | " | Dipper Harbour, N.B. | " | 3 | 0 | " |
| 1957 | " | Deadman Harbour, N.B. | " | 4 | 0 | " |
| 1957 | " | New River Beach, N.B. | " | 1 | 0 | " |
| 1957 | July | Saint John, N.B. | " | 1 | 0 | " |
| 1957 | " | Negro Harbour, N.B. | " | 1 | 0 | " |
| 1958 | " | Saint John, N.B. | " | 1 | 0 | Bond and Corbeil (MS 1958a, b) |
| 1961 | " | Blacks Harbour, N.B. | Clams and blue mussels | 1 | 0 | Boyd and Lachance (MS 1961) |
| 1961 | Aug. | Lepreau Basin, N.B. | Bar clams and soft-shell clams | 5 | 0 | " |
| | | Total (excluding partly confirmed cases): | | 80 | 3 | |

[a]Partly confirmed cases.

northeast (Vancouver 1798). Three members of one of Captain Vancouver's parties exploring Mathieson's Channel on the coast of British Columbia were stricken, probably from eating blue mussels. Mr Menzies' entry in his diary for June 17, 1793, gives the typical symptoms of PSP:

"Near the head of this arm they stopped to breakfast on the morning of the 15th where the people finding some good looking mussels about the rocks and shores, boiled a quantity of them . . . but unfortunately for them . . . these mussels proved to be of a deleterious quality as all those who had ate of them in any quantity were, soon after they embarked, seized with sickness, numbness about the mouth, face and arms, which soon spread over the whole body accompanied with giddiness and general lassitude; this was the case with three of the crew of the Discovery's boat. . . One of them, John Carter, puked a great deal, and found himself so much relieved by it that he kept pulling on his oar till about one o'clock when the whole party stopped to dine: but in attempting to get out of the boat he was so weak and giddy that he fell down and he and the other two were obliged to be carried to shore. On this, Mr. Johnstone instantly directed a fire to be kindled and plenty of warm water to be got ready as soon as possible, that each of them might drink a sufficient quantity of it to operate as an emetic . . .but, before it could be got ready, John Carter became very ill . . . his pulse becoming weaker and weaker, his mouth and lips appearing black and his face and neck becoming much swelled together with faintness, general numbness and tremor. Under these circumstances he gradually sank without much struggle and expired just as soon as they were offering him the first draught of warm water which he was unable to swallow, and this sad affair happened within five hours from the time of his eating the mussels."

## History of PSP on the Canadian Atlantic Coast

Attention of government agencies for public health and fisheries to the PSP problem on the Canadian Atlantic coast was precipitated by the 1936 epidemic in Digby County, N.S. (Table 1; Murphy 1936; Anon 1937). Research on the problem (Gibbard et al. 1939) went ahead quickly because, about that time, studies on the PSP problem in California were published along with methods of measuring the poison content of shellfish (Sommer et al. 1937). Surveys showed the distribution of toxic mussels in the Bay of Fundy region, and standards for safe use of toxic shellfish were agreed on.

In 1943, commercial shellfish processors asked permission to can mussels to meet heavy wartime demands and a wide survey was undertaken. This covered the Bay of Fundy region, the Atlantic coast of Nova Scotia, and the southern Gulf of St. Lawrence. Results showed that soft-shell clams as well as mussels were occasionally toxic in the Fundy region, but shellfish in the other areas surveyed were always poison-free. Strict harvesting and processing controls were instituted in the Fundy region to prevent toxic soft-shell clams from being marketed, and mussel fishing was prohibited.

Few people believed that these controls were necessary until 1945, when well documented epidemics of PSP on both the New Brunswick and Nova Scotia sides of the Bay of Fundy (Table 1) demonstrated the reality of PSP risks (Medcof et al. 1947). It was then that research and management programs

TABLE 2. Confirmed cases of paralytic shellfish poisoning in the St. Lawrence region, 1880–March 1970 (illnesses include total number of nonfatal and fatal poisonings).

| Year | Month | Source of shellfish | Shellfish eaten | Illnesses | Fatalities | Reference |
|---|---|---|---|---|---|---|
| 1880 | July | Douglastown | Clams | 1 | 1 | Stafford (1912); Medcof et al. (MS 1966) |
| 1884 | " | Douglastown | " | 1 | 1 | Stafford (1912); Medcof et al. (MS 1966) |
| 1902 | " | Baie-des-Capucins | " | 7 | 1 | Medcof et al. (MS 1966) |
| 1906–10 | Summer | Escoumins | " | 5 | 4 | " |
| 1914 | July | Baie-Comeau | " | 1 | 0 | " |
| 1914 | July | Barachois | " | 1 | 0 | " |
| 1924–25 | Aug. | Ste-Flavie | " | 2 | 0 | " |
| 1928 | Aug. | Ste-Flavie | Mussels | 1 | 0 | " |
| 1929–30 | ? | Baie des Anglais | Clams | 2 | 2 | " |
| 1935 | July | Havre-St-Nicolas | Clams | 1 | 0 | " |
| 1936–37 | ? | Godbout | Rough whelks | 12 | 0 | " |
| 1945 | July | Ste-Luce | Clams | 2 | 0 | " |
| 1945 | July | Bic | " | 1 | 1 | " |
| 1948 | Aug. | Baie-des-Capucins | " | 2 | 1 | " |
| 1948 | Aug. | Les Boules | Mussels | 5 | 2 | Medcof et al. (MS 1966); Medcof (MS 1949) |
| 1951 | Apr. | Grand-Métis | Clams | 3 | 0 | Medcof et al. (MS 1966) |
| 1954 | July | Metis-Beach | Clams and mussels | 9 | 2 | Medcof et al. (MS 1966); Tennant et al. (1955) |

| Year | Month | Source of shellfish | Shellfish eaten | Illnesses | Fatalities | Reference |
|---|---|---|---|---|---|---|
| 1960 | Spring | Latour | Clams | 1 | 0 | Medcof et al. (MS 1966) |
| 1962 | Aug. | Anse St. Pancrace | Clams | 1 | 0 | " |
| 1962 | Sept. | Mistassini | Mussels and clams | 9 | 1 | " |
| 1962 | " | Franquelin | Mussels | 3 | 0 | " |
| 1962 | " | St-Nicolas | Clams | 4 | 0 | " |
| 1963 | Apr. | Latour | Clams | 1 | 0 | " |
| 1966 | Sept. | Les Méchins | Mussels | 2 | 0 | Que. Ministry of Industry and Commerce (unpublished records) |
| 1966 | " | Ilets des Méchins | " | 1 | 0 | " |
| 1966 | " | Ste-Marthe | " | 2 | 0 | " |
| 1966 | " | Ste-Anne-des-Monts | " | 1 | 0 | " |
| 1966 | " | Cap-Chat | " | 1 | 0 | " |
| 1966 | Nov. | Les Méchins | " | 4 | 0 | " |
| 1966 | Nov. | Ilets des Méchins | " | 2 | 0 | " |
| 1967 | Mar. | Les Méchins | " | 1 | 0 | " |
| 1969 | Aug. | Godbout | Clams | 1 | 1 | " |
| 1969 | " | Ste-Anne-des-Monts | Mussels and periwinkles | 4 | 1 | " |
| 1969 | " | Pointe Metis-Beach | Clams | 8 | 1 | " |
| 1969 | Sept. | Petite Vallée | Mussels | 1 | 1 | " |
| 1970 | Mar. | Cap-Chat | Whelks | 4 | 1 | Dr A. Litalien (personal communication) |
| | | | Total: | 107 | 21 | |

gained full support and surveys and monitorings of toxicity were intensified. During all this period nothing was reported from the St. Lawrence region, but in 1948 a rash of illnesses and two deaths (Table 2) made newspaper headlines and broadened the area of concern. In 1949, a survey of the south shore of the St. Lawrence estuary showed the need for controls (Medcof et al. MS 1966; Vladykov 1950) and regulations like those used in the Fundy region were established.

There is little doubt that the controls were beneficial but they did not eliminate the PSP problem. In 1954 there was another outbreak in the St. Lawrence, and in 1957 another in the Fundy region. These led to a vigorous search for more effective controls. The aim was to discover how to predict, each year, just when shellfish would become toxic and what levels of toxicity could be expected. In 2 years of intensified studies, the source of poison was established beyond doubt, the causative organism was isolated and cultured, and environmental factors that regulate annual cycles of shellfish toxicity were identified (Prakash and Medcof 1962; Prakash 1962, 1963, 1967). A method of predicting times and levels of shellfish toxicity was not worked out because environmental variables were too complex. But increased knowledge of the causative organism and of its behaviour made more effective controls possible and made continuing research more meaningful. Since then a wide survey of the St. Lawrence estuary has revealed many unreported cases of PSP, and compilation of records of that region (Medcof et al. MS 1966) has shown that the St. Lawrence problem is greater than formerly supposed. Recent work has also revealed many new facets of the problem in the Fundy region and has helped to improve the control system.

In retrospect, it seems both strange and unfortunate that the PSP problem in eastern Canada was not appreciated earlier. Lescarbot (1609) stated in his writings that the Indians at Port Royal on the Annapolis Basin, N.S., did not eat mussels. When they were starving, they would eat their dogs and the bark of trees, but they would not eat these shellfish. To us this food taboo suggests that Port Royal Indians must have had traditional knowledge of the hazard of eating shellfish from the open coast of the Bay of Fundy, where mussels are the dominant species of shellfish and are to this day responsible for illnesses and occasional deaths. The story is the same for the St. Lawrence region. The deputy Postmaster General of British North America (Heriot 1807) described no specific instances of PSP, but it is clear from his writings that the malady was then well known among the inhabitants. Documented cases of PSP in the Fundy region (Ganong 1889) and in the St. Lawrence region (Stafford 1912; Uzmann 1952) were no more heeded than Lescarbot's, Vancouver's, and Heriot's reports. Fortunately the researches carried out by Dr H. Sommer and his associates (Sommer et al. 1937) did gain attention. The trail they blazed is now a well-trodden path.

## Features of Poisonings

### Symptoms and Causes

Most of the illnesses from eating shellfish are either allergic reactions or infections (bacterial or viral) of the digestive system. The former are not related

to the wholesomeness of the shellfish and the latter are caused by spoiled or sewage-contaminated shellfish. Paralytic shellfish poison is neither an allergy nor an infection. As mentioned before, it is caused by a true poison that some shellfish sometimes contain. Symptoms of PSP are distinctive, and readily separate it from the other types of shellfish-induced illnesses. According to Halstead (1965),

"Paralytic shellfish poisoning may be diagnosed readily by the presence of pathognomonic symptoms which usually manifest themselves within 30 minutes. Initially there is a tingling or burning sensation of the lips, gums, tongue and face, with gradual progression to the neck, arms, fingertips, legs and toes. The paresthesia later changes to numbness, so that voluntary movements are made with difficulty. In severe cases ataxia and general motor incoordination are accompanied in most instances by a peculiar feeling of lightness, 'as though one were floating in air.' Constrictive sensations of the throat, incoherence of speech, and aphonia are prominent symptoms in severe cases. Weakness, dizziness, malaise, prostration, headache, salivation, rapid pulse, intense thirst, dysphagia, perspiration, anuria, and myalgia may be present. Gastrointestinal symptoms of nausea, vomiting, diarrhea, and abdominal pain are less common. As a rule, the reflexes are not affected. Pupillary changes are variable, and there may be an impairment of vision or even temporary blindness. Mental symptoms vary, but most victims are calm and conscious of their condition throughout their illness. Occasionally, patients complain that their teeth feel 'loose or set on edge.' Muscular twitchings and convulsions are rare."

This symptomatology closely parallels John Carter's and that of Canadian Atlantic victims of PSP (Medcof et al. 1947: Tennant et al. 1955; Bond and Medcof 1958). Analysis of our case history records indicates that PSP cases show a typical sequence of symptoms, which may help in distinguishing the poisoning as mild, severe, or extreme, as follows:

| | |
|---|---|
| Tingling sensation or numbness around lips, gradually spreading to face and neck. Prickly sensation in fingertips and toes. Headache, dizziness, nausea. | MILD |
| Incoherent speech. Progression of prickly sensation to arms and legs. Stiffness and noncoordination of limbs. General weakness and feeling of lightness. Slight respiratory difficulty. Rapid pulse. | SEVERE |
| Muscular paralysis. Pronounced respiratory difficulty. Choking sensation. | EXTREME |

Many factors affect symptoms and severity of poisoning. These are discussed in later sections. In fatal cases, death is caused by respiratory paralysis and cardiovascular collapse, usually within 12 hr of consumption of toxic shellfish.

## Treatment and Prevention

Although much is known about how the poison behaves in the body, no one has discovered an antidote for it. Many have tried and are still trying (Anon



95480—2½

1970). Until one is discovered, the best protection against PSP is public education on what areas produce toxic shellfish, what species and what parts of their bodies are hazardous, how cooking affects toxicity, and where, and where not to seek advice on use of shellfish.

Various authors have recommended treatments (Meyer et al. 1928; Müller 1932; Sommer and Meyer 1937), but the fullest statement is given by Halstead (1965):

> "The treatment of paralytic shellfish poisoning is largely symptomatic. The poison has no specific antidote. Apomorphine is more effective than lavage in removing pieces of shellfish from the stomach. Since mussel poison is readily absorbed on charcoal, Lloyd's reagent and similar absorbents may be tried. Alkaline fluids are of value since the toxin is unstable in that medium. Diuresis may be instituted with 5 percent ammonium chloride.
>
> "Artificial respiration is an important adjunct and should be instituted promptly if there is any evidence of respiratory embarrassment. Experimentally this technique has been used with good results and is recommended (Sapeika, 1953; Murtha, 1960). Primary shock may be present and require attention.
>
> "Drug therapy has varying degrees of success. The anticurare drugs, such as neostigmine, are useful in aiding artificial respiration. DL-amphetamine, epinephrine, ephedrine, and DMPP (1,1-dimethyl-4-phenylpiprazinium iodide) are also recommended (Murtha, 1960). To counteract the acetylcholine esterase-like inhibitory effect, Pepler and Loubser (1960) advised using oximes, such as pyridinealdoxime methiodide. Digitalis and alcohol are not recommended."

R. M. Bond (Bond and Medcof 1958) confirmed earlier conclusions (Prinzmetal et al. 1932) that the poison is absorbed quickly from the digestive tract and that symptoms appear quickly. Bond chewed meats of highly toxic red mussels and within 5 min he experienced that prickling sensation in the lips that PSP victims describe as their first symptom. The first treatment, as Halstead recommends, should therefore be aimed at halting this rapid absorption by emptying the stomach of toxic shellfish as quickly as possible.

The digestive system does not seem to absorb all the poison that enters it. This is deduced from the fact that it requires less poison to kill a rat if the dose is administered by intraperitoneal injection than if it is given by mouth (Kao 1966). Victims of PSP should therefore receive no treatment that is likely to increase poison absorption by the digestive system. For example, they should not be given alcohol because alcohol readily dissolves the poison and hastens its absorption.

The poison is quickly eliminated in the urine (Prinzmetal et al. 1932). The "plenty of warm water" that Vancouver's men prepared for John Carter might have served well, not only as an emetic but also to induce urination.

## Case History Records

Much information on PSP in eastern Canada for the period 1880–1970 came from medical and public health journals, hospital records, and interviews with attending physicians or nurses-in-charge of health units. However, most of

the information came from personal interviews with PSP victims during or soon after their illnesses or from interviews with their next of kin. A case history form (Appendix I) was used to compile the fullest possible record of each illness and the circumstances leading to it. Wherever possible, each victim's case was confirmed by careful checking with physicians or, if the sufferer had no medical attention, with other persons. Any case that could not be unquestionably diagnosed as PSP was rejected or classified as "partly confirmed."

We confirmed records of 107 cases (21 fatal) of PSP in the St. Lawrence region and of 80 cases (3 fatal) in the Fundy region. Tables 1 and 2 summarize these chronologically by region, year, month, and locality within region. Besides these confirmed cases, physicians reported treating a total of 30–40 patients for PSP on the Nova Scotia side of the Bay of Fundy in 1936 without formally reporting them (Medcof and Gibbons MS 1948). We had no individual case history reports on these and listed them in Table 1 as partly confirmed. If they were included, they would raise the number of Fundy cases to about 115 and the total number of cases for the Canadian Atlantic coast to about 222.

## Frequency of Poisonings by Years, Months, and Day-of-week

Outbreaks of PSP in the Bay of Fundy region are rare, but when they occur they may affect many people (Table 1, Fig. 3). In the St. Lawrence region outbreaks are frequent but few people are involved in each (Table 2, Fig. 3). Total counts of poisonings in the period 1930–70 are slightly lower for the Fundy (79) than for the St. Lawrence (87) region; however, the count for fatal poisonings during this period was much lower for the Fundy (2) than for the St. Lawrence (14) region. Annual frequencies of poisonings in the two areas seem quite unrelated. For example, two of the worst years for poisonings in the Bay of Fundy were 1946 with 26 cases (none fatal) and 1957 with 33 cases (none fatal) (Bond and Medcof 1958). The corresponding counts for the St. Lawrence region in these years were three (one fatal) and zero. Conversely the worst PSP years for the St. Lawrence region were 1962 with 17 cases (one fatal) and 1969 with 18 cases (4 fatal). No poisonings were reported from the Fundy region in either of these years.

Poisonings occur as early as March and as late as November but PSP is essentially a summer malady (Fig. 4). The worst months in the Fundy region are June, July, and August and in the St. Lawrence region July, August, and September.

Sunday is the worst day of the week for poisonings in the Bay of Fundy region (Table 3). Sundays in the summer are favorite times for picnickers to visit beaches, to gather and eat shellfish, and to carry them home for their friends.

In the St. Lawrence region, although Sundays are bad, Fridays are worse probably because, until recently, they were meatless days for devout Roman Catholics.

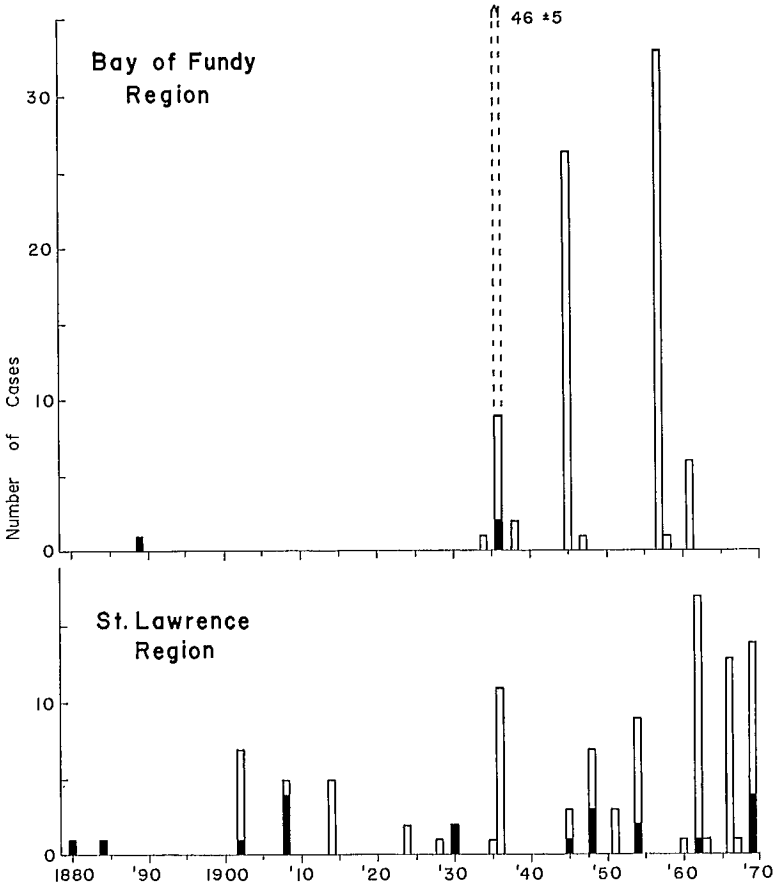


FIG. 3. Frequencies of PSP by years in the Bay of Fundy and St. Lawrence regions, 1880–1969 (solid bars, deaths; open bars, nonfatal poisonings; broken lines, partly confirmed nonfatal poisonings).

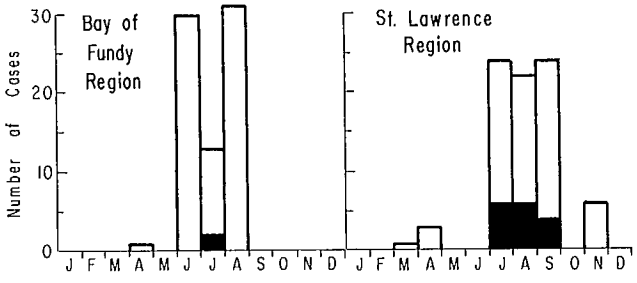


FIG. 4. Frequencies by months, of fatal (solid bars) and nonfatal (open bars) poisonings in the Bay of Fundy and St. Lawrence regions, 1880–1969.

TABLE 3. Frequencies of poisonings by day of week in the two regions.

| Region | No. of cases | Sun. | Mon. | Tues. | Wed. | Thurs. | Fri. | Sat. |
|---|---|---|---|---|---|---|---|---|
| St. Lawrence | 74 | 14 | 7 | 8 | 4 | 15 | 19 | 7 |
| Bay of Fundy | 63 | 48 | 2 | 5 | 2 | 2 | 1 | 3 |
| Total | 137 | 62 | 9 | 13 | 6 | 17 | 20 | 10 |

## HUMAN SENSITIVITY AND TOLERANCE

Since 1944 many records of consumption of toxic shellfish from the New Brunswick section of the Fundy region have been compiled. Of these, 131 refer to areas where shellfish toxicities were being monitored and included dates, and the number, size, and species of shellfish consumed. This information was combined with data on toxicity and meat yields of shellfish to estimate the dosage in micrograms (μg) of poison ingested by each person.

The 131 cases were grouped according to severity of poisoning: 82 persons showed no symptoms of illness; 21 suffered mild poisoning; 20 were severely poisoned; and 8 suffered extreme poisoning. The estimated average dosage for each group is shown in Fig. 5. Records from the 1945 epidemic (Medcof et al. 1947) and subsequent data (Fig. 5) indicate that mild, severe, and extreme poisonings result from ingestion of 1000, 1900, and 2000 μg, respectively. However the person-to-person variation in sensitivity to shellfish poison is so great that average dosages have little significance. From the public health standpoint, it is more important to know the limits of the range of sensitivity and particularly the minimum dosage that may induce illness.

Compared with residents, nonresidents of shore communities have lower tolerance for shellfish poison. Only one of the 31 residents became ill from eating poisonous shellfish, whereas 48 of the 100 nonresidents became ill (Fig. 5). This confirms deductions from earlier studies (Medcof et al. 1947; Bond and Medcof 1958).

Several records suggest that young children are more sensitive to shellfish poison than adults. The two young victims of PSP (2 and 8 years old) represented in Fig. 5 became ill from less than average doses. The two-year-old was poisoned during the New Brunswick epidemic in 1957 (Bond and Medcof 1958). She is reported to have eaten only two steamed clams (estimated dosage 96 μg) but exhibited symptoms of severe poisoning including prostration. In contrast, a 65-year-old man consumed 2 doz raw clams (estimated dosage 8300 μg) and suffered no more than the child. Susceptibility of rats to PSP is known to decrease with age even when differences in weight of old and young animals are allowed for (Kao 1966).

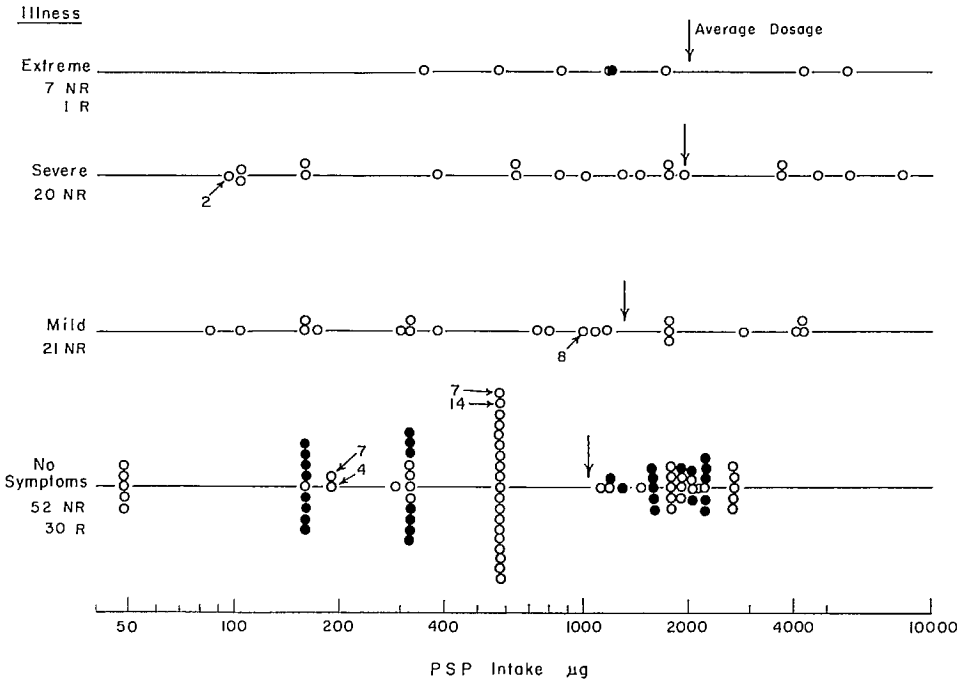


FIG. 5. Illness reactions, grouped according to severity, of 31 residents (R, solid circles) and of 100 nonresidents (NR, open circles) of Bay of Fundy communities to different poison intakes (numbers represent ages of the six children in the groups).

Sex may also affect susceptibility to shellfish poison. Table 4 compares dosage estimates for the adult men and women described in Fig. 5 who suffered equal severities of poisoning. Six of the seven comparisons made in the table indicate that on the average a man must take in one and one-half times as much poison as a woman to suffer the same symptoms. The comparisons provide strong evidence that woman are more sensitive to poison than men. Stephenson et al. (1955) have shown that, weight for weight, female mice are more susceptible to shellfish poison than males.

Some of our records suggest that PSP symptoms are more acute when shellfish are eaten alone on an empty stomach than when they are eaten as part of a normal meal and that consumption of alcohol along with shellfish accentuates symptoms.

Reliable records of fatal cases are so few, and sensitivity to shellfish poison is so variable, that estimates of the human lethal dose must be regarded as little more than educated guesses. The intravenous lethal dose has been estimated at 400 µg (Kao and Nishiyama 1965) and the oral lethal dose at 200 µg (Schantz 1970[1]). Other estimates have ranged from about 1000 µg (Tennant et al. 1955) to approximately 12,400 µg (Meyer 1953).

[1] In a recent article, Schantz (1970) has suggested that the oral lethal dose for man may be close to 0.5 mg (500 µg).

TABLE 4. Estimated poison intake by adult male (M) and female (F) patients who suffered various degrees of poisoning during 1945 and 1957 PSP epidemic in the Bay of Fundy region.

| Year | | No. of cases | Poison dosage (μg) Average | Range |
|---|---|---|---|---|
| | | Mild poisonings | | |
| 1945 | M | 4 | 1640 | 800–2880 |
| | F | 6 | 747 | 160–1760 |
| 1957 | M | 6 | 1175 | 304–4128 |
| | F | 2 | 4080 | 4032–4128 |
| | | Severe poisonings | | |
| 1945 | M | 3 | 3733 | 1760–5760 |
| | F | 4 | 1680 | 640–3680 |
| 1957 | M | 3 | 4816 | 1456–8272 |
| | F | 3 | 1285 | 880–1952 |
| | | Extreme poisonings | | |
| 1945 | M | 1 | 5760 | – |
| | F | 1 | 4320 | – |
| 1957 | M | 2 | 1152 | 576–1728 |
| | F | 2 | 1032 | 880–1184 |
| Pooled data for all levels of poisonings | M | 19 | 2491 | 304–8272 |
| | F | 18 | 1644 | 160–4128 |

## POISONINGS OF ANIMALS

Case history records show that many domestic animals (mostly cats and hens) die from eating discarded parts of shellfish that are being prepared for human consumption. In the Fundy region, discarded rims (body parts other than the adductor muscle) of scallops are the most common cause, but soft-shell clams and bar clams have also been implicated. In the St. Lawrence region, soft-shell clams are the only shellfish known to have killed domestic animals, but blue mussels have caused one nonfatal poisoning (Medcof et al. MS 1966).

Symptoms listed in case history records for domestic animals (Medcof et al. 1947, MS 1966; Tennant et al. 1955) suggest that in hens the legs are paralyzed before the wings and that, in cats, the hind legs are affected before the forelegs. Other investigators report that chickens are more sensitive than mice (Yndestad and Hauge 1969) and that homing pigeons are very sensitive to the poison (Coulson et al. 1968). All this agrees with the general opinion that birds are more susceptible to PSP than other warm-blooded animals (McFarren et al. 1960).

Cold-blooded vertebrates are considered to be relatively insensitive to shellfish toxin; however, there are reports that certain fishes, amphibians, bivalve molluscs, and starfishes are affected (Ray and Wilson 1957; Gates and Wilson 1960; Evans 1964; Adams et al. 1968; Coulson et al. 1968; Clark 1968).

In winter, Bay of Fundy fishermen often see herring gulls feeding on scallop rims that are thrown overboard when scallops are shucked at sea. The birds seem to eat enough to be poisoned because these rims are very toxic (see Table 7) but apparently they suffer no ill effects. This has given rise to a local belief that wild animals are immune to the poison. However, in 1968 there were heavy mortalities of several species of sea birds with symptoms of PSP on the Northumberland coast in England (Coulson et al. 1968). These included some herring gulls but no eider ducks, the latter of which regularly feed on mussels. On the Pacific coast of the United States, there have been similar mortalities of sea birds, including a few herring gulls, that have been attributed to PSP (McKernan and Scheffer 1942). Although susceptible to PSP, gulls are not as sensitive to it as some other species.

These observations in England and the United States do not support the wild-animal immunity theory, nor does the following case history from our own coast (Medcof et al. MS 1966, cases 16 and 77): *Wild ducks and a boy.* Following a severe storm in July 1914, George Cotton found 45 helpless young black ducks (*Anas rubens*) on the beach at Barachois, Que. He placed these in a poultry pen and made his 14-year-old son, Patrick, responsible for feeding them. One day Patrick dug some soft-shell clams from the river at Barachois, opened them raw, and fed the ducks the softer parts ("bellies") by hand. The boy ate some himself. After about 15 min Patrick noticed that some ducks were lying on their backs, dead, and that others were behaving strangely. At the same time he began to suffer from stomach ache, headache, numbness of the face and arms, and shortness of breath. He made his way to the house and was able to explain to his mother that he was sick from eating clams before he collapsed on the kitchen floor. Patrick's mother made him vomit by having him drink warm milk and mustard. Patrick recovered and gave us this case history in 1966.

## Toxicity of Shellfish

### Geographic Distribution

The distribution of toxic shellfish in eastern Canada conforms closely with that of areas affected by PSP. Poison has also been detected occasionally in places where PSP is unheard of. Thus a single positive toxicity record for one species in an area has limited meaning. It need not imply that all shellfish of that species or of other species in that area are sometimes or always toxic. Results for affected areas show that toxicity varies widely, from place to place within areas at any particular time, from season to season, from year to year, and from species to species.

The whole Canadian Atlantic coast has been searched for toxic shellfish except for Anticosti Island and the opposing north shore of the Gulf of St. Lawrence, the coast of Labrador, and the east coast of Newfoundland. Toxic

shellfish have been found only in two regions, the lower estuary of the St. Lawrence River and the middle and lower reaches of the Bay of Fundy (Fig. 2).

Shellfish on the Gulf of St. Lawrence coasts of New Brunswick, Prince Edward Island, Nova Scotia, the Magdalen Islands, and Newfoundland are consistently poison-free. Those on the Atlantic coast of Nova Scotia are also poison-free from Cape North on Cape Breton Island to Yarmouth Harbour near the mouth of the Bay of Fundy.

### Bay of Fundy region

On the Nova Scotia side of the Bay of Fundy, toxic shellfish occur regularly on a 180-mile stretch of coast from Brier Island northeast along the coast into Minas Channel (Fig. 6). The worst places for toxic shellfish in this section are Brier Island, Long Island, and Digby Neck. Toxic shellfish are found occasionally at Yarmouth, in parts of St. Mary's Bay, and in Annapolis Basin. They are rare in upper parts of the Bay of Fundy, none have been found in Minas Basin, and they have been found only during peak years in Chignecto Bay at Joggins.

On the New Brunswick side of the Bay of Fundy, no toxic shellfish are found east of St. Martins (near Saint John), but they are common in the 100-mile stretch of coast from St. Martins south to the Canada–United States boundary (Fig. 6). The worst places for toxic shellfish in this section lie between Point Lepreau and Head Harbour (Campobello Island).

Red mussels and whole scallops from Georges Bank are usually poison-free, but occasionally the digestive glands ("livers") of some scallops contain traces of toxin (Bourne 1965). Toxic shellfish have been reported at several places on the Maine coast, and as far west as Essex Beach, Massachusetts (Goggins 1961).

### St. Lawrence region

Almost every year toxic shellfish are found in the 300-mile stretch of the north shore of the St. Lawrence estuary from Tadoussac east to Sheldrake (Fig. 7). They seem to be even more frequent in the 150-mile stretch of south shore from Trois-Pistoles east to Ste-Anne-des-Monts. The worst places for toxic shellfish seem to be Mistassini on the north shore and Metis-Beach and Baie-des-Capucins on the south shore. So far, there has been no thorough survey of the 120-mile stretch of the Gaspé coast from Ste-Anne-des-Monts east to Grand Grève at the entrance to Gaspé Bay, but some 1969 samples from this section were extremely poisonous. Inside Gaspé Bay and at nearby Barachois, shellfish regularly become toxic but toxicity decreases rapidly to the southwest and west of Barachois. Traces of poison were detected only once at St-Omer on the north shore of the Bay of Chaleur but never west of St-Omer and never on the south shore of the Bay.

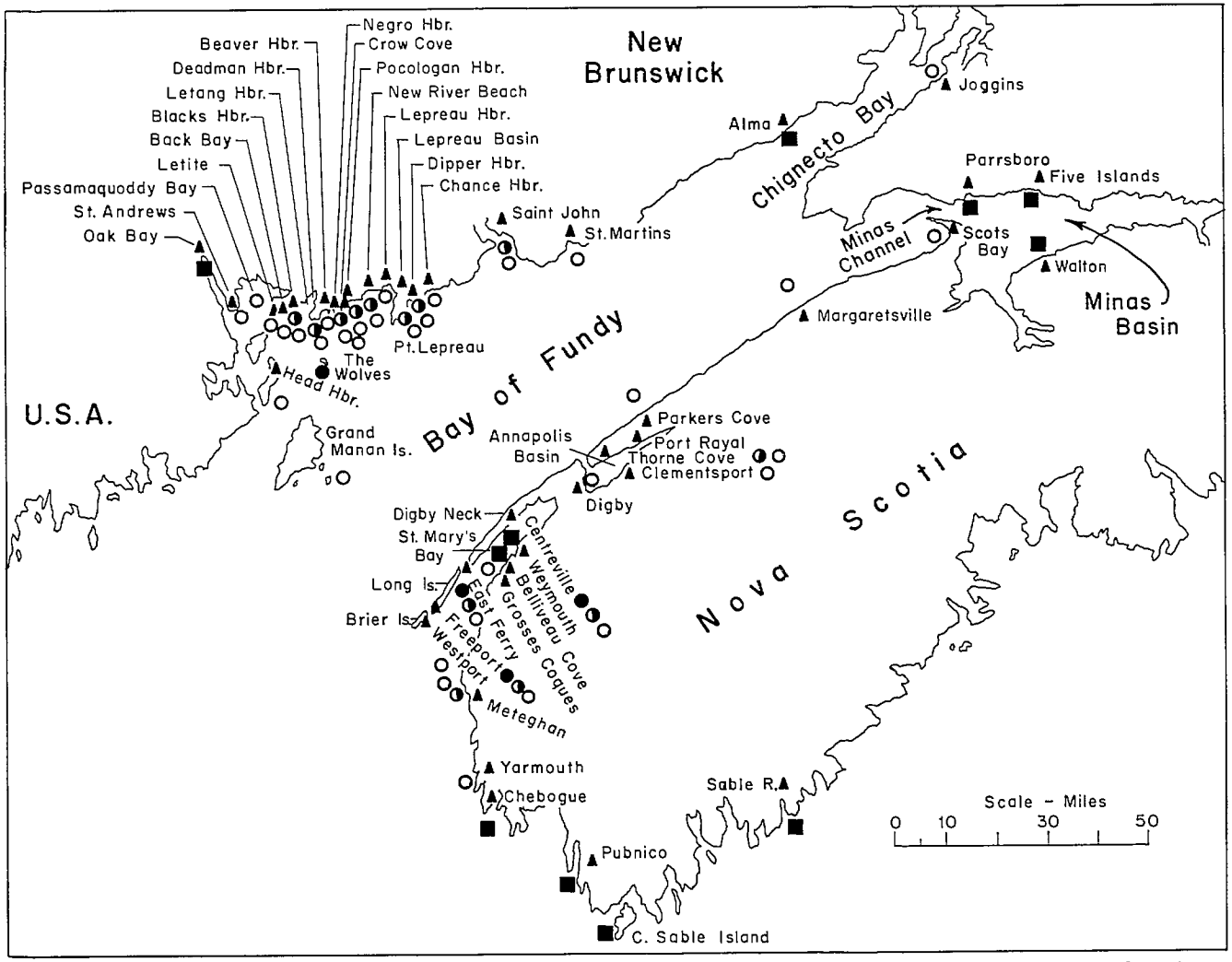


FIG. 6. Geographic distributions of toxic shellfish and poisonings in the Bay of Fundy region, 1889–1969 (■, shellfish always poison free; ○, shellfish sometimes toxic; ◑, nonfatal poisonings; ●, fatal poisonings).

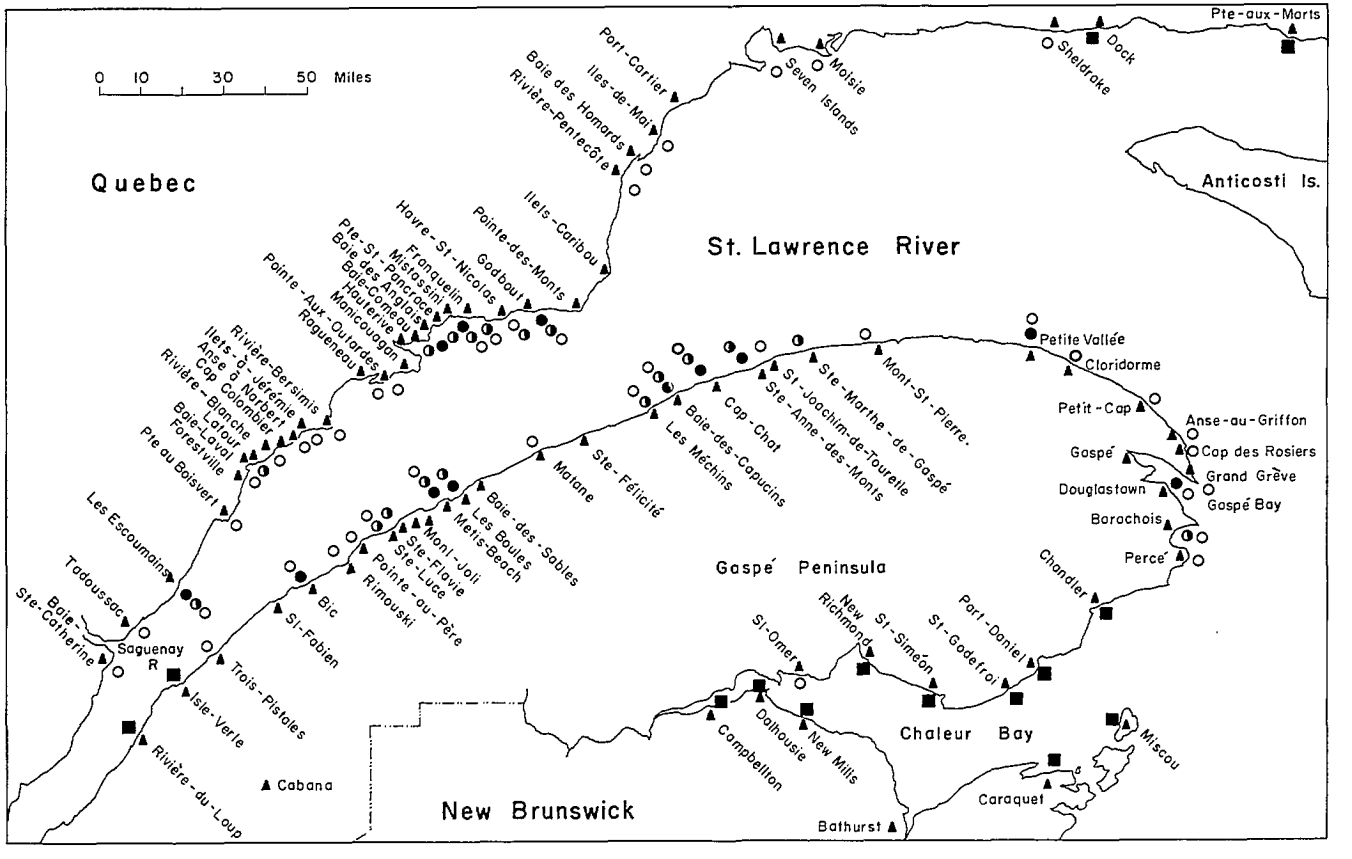


FIG. 7. Geographic distributions of toxic shellfish and poisonings in the St. Lawrence region, 1880-1969 (■, shellfish always poison free; ○, shellfish sometimes toxic; ◑, nonfatal poisonings; ●, fatal poisonings).

## Toxic Species

In the two regions concerned, the main shellfish that cause PSP in humans, in decreasing order of hazard, are soft-shell clams, mussels, and rough whelks. The percentages of all poisonings attributed to these range from 65 for clams to 9 for whelks, and of deaths from 65 for clams to 4 for whelks.

Shellfish that become toxic in the two affected regions are listed in Table 5 under their English and French common names and their scientific names. Some of these are not considered to be public health hazards. For example, the sea scallop has the highest year-round toxicity of all the Fundy shellfish species studied and is the frequent cause of deaths of cats and poultry. It is also a popular sea food, but has been responsible for only one recorded human illness in eastern Canada. This is because people seldom eat any part of a scallop except the adductor muscle, which is poison-free. Likewise, the tiny wedge clam is often toxic in the St. Lawrence region, but nobody eats it. Case history records are the best indicators of what species are hazardous.

*Clams* have been responsible for 65% of the documented illnesses and 65% of the deaths in the St. Lawrence and Fundy regions (Table 6). Soft-shell clams (*Mya arenaria,* Fig. 8A) are our most commonly used shellfish seafood. Their meats are eaten whole, sometimes raw, but more often cooked. They can store large amounts of toxin; the highest recorded scores for whole raw soft-shell clams in the Fundy and St. Lawrence regions are 7700 and 6600.

Bar clams (*Spisula solidissima,* Fig. 8C) are sometimes very toxic in the Fundy region and may remain toxic for long periods. They have been responsible for less than 1% of the documented poisonings and for none of the deaths. They are abundant on some intertidal beaches and available to picnickers, but we do not consider them a serious PSP hazard.

Other clams become toxic in affected areas, e.g., razor clams (*Ensis directus,* Fig. 8F); wedge clams (*Mesodesma arctatum,* Fig. 8D); ocean clams (*Arctica islandica,* Fig. 8E); and bay quahaugs (*Mercenaria mercenaria,* Fig. 8B). None of these have been implicated in poisonings and none are rated as PSP hazards.

*Mussels* are next in importance to clams as PSP hazards and have been responsible for approximately 24% of the recorded illnesses and 30% of the fatalities (Table 6). Blue mussels (*Mytilus edulis,* Fig. 9A) are more popular than red mussels (*Volsella modiolus,* Fig. 9B) and are equally toxic. They are eaten whole and are almost always cooked before being eaten. They are often more toxic than clams, and in proportion to quantities consumed, they seem to have caused more poisonings. The highest score recorded for raw blue mussels is 28,000 (Head Harbour, N.B., September 18, 1944) and the second highest is 23,000 (St-Joachim-de-Tourell, Que., September 12, 1969).

*Sea scallops* (*Placopecten magellanicus,* Fig. 9C) have caused less than 1% of the recorded illnesses and no deaths. They are deepwater forms not ordinarily available to picnickers and, since only their adductor muscles are marketed commercially, there is no danger of PSP to the consumer. Scores for whole raw scallop meats range well above 1000 in many parts of the Fundy region but most

of the toxin is concentrated in the rim (Table 7), which is discarded at sea by fishermen during shucking operations. There is no evidence that it would be hazardous to eat the whole meats of scallops fished outside the affected regions shown in Fig. 2.

TABLE 5. Species of shellfish found to be toxic in the Bay of Fundy and St. Lawrence regions.

| Common names | | Scientific name | Reference | Fig. no. |
|---|---|---|---|---|
| English | French | | | |
| | | **Clams** | | |
| Clam, soft-shell clam, sand clam | coque, mye | *Mya arenaria* | Pugsley (1939); Medcof et al. (1947) | 8A |
| Bar clam, Atlantic surf clam, sea or hen clam | palourde de dune, fausse-praire | *Spisula* (*Mactra*) *solidissima* | Medcof et al. (1947) | 8C |
| Bay quahaug, little-neck clam | palourde, quahaug commune | *Mercenaria* (*Venus*) *mercenaria* | Bond and Lachance (MS 1959) | 8B |
| Arctic wedge clam | clovisse arctique | *Mesodesma arctatum* | Medcof (MS 1952) | 8D |
| Common razor clam | couteau, rasoir | *Ensis directus* | Medcof et al. (1947) | 8F |
| Ocean clam, ocean or black quahaug | palourde (quahaug) de mer | *Arctica* (*Cyprina*) *islandica* | Boyd and Lachance (MS 1970) | 8E |
| | | **Mussels** | | |
| Blue or black mussel, common mussel | moule, mouque | *Mytilus edulis* | Pugsley (1939); Medcof et al. (1947) | 9A |
| Red or horse mussel | moule géante, modiole du nord | *Volsella* (*Modiolus*) *modiolus* | Gibbard et al. (1939) | 9B |
| | | **Scallops** | | |
| Sea scallop, scallop | pétoncle | *Placopecten magellanicus* | Medcof et al. (1947); Bourne (1965) | 9C |
| | | **Whelks, moon-shells, dogwinkles** | | |
| Rough or common whelk | buccin, bourgot bourgeau | *Buccinum undatum* | Medcof (MS 1952) | 9D |
| Spindle shell, Stimpson's whelk | fuseau de Stimpson | *Colus stimpsoni* | " | 9E |
| Ten-banded whelk, ten-ridged neptune | neptunée à dix côte | *Neptunea decemcostata* | " | 9F |
| Greater clam drill, moon-shell | lunatie de l'Atlantique | *Lunatia* (*Polinices*) *heros* | " | 9G |
| Atlantic dogwinkle | bigorneau | *Thais* (*Purpura*) *lapillus* | " | 9H |

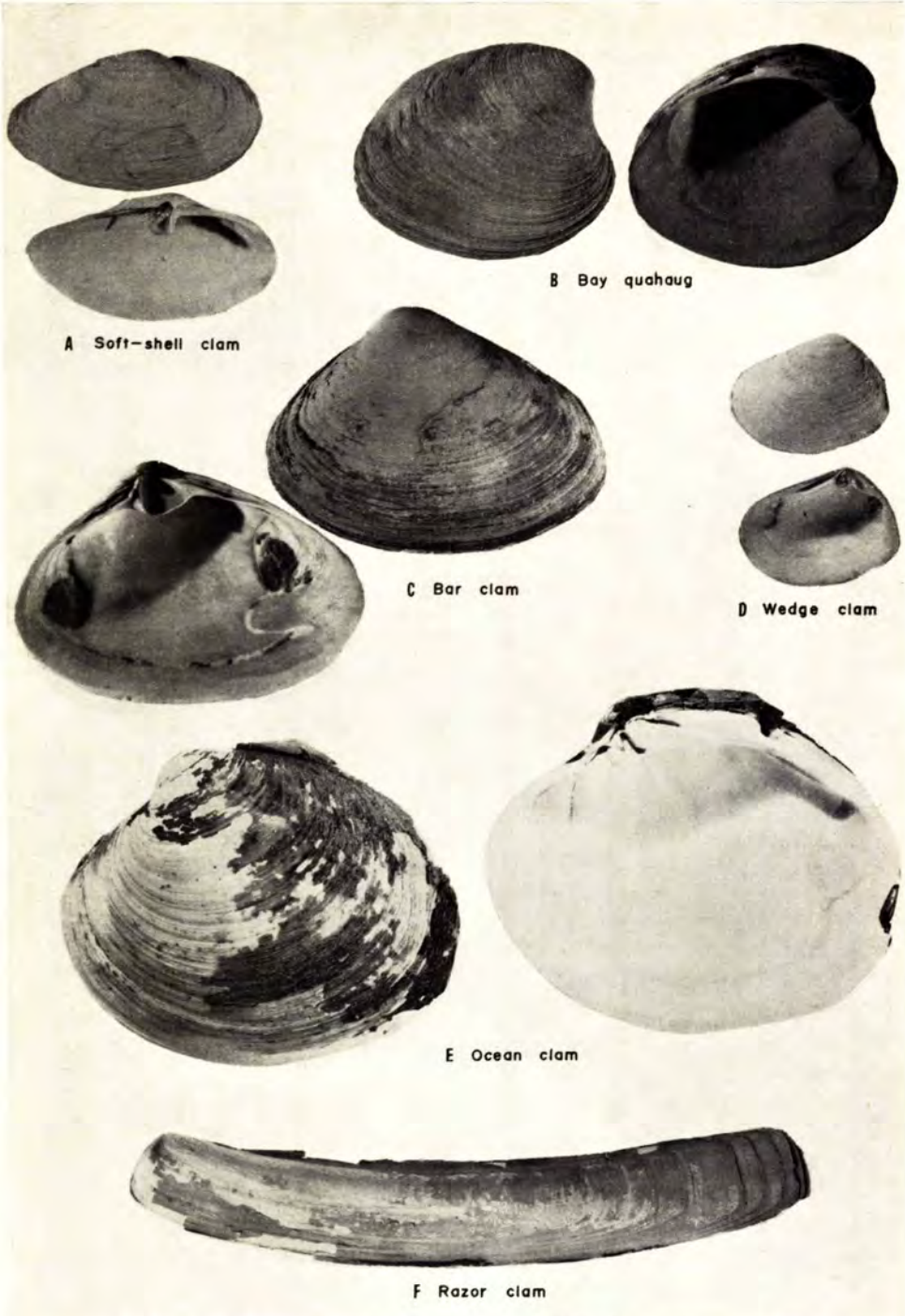


FIG. 8. Shellfish found to be toxic in affected areas of eastern Canada: (A) soft-shell clam; (B) bay quahaug; (C) bar clam; (D) wedge clam; (E) ocean clam; (F) razor clam.

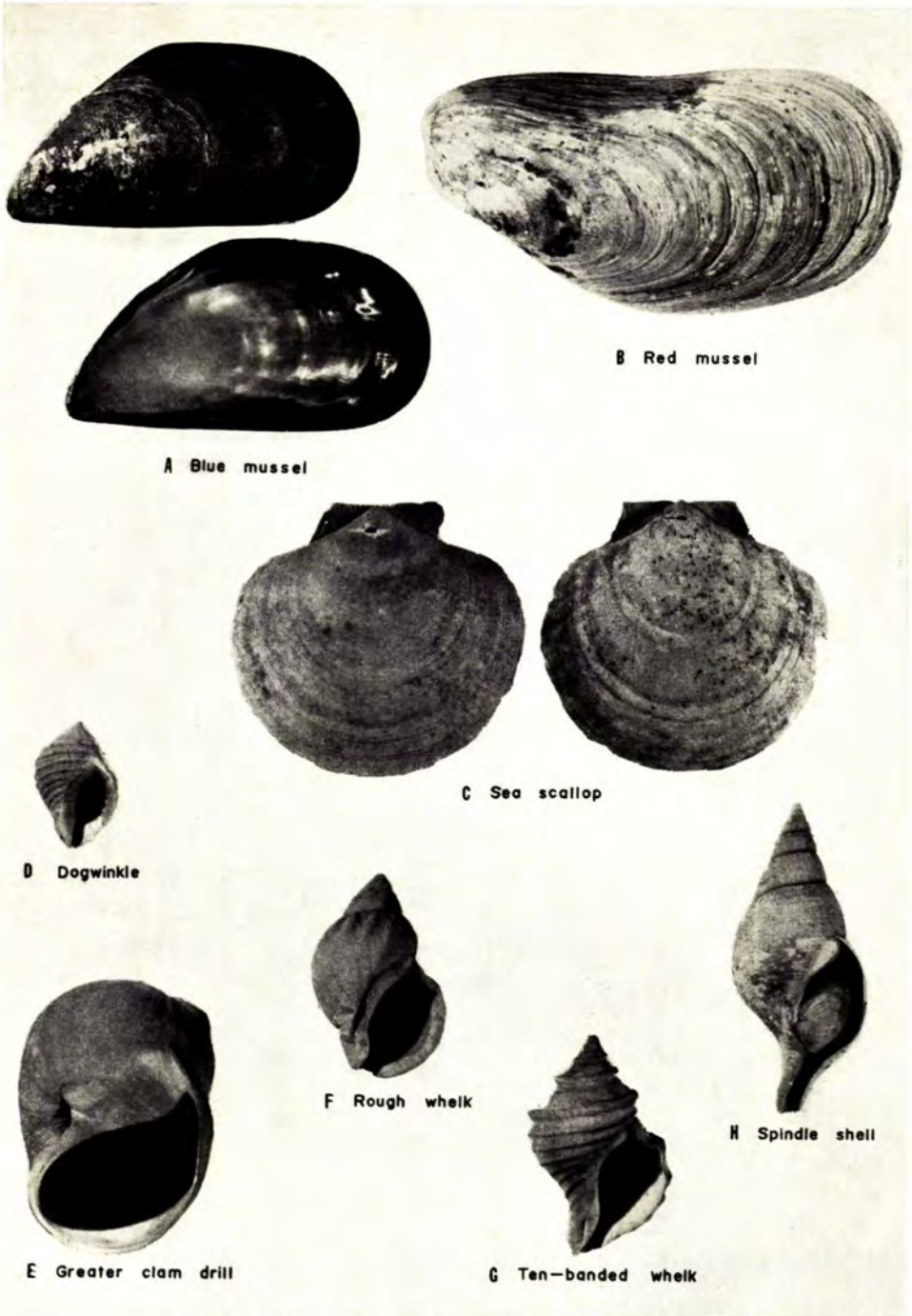


FIG. 9. Shellfish found to be toxic in affected areas of eastern Canada: (A) blue mussel; (B) red mussel; (C) sea scallop; (D) dogwinkle; (E) greater clam drill; (F) rough whelk; (G) ten-banded whelk; (H) spindle shell.

TABLE 6. Numbers of fatal and nonfatal poisonings (P) and deaths (D) attributed to various shellfish species in the two regions, 1880–March 1970. (Poisonings by more than one species eaten at the same meal or by unidentified species of shellfish are not included in this table.)

| | Total | | Clams | | Mussels | | Whelks | | Scallops | |
|---|---|---|---|---|---|---|---|---|---|---|
| Region | P | D | P | D | P | D | P | D | P | D |
| St. Lawrence estuary | 99 | 20 | 52 | 15 | 31 | 4 | 16 | 1 | 0 | 0 |
| Bay of Fundy | 71 | 3 | 60 | 0 | 10 | 3 | 0 | 0 | 1 | 0 |
| Total | 170 | 23 | 112 | 15 | 41 | 7 | 16 | 1 | 1 | 0 |
| % of total poisonings | | | 65 | | 24 | | 9 | | <1 | |
| % of total deaths | | | 65 | | 30 | | 4 | | 0 | |

TABLE 7. Anatomical distributions of poison in some Canadian Atlantic shellfish. Except where otherwise indicated, toxicity scores (μg/100 g) are based on raw shucked meats.

| Species | Whole animal | Digestive gland | Gill | Adductor muscle | Gonad ("roe") | Siphon ("neck") | Remaining parts |
|---|---|---|---|---|---|---|---|
| Soft-shell clam (Sept.) | 540 | 3450 | 1680 | – | – | <32 | 255 |
| Soft-shell clam (Oct.) | 67 | 96 | 1600 | <32 | – | – | 32 |
| Bar clam | – | 4700 | 9450 | 74 | – | <32 | 160 |
| Blue mussel | 1250 | 1740 | – | – | – | <44[a] | 70 |
| Blue mussel (steamed) | 550 | 1342 | – | – | – | 44[a] | 90 |
| Red mussel | <44 | 90 | – | – | – | <44[a] | <44 |
| Red mussel (steamed) | 44 | 50 | – | – | – | <44[a] | <44 |
| Sea scallop | 1220 | 6400 | 450 | <32 | 150 | – | 540 |
| Rough whelk | 512 | 1195 | – | – | – | – | 50[b] |

[a]Byssus, not siphon.
[b]Includes foot and columellar muscle.

*Rough whelks* (*Buccinum undatum,* Fig. 9F) are carnivorous snails, and are a popular domestic and commercial seafood in the St. Lawrence region. They are the third most hazardous shellfish and, although they have been implicated in only two PSP outbreaks, they are responsible for 9% of all illnesses and 4% of all deaths. The first outbreak occurred in 1937 at Godbout, when 12 persons suffered mild poisoning (Table 2). The second was in March 1970 at Cap-Chat,

where four persons were poisoned, one of them fatally. There was some early controversy as to whether whelks could be "vectors" of paralytic shellfish poison. But toxicity assay data and the detailed case histories compiled by Dr Litalien of Ste-Anne-des-Monts in 1970 strongly suggest that they can be and are vectors. Toxicity assays have shown that most of the toxin is concentrated in the dark-coloured digestive gland that occupies the uppermost body whorls. These are always trimmed away in commercial processing and usually trimmed away in home cooking. The remaining muscular parts are relatively free of toxin (Table 7). Scores for raw whelks are usually low but may rise to at least 600 (Medcof et al. MS 1966). Whelks are always cooked before eating and this undoubtedly reduces risks of poisoning. Rarity of poisonings from rough whelks suggests that they are not a serious PSP hazard when shucked and trimmed in the regular way, but can be hazardous if eaten whole, i.e., without trimming away the digestive gland.

Other carnivorous snails like the ten-banded whelk (*Neptunea decemcostata,* Fig. 9G) and spindle shell (*Colus stimpsoni,* Fig. 9H) are attracted to lobster traps and are occasionally eaten by fishermen. These may be highly toxic but, because they are deepwater forms and unpopular sea foods, they are not regarded as true PSP hazards. Similarly, moon-shells or greater clam drills (*Lunatia heros,* Fig. 9E) and dogwinkles (*Thais lapillus,* Fig. 9D) are toxic at times but are not used as food on our coast and cannot be regarded as PSP hazards.

Periwinkles (*Littorina littorea*) from both the St. Lawrence and the Fundy regions have been sampled hundreds of times but are consistently poison-free even when taken from the worst affected areas. Periwinkles are herbivorous snails and feed on plants that encrust rocks, and for this reason, they have no access to poison. They are regularly marketed from the Bay of Fundy.

Atlantic oysters (*Crassostrea virginica*), unlike their Pacific counterparts, have never been implicated in PSP. The Atlantic oyster does not occur naturally in affected areas but is capable of accumulating small amounts of poison if transplanted to these areas at the appropriate time (Bond and Corbeil MS 1958a). We do not consider them a PSP hazard.

## Anatomical Distribution of Poison

Poison is not evenly distributed in the bodies of toxic shellfish. It is concentrated in different organs depending on the species and the season of the year (Table 7). In summer, the digestive gland is the main site of poison for most species. Gills and gonads are secondary sites, and muscular parts, including siphons of soft-shell clams, contain relatively small amounts.

In autumn the scores of digestive glands are usually low and the gills are sometimes the main sites of poison in shoft-shell clams and bar clams. The gill scores are about the same in late summer and early autumn. This suggests that digestive glands lose poison more rapidly than gills (Medcof et al. 1947).

Siphons of soft-shell clams apparently have a low capacity for poison accumulation, although in the Pacific coast butter clam (*Saxidomus giganteus*) they are

the main accumulation site (Pugsley 1939). Digestive glands are the main poison accumulation sites in red and blue mussels, as in California mussels (*Mytilus californianus*) (Sommer and Meyer 1937).

There is a popular belief that mussels accumulate their poison in the byssus threads ("beards"), but repeated tests have shown that beards are poison-free. The same tests show that, although cooking reduces total amounts of poison in mussels, the relative toxicities of various organs are not altered by cooking.

The digestive glands ("livers") of toxic sea scallops contain most of the poison. The mantle is a secondary site. Scores for gonads ("roes") and gills are generally low (Bourne 1965), although a few high scores for gills have been reported (Medcof et al. 1947). Adductor muscles of sea scallops from the Bay of Fundy have always scored poison-free, but in 1970, several samples from the Bay of Chaleur of what were assumed to be sea scallop muscles had low, positive scores (Boyd and Turgeon MS 1971). These have yet to be confirmed because Iceland scallops (*Chlamys islandicus*) and sea scallops occur together in this area.

In toxic rough whelks the digestive glands contain most of the poison (Table 7). The rest of the body is mostly muscular and usually tests poison-free or only mildly toxic (Medcof et al. MS 1966).

### Individual Variation

Toxicity scores of individual shellfish in samples of the same species, taken at the same time and place, vary somewhat and their variation has been studied on both the Atlantic and Pacific coasts. Results for soft-shell clams and blue mussels from the Bay of Fundy (Medcof et al. 1947) and for butter clams from British Columbia and Alaska (Neal MS 1967; Quayle 1969) all show little variability. When one animal in a sample contains appreciable amounts of poison, the others are likely to be toxic to more or less the same degree. It is not one bad clam or one bad mussel that is responsible for PSP.

The capacity for poison accumulation by individual shellfish varies with size (age) as would be expected; however, experiments carried out with different size-groups of soft-shell clams indicate that, weight for weight, small shellfish are as hazardous, if not more so, than large (Medcof et al. 1947).

### Within-Area Variation

The toxicity of shellfish in any affected area varies from place to place and with position in the intertidal zone. It is therefore important that sampling sites for monitoring toxicity be carefully chosen. Results for both the Fundy and the St. Lawrence regions show that shellfish in beds near the mouths of inlets accumulate poison earlier in the season, and accumulate more poison than those in beds near the heads of inlets. For example, scallop toxicities are high for samples fished off the mouth of Passamaquoddy Bay; they are lower for samples from just inside the Bay; still lower for the middle of the Bay; and zero in the estuary

of the St. Croix River, which is a tributary to the Bay. The distance involved here is 11 miles. A similar trend for soft-shell clams appears over shorter distances, e.g., over 1½ miles in Havre-St-Nicolas, on the north shore of the St. Lawrence, and over only ¾ mile in Lepreau Basin, N.B. (Table 8).

TABLE 8. Differences in toxicity of shellfish in different parts of inlets.

| Collection station | Toxicity ($\mu g/100\ g$) |
|---|---|
| **Scallops** | |
| (*Passamaquoddy Bay, N.B., May 3, 1946*) | |
| 1 In Bay of Fundy 3 miles off the mouth of Passamaquoddy Bay | 210 |
| 2 Inside Passamaquoddy Bay 2 miles from the mouth | 190 |
| 3 Inside Passamaquoddy Bay 3 miles from the mouth | 105 |
| 4 Inside Passamaquoddy Bay 8 miles from mouth and in St. Croix estuary | <32 |
| **Soft-shell clams** | |
| (*Lepreau Basin, N.B., Aug. 9, 1945*) | |
| 1 Mouth of Basin (large clams) | 2350[a] |
| 2 One-quarter mile upstream | 2450 |
| 3 One-half mile upstream (small clams) | 1120 |
| 4 Three-quarter mile upstream (small clams) | 240 |
| (*Havre-St-Nicolas, Que., Sept. 3, 1963*) | |
| 1 Mouth of the Harbour | 608 |
| 2 Three-quarter mile up from mouth | 512 |
| 3 One-half mile up from mouth | 240 |

[a]A comparable score for small clams would be about 2600.

Besides this, toxicities of intertidal shellfish decrease from low tide level to high tide level on the same beach. Results for the Fundy region show that, on the average, the scores of soft-shell clams fished near spring-tide low water mark are 27% greater than those for clams taken at the half-tide level (Medcof et al. 1947). This pattern is consistent on the Canadian Atlantic coast for both soft-shell clams and other species.

Our findings on variations in toxicity with position in the intertidal zone parallel those for California mussels (Sommer and Meyer 1937). They differ from those for butter clams in British Columbia and Alaska (Chambers et al. MS 1952; Neal MS 1967; Quayle 1969), where no clear relations were found.

## Seasonal and Annual Variation

Areas affected by PSP usually show great seasonal and annual variations in shellfish toxicity. Typically, inshore species like soft-shell clams and mussels are poison-free, or nearly so, during winter and spring, but their scores rise quickly to a peak in summer and then decline in autumn (Fig. 10). There is little consistency in annual variations.

### Bay of Fundy Region

In the Fundy region, scores generally follow the typical pattern with one major annual peak in July, August, or September (Fig. 10), although occasionally this peak may appear as early as June and as late as October. Peak scores for the same area may vary greatly from year to year even when the pattern of seasonal changes is typical. As a rule, the whole Bay of Fundy behaves as a unit with increases and decreases in most areas occurring at about the same time. The explanation of this condition probably is that the vigorous mixing by the extreme tides in the Bay prevents establishment and persistence of local hydrographic conditions that may be favourable or unfavourable for toxin accumulation in shellfish. Whatever the cause, there is a degree of seasonal regularity in toxicity changes in the entire Fundy region as illustrated by data for Head Harbour in Fig. 11, which summarizes 15 years' records.

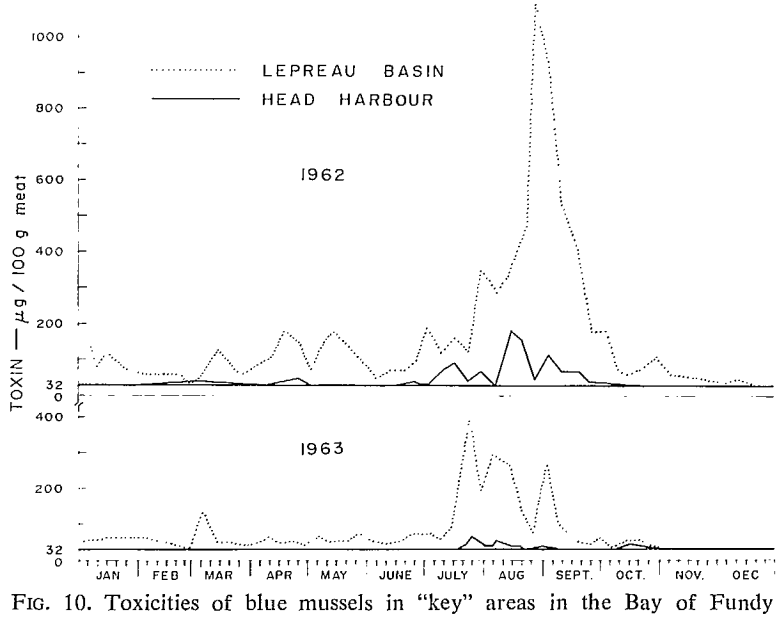


FIG. 10. Toxicities of blue mussels in "key" areas in the Bay of Fundy region 1962–63. In the key areas, shellfish begin accumulating poison about a week earlier than those in other areas and help in forecasting increases in toxicity above the danger level (80 µg) in the other areas.

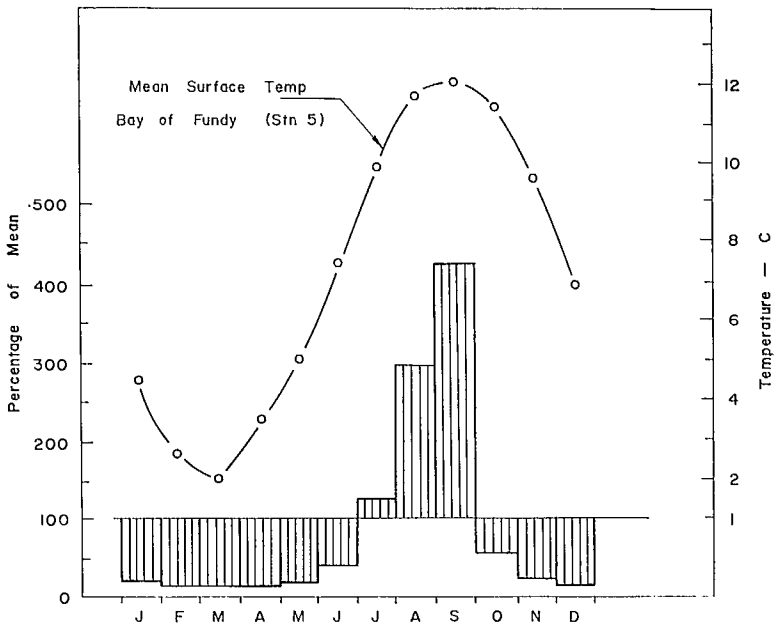


FIG. 11. Monthly toxicities of blue mussels at Head Harbour, N.B., as percentages of the mean (290 µg) for the years 1944–58 (from Prakash and Medcof 1962).

## ST. LAWRENCE REGION

In the St. Lawrence region the patterns of toxicity changes (Fig. 12) are not fixed. They may follow the typical pattern, with one sharp summer or autumn peak and very low values during the rest of the year, as seen in Baie des Homards, or the scores may range well above zero the year round with one or more peaks superimposed on the background score as in Baie-des-Capucins, or they may rise to a summer high and even continue to rise until late autumn as seen at Pointe au Boisvert. Because of ice conditions no scores are on record for late winter, but spring scores taken when ice clears are frequently high. For instance, mussels from Les Méchins, Que., that scored 1804 on November 10, 1969, still scored 1012 on March 23, 1970. It appears that low autumn and winter temperatures retarded poison losses and that the 1970 spring scores were carryovers from the late autumn toxicity maximum of 1969.

Compared with those in the Fundy region, toxicity changes in the St. Lawrence are less regular and less predictable; periods of high toxicity are more protracted; annual differences in peak scores are smaller and patterns of change in different areas within the region are less consistent. Regularity of seasonal changes in the Fundy region simplifies control programs, and irregularity of cycles in the St. Lawrence region makes them difficult. The contrast between the Fundy and St. Lawrence regions is heightened by the fact that average scores may

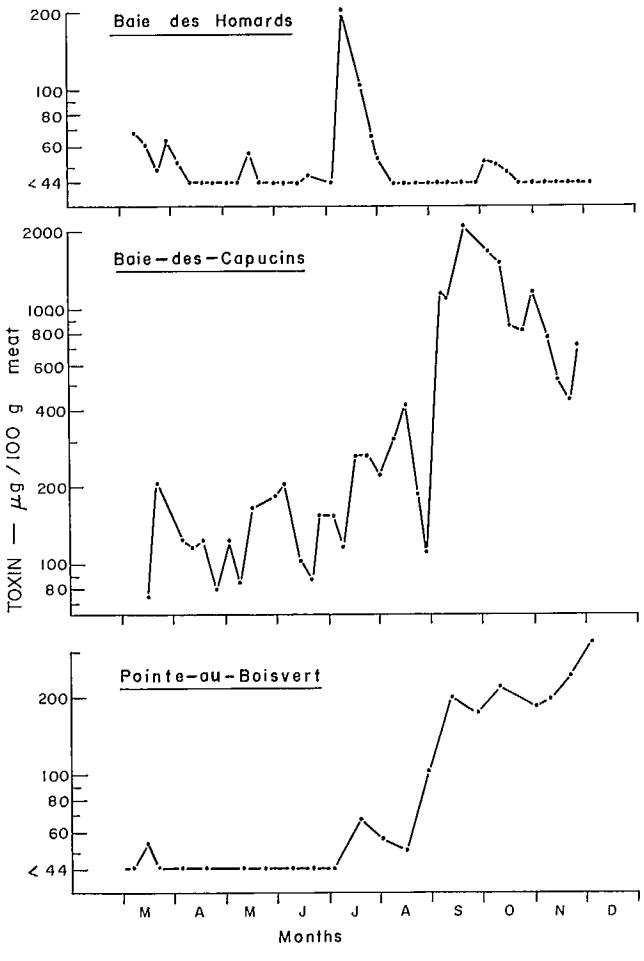


FIG. 12. Seasonal changes in toxicity of soft-shell clams in three neighbouring areas in the St. Lawrence region, 1966.

differ greatly in particular years. For example, 1966 and 1969 toxicity scores were high in the St. Lawrence but low in the Fundy region. The reverse was true in 1957.

## Causative Organism

The dinoflagellates from which shellfish acquire the poison are members of the group Pyrrhophyta, which includes mainly planktonic, single-celled, microscopic organisms. Dinoflagellates occur in both fresh and salt waters and are among the microscopic organisms comprising the phytoplankton. Dinoflagellates implicated in PSP are almost exclusively marine and most of them belong to the genus *Gonyaulax*.

The dinoflagellate from which shellfish acquire the poison in eastern Canada is *Gonyaulax tamarensis*. Conclusive evidence that this species is the causative organism was not obtained until 1961. *G. tamarensis* has now become a prime suspect in several outbreaks of PSP in Scotland (Gemmill and Manderson 1960), in Portugal (Silva 1964), in Norway (Oftebro and Bøhle 1965) and off the north-east coast of Britain (Wood 1968).

On the Atlantic coast of Canada, particularly in the Bay of Fundy, the accumulation of paralytic toxin in molluscan shellfish is a seasonal phenomenon. The simultaneous appearances of high toxicities in shellfish and large numbers of dinoflagellates in plankton during the summer months led Medcof et al. (1947) and Needler (1949) to the conclusion that *G. tamarensis* was the source of the shellfish poison in the Bay of Fundy. Studies carried out in 1961 showed that rises in toxicity of soft-shell clams and blue mussels coincided with increases of *G. tamarensis* in surface waters of the Bay of Fundy (Prakash 1963). *Gonyaulax tamarensis* appeared as an occasional plankter in June and became abundant in July and August, and during the same period, toxin was also detected in extracts of plankton (Table 9, see also Sullivan MS 1946). Similar trends in toxicity increase in shellfish and abundance of *G. tamarensis* in plankton were observed by Prakash along the Gaspé coast from Metis-Beach to Gaspé in 1962.

TABLE 9. Summer concentrations of toxin (μg/100 ml) in the extracts of plankton collected in the Bay of Fundy in 1961 (data from Prakash 1963).

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| May | 30 | <32 | July | 14 | 32 | Aug. | 14 | <32 |
| June | 13 | <32 | | 24 | 154 | | 21 | <32 |
| | 26 | <32[a] | | 31 | 2560 | Sept. | 7 | <32 |
| | 29 | <32 | Aug. | 10 | <53[a] | | 18 | <32 |

[a]Samples from Lepreau Basin; all others are from Head Harbour.

Although these observations provided important guidelines, proof of the source of toxin in shellfish was still lacking. In July 1961, *G. tamarensis* was isolated from the Bay of Fundy plankton and since then unialgal cultures of this species have been maintained in the laboratory in enriched seawater medium as well as in completely synthetic marine culture media. Of the various media tested, only two, Erd-Schreiber and $ASP_7$ (Appendix II), supported dense growths of *G. tamarensis* and these have been used for growing mass cultures for experimental demonstration of the toxin.

Live cells of *G. tamarensis* are spherical with dimensions ranging from 30 to 40 μ. They are yellowish green to yellowish brown. Older cells are usually larger than young ones and tend to be deeply pigmented. Each cell (Fig. 13, 14) has a well marked equatorial girdle and a smooth cellulose theca composed of several plates arranged in a definite pattern. The arrangement of these plates is distinctive of the species. The theca ruptures (Fig. 14B) when live cells are exposed to abrupt temperature changes or to strong chemicals like acids and formaldehyde.

The organism multiplies by asexual fission. In a healthy culture, "duplets" (Fig. 14A) and short chains of cells are common. Growth of cultures is affected by temperature, light intensity, and nutrient concentration and, under conditions in our laboratory, *G. tamarensis* attained densities as high as 2500/ml in 4 weeks from an initial inoculum of 1-3 cells/ml.

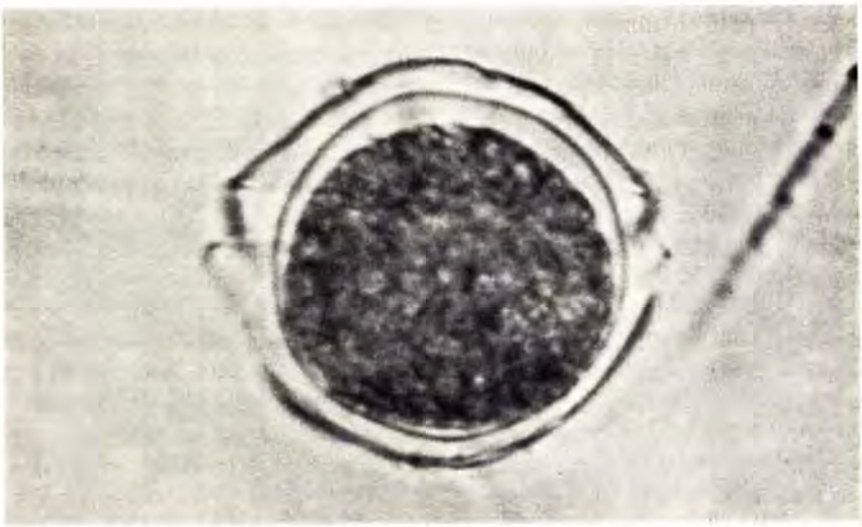

FIG. 13. The causative organism, *Gonyaulax tamarensis,* from the Bay of Fundy.

During the early phases of our work we found that unialgal cultures of *G. tamarensis*[2] isolated from the Tamar River estuary near Plymouth, Great Britain, were nontoxic to mice, whereas those from the Bay of Fundy were always highly toxic. This raised doubts as to whether they were the same species. However, in a comparative morphological study of typical specimens of the two forms, the Bay of Fundy form did not show sufficient morphological distinctions to qualify as a separate species or subspecies. We have therefore considered the English and Canadian forms of *G. tamarensis* as merely two different strains.

Toxicity of the Bay of Fundy strain of *G. tamarensis* was demonstrated by extracting toxin from mass cultures and testing it on mice. Additional evidence of its toxic nature was provided when nontoxic and mildly toxic clams were fed on mass cultures. Their scores rose, and the rise in toxicity was associated with the number of cells consumed (Table 10, Fig. 15).

## FACTORS AFFECTING ABUNDANCE OF GONYAULAX TAMARENSIS

Abundance and seasonal distribution of marine dinoflagellates are intimately realted to the temperature, salinity, light, nutrient, and current regimes in the sea.

[2]Kindly supplied by Dr L. Provasoli, Haskins Laboratories, New York, N.Y., U.S.A.

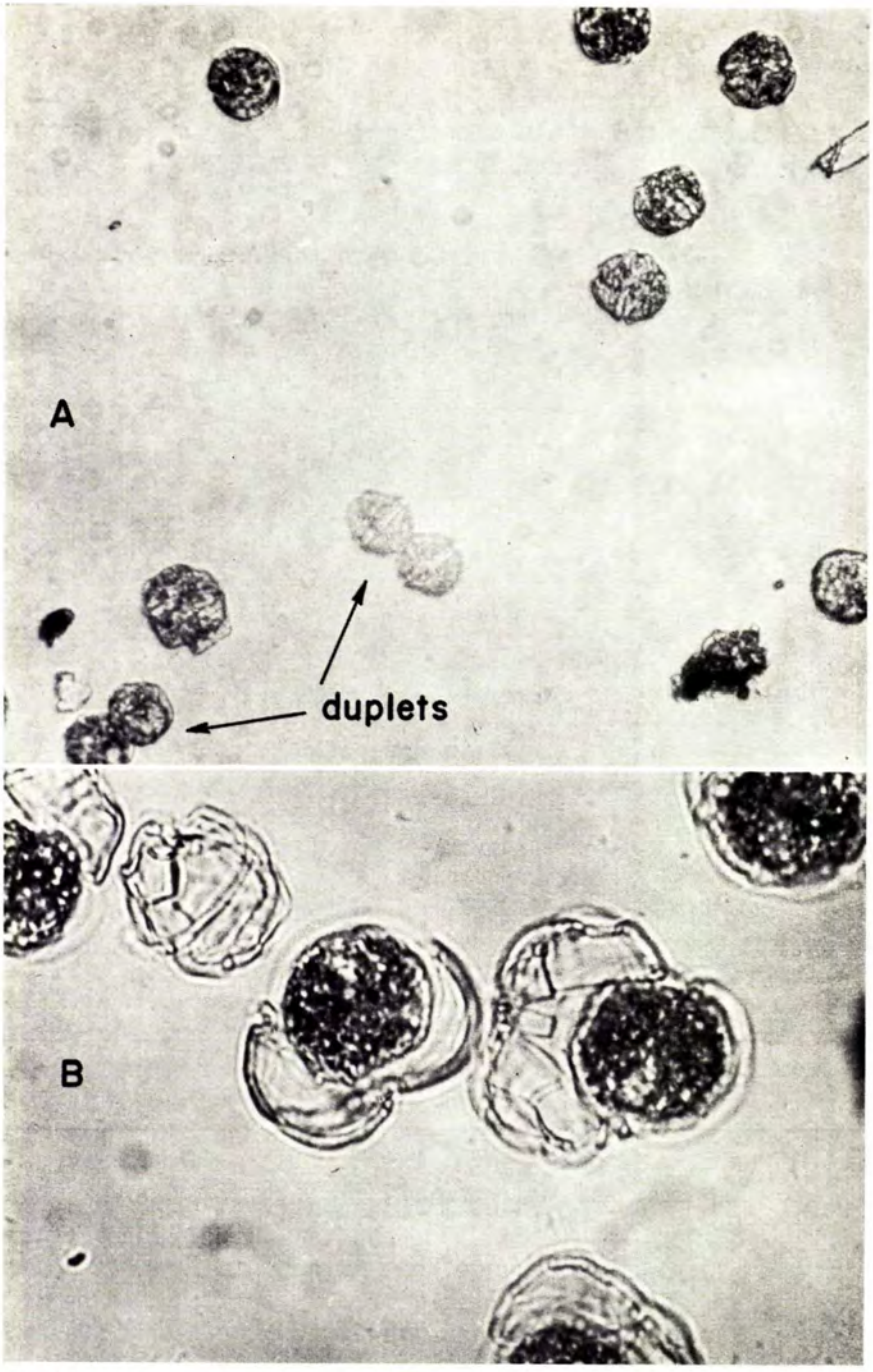


FIG. 14. Photomicrographs of Bay of Fundy isolates of *G. tamarensis*. (A) "Duplets" are common in growing cultures. (B) Ruptured cells showing two halves of the enclosing theca and its characteristic arrangement of plates.

High temperature, high light intensity, and a relatively stable water column are some of the factors that stimulate their growth. They can flourish in low concentrations of nitrogen and phosphorus as shown by the relatively large populations of dinoflagellates in temperate coastal waters during summer, when these nutrients are at their lowest level.

TABLE 10. Accumulation of toxin by soft-shell clams as a function of number of *Gonyaulax tamarensis* consumed (data from Prakash 1963).

| Group[a] | Vol culture fed (*ml*) | Estimated no. cells eaten | Toxin level (*μg/100 g*) |
|---|---|---|---|
| A | 0 | 0 | 176 |
| B | 1200 | $2.28\times10^7$ | 200 |
| C | 1400 | $2.66\times10^7$ | 192 |
| D | 2000 | $3.80\times10^7$ | 240 |

[a]Each group represents 25 clams of nearly uniform size dug from the same bed.

Occasionally, certain dinoflagellates produce "blooms" that impart a reddish or yellowish colour to the sea water. This phenomenon, commonly known as "red tide," is spectacular in the sea, but the causal factors and exact mechanism of its development still remain very much an enigma. A red water bloom of dinoflagellates is not a necessary prelude to a PSP outbreak, although a few occurrences of shellfish toxicity have coincided with sighting of discoloured water in the general area (Silva 1964; Prakash and Taylor 1966; Adams et al. 1968). No red water blooms have ever been reported from the Bay of Fundy or the estuary of the St. Lawrence. Likewise, no spectacular phosphorescence that could be linked with the onset of shellfish toxicity has been observed in either region.

Seasonal maxima in abundance of *G. tamarensis* is often associated with low salinities and high water temperatures. In 1962, e.g., it appeared in Bay of Fundy plankton in June, when salinities were low, and reached peak abundance during August, when water temperatures were high (Table 11, 12). Towards the end of September, it decreased markedly in numbers, and still later in the season it disappeared entirely from the plankton.

Of the various factors that influence seasonal and annual abundance, temperature has received most attention. Needler (1949) concluded that it was the principal factor in the Bay of Fundy. Temperature certainly affects abundance of *G. tamarensis*, both in nature and in the laboratory, but there is strong evidence that it is not the principal factor. For example, in the Bay of Fundy, abundance is low in September, when water temperatures are highest (Table 11). Prakash and Medcof (1962) found that shellfish toxicities are more closely associated with hours of sunshine than with water temperatures.

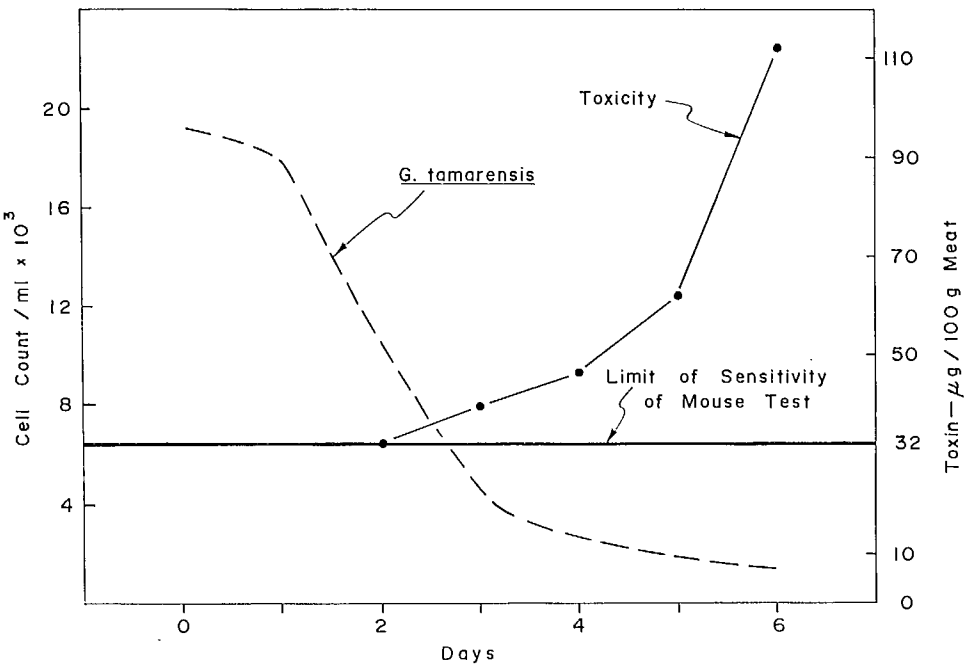


FIG. 15. Decreases in cell density of *G. tamarensis and* increases in toxicity of soft-shell clams during a six-day feeding experiment (from Prakash 1963).

TABLE 11. Mean monthly temperatures, salinities, and abundance of *Gonyaulax tamarensis* in surface waters in the Bay of Fundy (data from Prakash 1967).

| Month | Temp (C) | Salinity (‰) | Abundance of *Gonyaulax*[a] (*cell count/liter*) |
|---|---|---|---|
| Jan. | 4.3 | 32.18 | – |
| Feb. | 2.4 | 32.06 | – |
| Mar. | 1.9 | 31.85 | – |
| Apr. | 3.0 | 31.22 | – |
| May | 4.9 | 30.01 | – |
| June | 7.1 | 30.99 | 90 |
| July | 9.6 | 31.55 | 6300 |
| Aug. | 11.3 | 31.87 | 19000 |
| Sept. | 11.8 | 32.18 | 4000 |
| Oct. | 10.9 | 32.40 | – |
| Nov. | 9.2 | 32.35 | – |
| Dec. | 6.9 | 32.20 | – |

[a]Based on maximum counts at Head Harbour, N.B. in 1962.

Salinity may also be more important than temperature (Prakash 1967). *Gonyaulax tamarensis* cultures grow best at salinities between 15 and 23‰ and considerably less rapidly at higher salinities (Fig. 16). Salinities higher than 32‰ prevail in the Bay in fall and winter months, when this organism is rare in the plankton (Table 11).

TABLE 12. Seasonal abundance of *Gonyaulax tamarensis* in "key" areas of the Bay of Fundy in 1962 (data from Prakash MS 1967).

| Head Harbour | | | Lepreau Basin | | |
|---|---|---|---|---|---|
| Date | Temp (C) | Cell count/liter | Date | Temp (C) | Cell count/liter |
| June 15 | 8.9 | – | June 11 | 8.8 | – |
| 22 | 8.4 | – | 26 | 9.4 | – |
| 29 | 9.0 | <100 | July 9 | 9.8 | – |
| July 6 | 8.9 | 1400 | 17 | 10.5 | <200 |
| 12 | 9.5 | 3600[a] | 25 | 10.3 | 1100 |
| 17 | 9.4 | 3800 | Aug. 1 | 11.2 | 2800 |
| 20 | 9.0 | 3300 | 9 | 10.5 | 1200 |
| 23 | 10.0 | 6300[a] | 16 | 13.0 | 5400[a] |
| 27 | 10.3 | 5600[a] | 23 | 12.8 | 18000[a] |
| Aug. 3 | 10.5 | 1600 | 30 | 13.1 | 12000[a] |
| 15 | 10.8 | 13500[a] | Sept. 6 | 12.4 | 5400 |
| 20 | 11.5 | 4400 | 14 | 11.8 | 4200 |
| 24 | 11.6 | 19100[a] | 20 | 11.2 | 3000 |
| 31 | 11.2 | 2000 | 26 | 11.8 | 2000 |
| Sept. 10 | 11.4 | 4000 | Oct. 5 | 11.5 | – |
| 14 | 11.0 | 3000 | 11 | 10.7 | – |
| 21 | 10.8 | <100 | 18 | 10.5 | – |
| 28 | 10.5 | – | 25 | 9.9 | – |
| Oct. 5 | 10.5 | – | 30 | 9.5 | – |
| 19 | 10.3 | – | Nov. 2 | 8.8 | – |
| 30 | 9.5 | – | 9 | 8.6 | – |
| Nov. 2 | 8.8 | – | | | |

[a]Toxin detectable in plankton extracts.

It appears that *G. tamarensis* encysts during fall and winter months and, because of loss of buoyancy, the encysted cells sink to the bottom and provide a seed population for the next summer. Temperature appears to be the most important of the various factors that induce cyst formation. *Gonyaulax tamarensis* retains its toxicity while encysted, and possibly high toxicity levels encountered in winter in sea scallops may in part be due to their feeding on such cysts on the sea bottom (Bourne 1965).

*Gonyaulax tamarensis* does not require high concentrations of inorganic nutrients like nitrogen and phosphorus, but it does require organic growth-promoting factors like vitamins. Laboratory experiments with unialgal cultures have shown that vitamin $B_{12}$, thiamine, and biotin are essential for the growth of this dinoflagellate (Prakash 1967) and these organic factors are likely adequately available in surface waters of the Bay of Fundy and the St. Lawrence estuary during summer months.

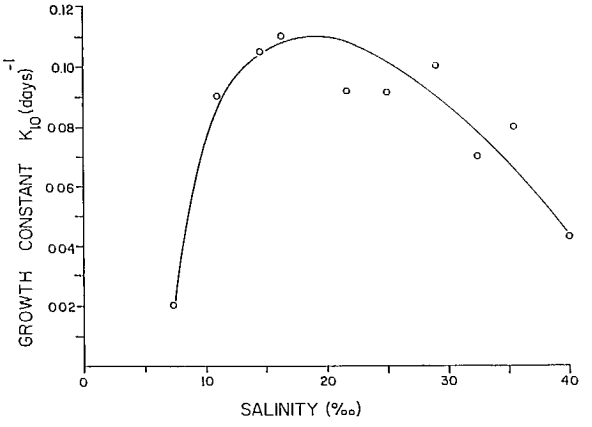


FIG. 16. Influence of salinity on growth rate of *G. tamarensis* (from Prakash 1967).

Laboratory evidence (Prakash and Rashid 1968) indicates that small amounts of humic and fulvic acids act as growth stimulants for *G. tamarensis*. This stimulation is independent of nutrient concentration and appears to occur in nature. Substantial amounts of humic matter enter the Bay of Fundy in runoff from land every spring and may play an important role in influencing seasonal abundance of *G. tamarensis*.

This evidence that seasonal abundance of *G. tamarensis* is dependent on low salinity and organic enrichment of surface waters is supported by the fact that heavy growths of *G. tamarensis* in the inner Oslo Fjord in Norway are linked with organic enrichment from sewage (Oftebro 1965). Recent study on seasonal abundance of *G. tamarensis* in Trondheimsfjord, Norway provides further evidence of good growth conditions created by heavy input of humusladen river water in the fjord (Sakshaug et al. MS 1971).

A factor limiting summer abundance of *G. tamarensis* is grazing by zooplankters. Needler (1949) observed the simultaneous appearance of *G. tamarensis* and a large ciliate (tintinnid) that she identified as *Favella ehrenbergii* in Bay of Fundy plankton. She concluded that *F. ehrenbergii* was the principal predator and controlled the abundance of the dinoflagellate. Prakash (1963) observed the same phenomenon (Table 13), but was unable to find any ciliate recognizable as *F. ehrenbergii*. The most common ciliate associated with *G. tamarensis* in the Bay of Fundy was a species of *Favella* not identical with but similar to *Favella panamensis*. On several occasions this species was found gorged with *G. tamarensis*

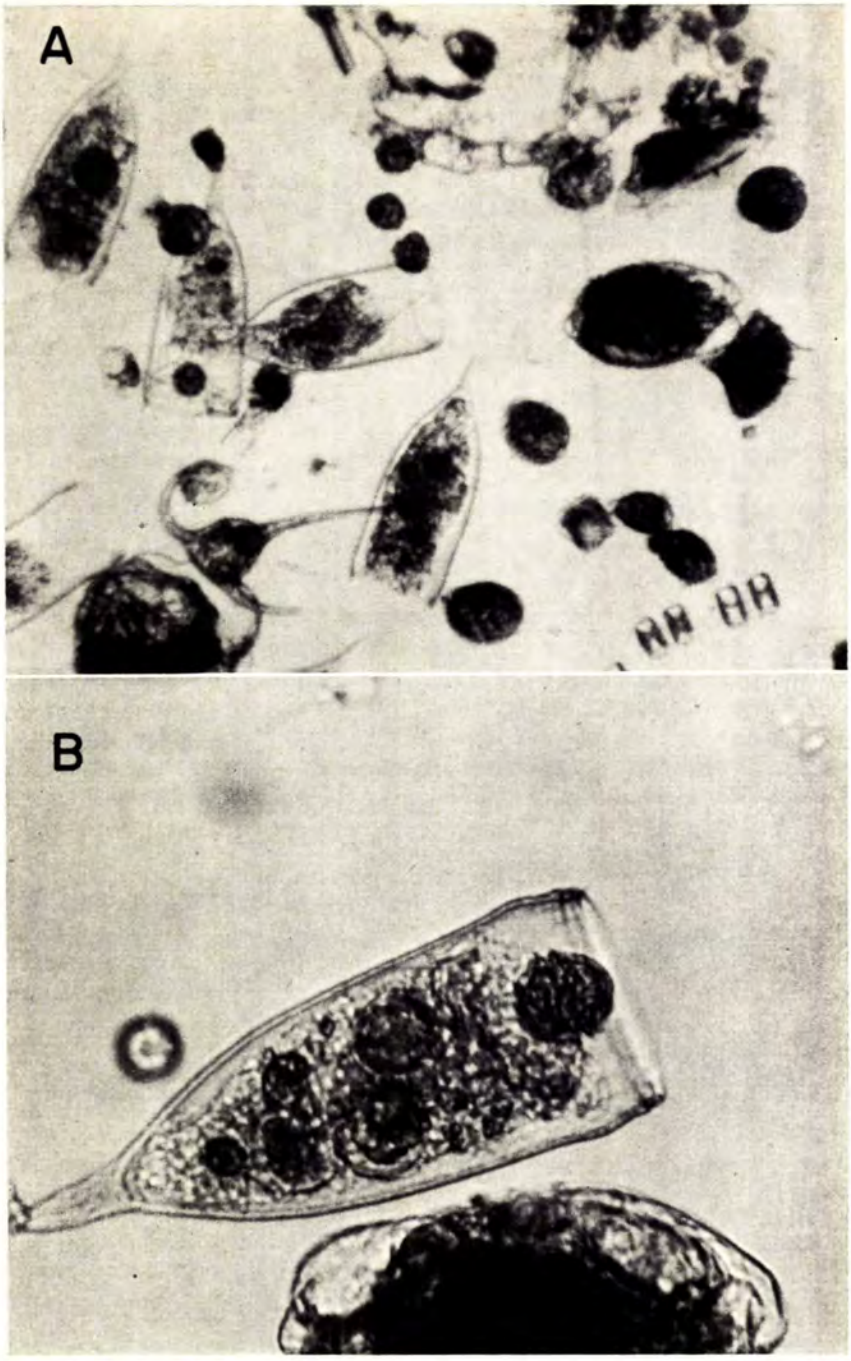


FIG. 17. (A) Typical summer plankters in the Bay of Fundy. The urn-shaped organisms are ciliates (tintinnids) and the spherical ones *G. tamarensis.* (B) A tintinnid (*Favella* sp.) preying on *G. tamarensis.*

TABLE 13. Seasonal abundance of dinoflagellates and tintinnids in Bay of Fundy plankton (+, occasional; ++, common; +++, numerous) (data from Prakash 1963).

| | Dinoflagellates | | | | | *Favella* (tintinnid) |
|---|---|---|---|---|---|---|
| | *Ceratium* | *Peridinium* | *Exuviaella* | *Dinophysis* | *Gonyaulax* | |
| Jan. | +++ | – | – | + | – | – |
| Feb. | | | (No observations) | | | |
| Mar. | + | – | – | + | – | – |
| Apr. | – | – | – | + | – | – |
| May | + | + | – | + | – | – |
| June | – | ++ | – | – | + | + |
| July | ++ | ++ | – | + | +++ | ++ |
| Aug. | + | +++ | + | – | + | ++ |
| Sept. | + | + | + | + | ++ | +++ |
| Oct. | ++ | + | + | – | – | + |
| Nov. | ++ | + | – | – | – | + |
| Dec. | + | + | – | – | – | + |

in different stages of digestion (Fig. 17). Observations on the feeding behaviour of this ciliate indicated that it feeds selectively on and limits the late summer populations of *G. tamarensis* (A. Prakash, unpublished data).

## Poison Accumulation and Elimination

### ACCUMULATION BY FILTER FEEDERS

Conceivably any kind of animal that feeds on *Gonyaulax tamarensis* could accumulate poison. However, the animals that are most famous for poison storage are filter-feeding molluscs. Bivalves (clams and mussels) accumulate most of their toxin during summer (Fig. 11). This is explained by their efficient removal of *G. tamarensis* from the water they filter (Needler 1949; Prakash 1963, MS 1967). Their rate of toxin accumulation apparently depends partly on the concentration of *G. tamarensis* and partly on their efficiency in filtering. Marked differences are often observed among the various species of shellfish, not only in the rates of toxin accumulation but also in the rates of toxin loss.

Blue mussels and red mussels in the Bay of Fundy seem to accumulate the poison more quickly than other shellfish. During summer, it is not unusual for mussel scores to increase by 1000 every day for several successive days. This rapid uptake appears to be largely due to their almost continuous and selective feeding on *G. tamarensis*. Blue mussels in their natural surroundings are reported to feed 97–99% of the time at suitable temperatures and their filter feeding is apparently not affected by tidal cycles (Loosanoff 1942; Theede 1963). They apparently feed selectively on dinoflagellates in the same manner as California mussels, which show this preference even when the water contains only about 2% dinoflagellates and 98% diatoms (Buley 1936).

Scores of soft-shell clams change less rapidly but they behave in much the same way as mussel scores. In badly affected areas of both the St. Lawrence and the Fundy regions, clam scores may rise from less than 100 to 2000 or more and fall again to less than 100 within 6 weeks. Bar clams of the Bay of Fundy region are highly toxic and may remain toxic the year round in affected areas. The toxicity of bar clams may be 5–12 times as high as that of soft-shell clams, as indicated by the following scores recorded from Lepreau Basin, N.B., in August and September 1961:

| | Aug. | | | | | Sept. | |
|---|---|---|---|---|---|---|---|
| | 25 | 28 | 29 | 30 | 31 | 6 | 7 |
| Soft-shell clams | 464 | 336 | 320 | 384 | 400 | 304 | 416 |
| Bar clams | 2400 | 1696 | 2720 | 2240 | 2720 | 3740 | 3040 |

Records for ocean clam samplings in 1969 and 1970 from the Bay of Fundy (Medcof MS 1970; MS 1971) suggest that this species is not an efficient accumulator. Sea scallops in the Bay of Fundy behave very much like bar clams, i.e., they accumulate poison quickly and may remain toxic throughout the year. Uptake of toxin by the Atlantic oyster is very limited and apparently this species avoids taking in dinoflagellates (Loosanoff 1949).

Information on toxin accumulation in different species has been obtained by transplanting nontoxic or mildly toxic shellfish to affected areas and following their trends in toxin uptake. Medcof (MS 1949) compared winter scores of toxic native soft-shell clams from Lepreau Basin with those of poison-free clams from another area transplanted to the same bed. The transplanted stock remained poison-free, whereas the native stock remained toxic throughout the winter. He concluded that the poison present in the native population was carried over from the previous summer and that there was no poison-bearing organism in winter plankton to permit poison accumulation by the transplanted clams.

The 1959 Bond-Medcof tray experiments (Table 14; Bond and Lachance MS 1959) showed that within 12 days scores of blue mussels increased fourfold, whereas scores of European oysters held in the same trays showed no increase and those of quahaugs and soft-shell clams showed only modest increases.

TABLE 14. Toxicities (μg/100 g) of various species of shellfish held in trays at Head Harbour, N.B. (data from Bond and Lachance MS 1959).

| | European oyster | Bay quahaug | Soft-shell clam | Blue mussel |
|---|---|---|---|---|
| July 15 (as fished) | 33 | <32 | 96 | 288 |
| July 20 | 32 | 67 | 91 | 512 |
| July 27 | 32 | 272 | 304 | 1168 |

To study poison uptake in relation to abundance of *G. tamarensis*, stocks of nontoxic soft-shell clams and blue mussels were held from May to December 1962 at Head Harbour and Lepreau Basin, New Brunswick. The shellfish were placed in cages anchored to the bottom so that the Head Harbour cages were constantly submerged and those at Lepreau Basin were submerged except at low tide (Prakash MS 1967). Weekly samples of shellfish were taken from each cage and from native stocks on adjacent beaches, and weekly plankton samples were taken above the cages to monitor abundance of *G. tamarensis*.

In both natural beds and the cages (Fig. 18), mussels accumulated more toxin than clams. This difference between mussels and clams appears to be due partly to differences in filter-feeding efficiency. Jorgensen (1966) found that mussels filter at higher rates than clams and it is reasonable to assume that in the transplanting experiments our mussels filtered larger numbers of *G. tamarensis* than our clams.

The blue mussels that were constantly submerged at Head Harbour accumulated more poison than those that were intermittently submerged at Lepreau Basin (Fig. 18). Their greater accumulation of toxin is attributed to their uninterrupted feeding on *G. tamarensis*. This also explains why mussels from intertidal ledges have lower scores than those from mooring poles (Medcof MS 1952) and why shellfish taken near low-water mark are more toxic than those taken near high-water mark. Duration of submergence and of feeding therefore, is one of the main factors affecting accumulation of toxin in shellfish.

## Rate of Toxin Accumulation in Relation to Abundance of G. tamarensis

There is a direct relation between seasonal fluctuations in shellfish toxicity and the abundance of *G. Tamarensis* (Needler 1949; Neal MS 1967; Sakshaug et al. MS 1971). The 1962 transplanting experiments at Head Harbour and Lepreau Basin (Fig. 19) showed this. The soft-shell clams increased in toxicity rapidly after July. At Lepreau Basin, their scores rose from 152 on August 23 to 464 on August 27 — an increase of 78 µg per day. From these figures it was calculated that each clam increased in toxicity by approximately 3 µg/day. Experiments with cultures of *G. tamarensis* indicate that approximately 5000 cells are required to produce 0.16 µg toxin (Prakash 1967). This would mean that each of the Lepreau Basin clams consumed not less than 100,000 cells/day. Judging from the density of *G. tamarensis* during the experiment (Table 12), and assuming 100% efficiency in filter feeding, each clam must have filtered at least 5 liters of sea water per day. This is well within the known limits since soft-shell clams can filter as much as 3 liters/hr (Sullivan MS 1946).

Although scores of Head Harbour clams followed the peaks in abundance of *G. tamarensis* (Fig. 19), the rate of increase in the poison content of individual clams was of the order of only 1 µg/day. The reason for the difference between the rates in the two areas is obscure. A possible explanation is that water temperatures were higher at Lepreau Basin (Table 12) and favoured higher filtering rates.

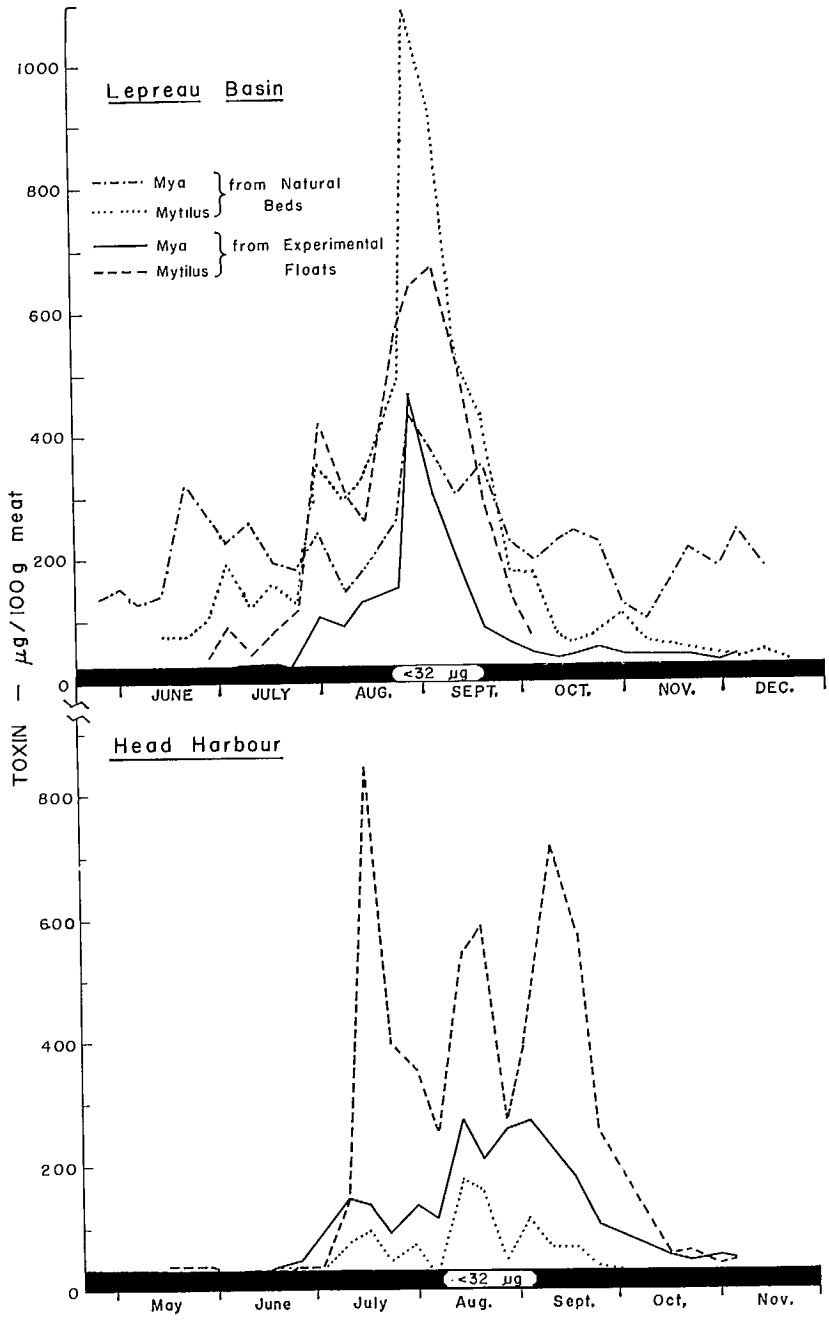


FIG. 18. Seasonal toxicities of shellfish from natural beds at Head Harbour and Lepreau Basin and of nontoxic shellfish transplanted from other areas and held in cages in 1962. *Mya,* soft-shell clam: *Mytilus,* blue mussel.

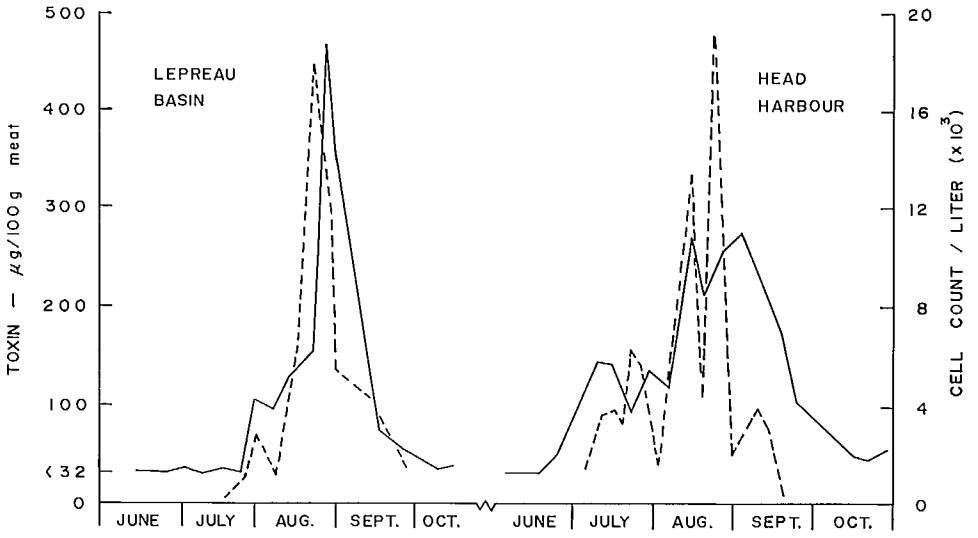


FIG. 19. Seasonal abundance of *G. tamarensis* (broken line) and toxicities of transplanted soft-shell clams (solid line) at Head Harbour and Lepreau Basin 1962.

These results indicate that (1) accumulation of toxin by shellfish results from feeding on *G. tamarensis,* (2) changes in shellfish toxicity are closely associated with changes in abundance of *G. tamarensis,* (3) some species of shellfish accumulate toxin faster than others because they filter more water, or feed more selectively on *G. tamarensis,* (4) rate of toxin accumulation depends also on feeding time (submergence), and (5) any environmental factor such as temperature or salinity that affects pumping rates of shellfish must also affect rates of feeding and toxin accumulation.

## LOSS OF TOXIN

The rate of decrease in shellfish toxicity varies with species and also with season. Since the accumulation of toxin in shellfish is largely dependent on the abundance of *G. tamarensis* (Fig. 19), estimates of the rates of toxin loss would be more meaningful if toxicity assays are conducted when the causative organism is absent in plankton. However, this is not possible for all shellfish species. Apparently some species like Atlantic oysters and ocean clams can eliminate all their poison as soon as *G. tamarensis* disappears from the plankton. Others seem to be less efficient in eliminating their poison and remain toxic for long periods after *G. tamarensis* disappears. Because of this, field observations give only approximate rates of toxin loss.

Blue mussels, ocean clams, and red mussels eliminate the poison quickly, but Atlantic oysters and bay quahaugs are slower in eliminating their poison.

Medcof (MS 1958; MS 1971) reported on toxin loss in these species of shellfish held in cages at Head Harbour, New Brunswick, in 1957 and 1970. The toxicities (μg/100 g) of the shellfish decreased as follows:

| | 1957 | | | | 1970 | | | |
|---|---|---|---|---|---|---|---|---|
| | July | | | Toxin loss | Aug. | | | Toxin loss |
| | 4 | 11 | 18 | per day | 5 | 10 | 17 | per day |
| Blue mussel | 768 | 256 | 112 | 47 | 530 | 220 | 97 | 36 |
| Red mussel | – | – | – | – | 640 | 640 | 280 | 30 |
| Ocean clam | – | – | – | – | 530 | 230 | 79 | 38 |
| Bay quahaug | <32 | 93 | 69 | 4 | – | – | – | – |
| Atlantic oyster | <32 | 46 | 32 | 2 | – | – | – | – |

Elimination of toxin appears to be slowest in the Bay of Fundy bar clams (Table 7) and sea scallops (Bourne 1965). These shellfish accumulate poison quickly and eliminate it so slowly that they often remain toxic the year round, like some British Columbia butter clams as reported by Quayle (1969). Soft-shell clams are somewhere between mussels and bar clams in their efficiency in poison elimination.

Low water tempreatures apparently retard poison release. Blue mussels at Les Méchins, Que. scored 1804 on November 10, 1969 (Boyd and Lachance MS 1970), and still scored 1012 on March 23, 1970 (Boyd and Turgeon MS 1971). This corresponds to an average daily increase in score of 6 μg. In contrast, scores for mussels from the same sampling station decreased from 21,700 on September 9, 1969, to 2700 on October 14, 1969 — a daily decrease of 550 μg. It is unlikely that these mussels maintained their high scores from November 1969 to March 1970 by feeding on *G. tamarensis* in the water under the ice. Presumably, low winter water temperatures reduced their rate of poison excretion to about 1% of that at high, late-summer water temperatures.

## Accumulation by Nonfilter Feeders

Several cases of human poisonings have been attributed to consumption of whelks (Table 6). It is unlikely that whelks and other species of carnivorous snails accumulate toxin in the same way as mussels and clams, i.e., by direct feeding on toxic dinoflagellates. Whelks are not filter feeders but feed on other molluscs and accumulate toxin "third hand" by feeding on toxic bivalves. Experiments on rough whelks reported by Caddy and Chandler (1968) and Ingham et al. (1968) confirmed this.

The ten-banded whelk tests poison-free in unaffected areas, but samples taken from the Bay of Fundy scallop beds in September 1948 had a score of 1060 when steamed (equivalent score when raw 3000–4000). In April 1952, when toxicities of other shellfish were low throughout the Fundy region, these whelks still had a score of 106 when raw. Samples of the spindle-shell snail taken at the same time and place had scores of 87 when raw (Medcof MS 1949, MS 1953). We believe that these snails accumulate toxin by feeding on prey that are toxic the year-round, e.g., scallops and bar clams.

Besides accumulating poison from their food, some snails synthesize toxins and store them in well-developed poison glands. The effects of these toxins on mice and humans are similar to those of paralytic shellfish poison. Fänge 1957; Asano and Itoh 1960; Whittaker 1960). The greater clam drill, another carnivorous snail, is always toxic (Medcof MS 1952; Goggins 1961), even when it is taken from areas where bivalve prey are never toxic. Greater clam drills also likely synthesize a poison similar to shellfish toxin. If these snails were to feed on toxic bivalves, they could probably transmit shellfish toxin as well as their natural toxin to consumers.

## The Toxin

Paralytic shellfish toxins acquired from marine dinoflagellates are among the most potent nonprotein poisons known. The toxin from *G. tamarensis* has not been studied in great detail or isolated in pure form, and its nature can only be inferred from what is known about those that have been studied in this way, mainly *G. catenella.*

### Characterization

The lethal dose of *G. catenella* toxin for man has been estimated from information on human fatalities resulting from consumption of toxic shellfish and on the relation of lethal dose to weight in experimental animals. As little as 200 μg of poison is considered a lethal oral dose (E. J. Schantz 1970) and 400 μg a lethal intravenous dose (Kao and Nishiyama 1965) for human beings.

Although procedures for isolating shellfish toxins were developed by 1885, most of our knowledge of their chemistry and pharmacology has been acquired during the last 30 years (McFarren et al. 1960; Courville 1965). Much of the work on isolating, purifying, and characterizing the *G. catenella* toxin was done by Dr E. J. Schantz and his associates at the U.S. Army Biological Laboratories, Fort Detrick, Maryland. Their early work on crude extracts of Alaska butter clams and California mussels showed striking similarities between these two poisons (Mold et al. 1957; Schantz et al. 1957).

Recently, investigators have studied toxin obtained directly from dinoflagellate cultures, especially of *G. catenella*. Burke et al. (1960) demonstrated similarities in physical and chemical properties between toxins from California mussels and those from cultures of *G. catenella*. They concluded that *G. catenella* was the source of poison in California mussels. Schantz et al. (1966) isolated the toxin in pure form from bacteria-free cultures of *G. catenella* and showed that it was identical in structure with toxins from Alaska butter clams and California mussels. They concluded that *G. catenella* toxin undergoes no chemical change while it is stored in the digestive glands or in the siphon of these shellfish.

These studies show conclusively that shellfish toxins originate from dinoflagellates and that the toxins referred to as "mytilotoxin" from mussels and "saxitoxin" from butter clams are structurally similar.

## Physical and Chemical Properties

Toxin from *G. catenella* is a dibasic nitrogenous substance. Its dihydrochloride salt, in purified form, is a white hygroscopic solid that is highly soluble in water and to a lesser extent in lower alcohols but insoluble in lipid solvents. The toxin is stable in acid solutions but decomposes in basic solutions. It has no ultraviolet absorption, but its infrared spectra show strong absorption of 3, 6, and 9μ. Its empirical formula, $C_{10}H_{17}N_7O_4 \cdot 2HCl$, and molecular weight at 372 appear to have been well established (Schantz 1960)[3]. The toxin in its dihydrochloride form can be completely detoxified by catalytic reduction in the presence of hydrogen to the dihydro compound $C_{10}H_{19}N_7O_4 \cdot 2HCl$. One mole of hydrogen per mole of toxin is taken up at atmospheric pressure and the decrease in toxicity is proportional to the degree of hydrogenation. Chin (1970) neutralized the poison by using strong oxidizing agents like sodium hypochlorite at a concentration of 3 ppm/μg poison. In a highly purified state, the toxin has a concentration of over 880 μg/mg of the dihydrochloride salt.

## Pharmacological Properties

Studies of crude toxin extracts and of purified toxin show that *G. catenella* poison is a neuromuscular toxin. It blocks both peripheral nerve and reflex transmission and affects respiratory and vasomotor centers of the central nervous system, leading to respiratory depression and eventually death Kellaway (1935).

[3]A recent study, however, suggests that the compound may be $C_{10}H_{15}N_7O_4$ (unpublished data by H. Rapoport, M. S. Brown, R. Oesterlin, and W. Schuett. Quoted by H. S. Mosher 1966. Non-protein neurotoxins. Science 151: 860–861).

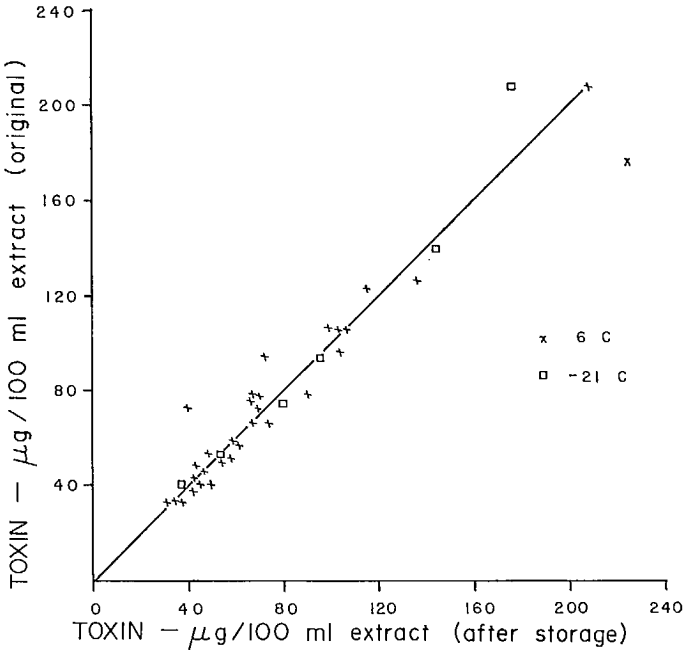


FIG. 20. Toxicities of extracts of *G. tamarensis* cultures before and after storage at two temperatures for 1 year (from Prakash 1967).

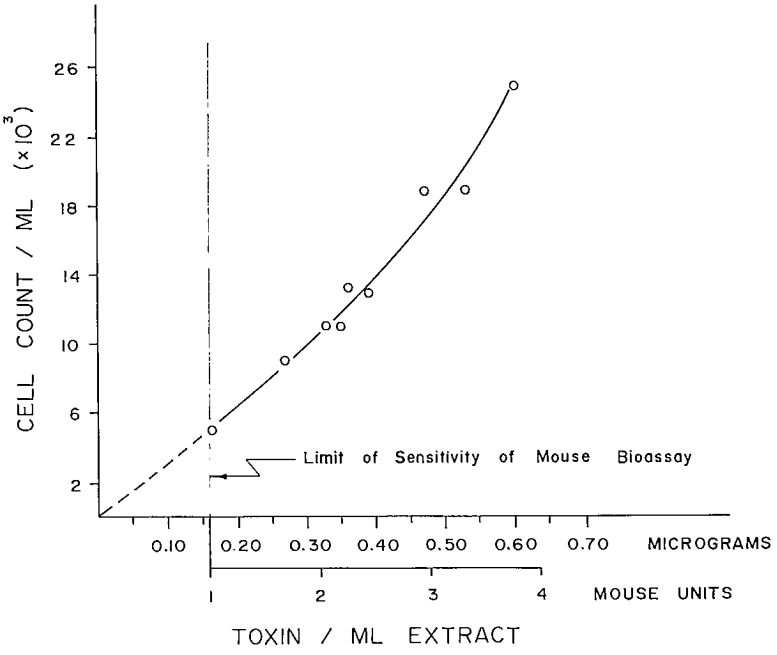


FIG. 21. Relation between toxin yield and number of *G. tamarensis* cells (from Prakash 1967).

The poison produces a direct depression of the myocardium, which in extreme cases leads to cardiovascular collapse (Murtha 1960).

Early studies suggested that physiological responses to the toxin are like those to the paralytic poison, curare, but recent work shows that this is not true. The toxin blocks neuromuscular transmission in motor axons and acts on the muscle membrane, rather than on the end plate, without depolarization (Evans 1964). The action is similar to that of puffer fish poison, since it does not affect potassium and chloride conductances of the excitable membrane and does not interfere with the increase in potassium conductance that usually follows depolarization (Kao and Nishiyama 1965). The neuromuscular transmission block is attributed to some specific interference with the increase in sodium permeability normally associated with excitation. Evans (1965) has shown that the toxin reduces the permeability of the muscle membrane to sodium ions, thereby preventing buildup of muscle action potentials and thus producing paralysis. Other aspects of pharmacological effects of shellfish toxin are reviewed by Halstead (1965) and Kao (1966).

## Toxin from G. tamarensis Cultures

Studies of the purified poison from *G. tamarensis* indicate that it is similar to *G. catenella* toxin in its biological action, but differs slightly in its chemical and physical properties (E. J. Schantz 1970). The differences may be attributable to impurities in the extracts that are known to interfere with the ion exchange columns. *Gonyaulax tamarensis* toxin appears to be similar, if not identical in chemical structure to *G. catenella* toxin, but more potent. Evans (1970) suggested that *G. tamarensis* toxin is composed of a minor fraction resembling *G. catenella* toxin and a major fraction resembling puffer fish poison in its biological effects. The toxin from *G. tamarensis* cultures resembles *G. catenella* toxin in being stable at low *p*H and in producing identical neurotoxic symptoms in mice. Acid extracts of *G. tamarensis* toxin are stable for long periods, and storage even for a year does not alter their potency (Fig. 20).

Toxin production by cultures of *G. tamarensis* depends on the number of cells per unit of volume (Prakash 1967). Approximately 4500–5000 cells yield 0.16 μg of toxin (Fig. 21). This suggests that the toxin producing potential of *G. tamarensis* is one of the highest of the known toxic species of *Gonyaulax*.

In young cultures of *G. tamarensis* the poison remains within the cells. However, as cultures age there is a progressive release of toxin from the cells into the surrounding medium. This is attributed to death and break up of cells (Prakash 1967).

There is no apparent difference in toxin yields between bacterized and bacteria-free cultures, and there is no reason to believe that the toxin results from interactions between bacteria and *G. tamarensis*.

# Measuring Shellfish Toxicity

## Development of Standard Procedure

There are chemical and serological tests for the toxin but these are complex and have not gained wide acceptance. The bioassay (mouse test) remains the widely preferred procedure for detecting and measuring the poison in shellfish.

The mouse test was first used by Sommer and Meyer (1937), with alcoholic extraction procedures. Later they found that the poison is stable in acid solution, and developed a quicker and simpler test using acid aqueous extraction. This was the first practical method of quantitative bioassay procedure for the toxin. They found that the optimum *p*H range of extracts for the bioassay was between 3 and 4 and they defined the mouse unit as the minimum amount of poison that is required to kill a 20-g mouse in 15 min when 1.0 ml of acid–aqueous shellfish extract is injected intraperitoneally. The time to death is defined as the time from injection to the last gasping breath of the mouse, and is measured to the nearest 5 sec. Larger doses kill mice more quickly, and a curve for the relation between time to death and mouse units may be constructed from the following data. Times of 4, 5, 6, 7, and 8 min are equivalent to 2.5, 1.9, 1.6, 1.4, and 1.3 mouse units, respectively. When the logarithm of the dose is plotted against the reciprocal of the time, a straight line is obtained. When death occurs between 240 and 480 sec, the dose may be calculated directly from the equation $\log \text{dose} = (145/t) - 0.2$, where $t$ is time to death in seconds. Sommer and Meyer reported the toxicity of shellfish samples in terms of the number of mouse units in 100 g of shellfish flesh.

Improvements in the Sommer–Meyer test were made by Mr J. Gibbard and his colleagues at the Laboratory of Hygiene, Department of National Health and Welfare, Ottawa (NHW), in collaboration with Dr J. P. Tully and others of the Fisheries Research Board of Canada (Medcof et al. 1947; Gibbard and Naubert 1948). Their acid–aqueous extraction procedure gives toxicity estimates, in mouse units per 100 g of shellfish meat, which are directly similar to those obtained by the Sommer–Meyer alcoholic extraction procedure, and forms the basis of the bioassay method in use today.

Stephenson et al. (1955) and Wiberg and Stephenson (1960) indicated how test conditions affect bioassay results. They demonstrated a highly significant inverse relation between the toxicity measured in mouse units (based on the mean time to death of test animals) and the median lethal dose ($LD_{50}$) per kilogram of mouse determined by an assay with an all-or-none response. They found that female mice are slightly more susceptible to the poison than male mice and that salt concentrations greater than 0.1 M reduced intraperitoneal toxicity of the poison. Later, Schantz (1960) estimated that 1% of sodium chloride in the assay solution injected into the mice increases the time to death sufficiently to reduce poison assay estimates by 50%.

The bioassay procedure was further refined by Schantz, in cooperation with Lewis, McFarren, and Schafer of the United States Public Health Service. They used purified shellfish poison as a reference standard, thus permitting the expression of assay results in weight (μg) of poison on the basis of the reference standard (Schantz et al. 1958; McFarren 1959) rather than in mouse units. Collaborative assays of standard toxic extracts by various laboratories in the United States and Canada showed reasonably close agreement, and the bioassay procedure was published as an official method of the Association of Official Agricultural Chemists (1965). This procedure is given, in slightly modified form, in Appendix III.

Publication of the official procedure has improved comparability of bioassay results from various laboratories. However, the various official instructions ignore the fact that in practice the toxicities of samples of living shellfish change after collection (Medcof et al. 1947). It is not unusual for their scores to increase by 100% during 3 days' air storage at room temperature, at temperatures just above freezing, or at freezing temperatures (–15 to –20 C). Similar increases occur in living shellfish that are placed in aquaria of flowing sea water or transplanted to new beds after harvesting from their native areas.

This phenomenon has not been explained, but the official procedure may be further standardized to reduce variability in bioassay results. For instance, in PSP control programs, it is important to have long sequences of strictly comparable scores for key area samples (see Control Programs). These enable management officers to detect onsets of toxicity seasons in their incipient stages.

It seems desirable to standardize the method of handling samples from the moment they are collected in the field and to standardize the interval between collection and extraction of samples.

### Scoring — Mouse Units and Micrograms

The amount of toxin (in micrograms) equivalent to one mouse unit depends on the assay technique and strain of mice used. For example, the mouse unit for the mice used by NHW at Ottawa up to May 18, 1966 (Swiss Webster; Connaught Laboratories, Toronto), is equivalent to 0.16 μg of the purified poison, whereas the mouse unit for the slighty less sensitive strain of mice used by NHW since then (Charles River; Canadian Breeding Laboratories, Montreal) is equivalent to 0.22 μg. These equivalents are used as factors (CF values) for converting the mouse units obtained in the tests to micrograms of the purified poison. This allows the results at any laboratory to be compared directly with those obtained at other laboratories that use the CF system of conversion.

With the strains of mice used before May 18, 1966, 32 μg poison/100 g shellfish meat is the lower limit of sensitivity of bioassay; for mice used after May 18, 1966, the corresponding value is 44 μg/100 g. Accordingly, negative results for these two strains are expressed as <32 and as <44 μg.

Results of bioassays conducted by NHW before 1955, and reported in mouse units, may be converted to micrograms by applying the CF 0.16.

## Bioassay of East Coast Shellfish

The extraction procedure for east coast shellfish is identical to that specified in the Association of Official Agricultural Chemists method (1965) except that the normality of the hydrochloric acid solution used is varied with the species of shellfish extracted. For example, 100-g samples of soft-shell clam meat are extracted with 100 ml of 0.18 N acid, and blue mussel samples with 80 ml of 0.18 N HCl plus 20 ml of distilled water. With this modification, *p*H adjustment of the extract is seldom required. All extracts are forwarded by mail or air express to the Public Health Engineering Division, Environmental Health Centre, NHW, Ottawa, for bioassay. The tests are normally completed and reported to the control agencies on the day of specimen receipt.

Six mice are used for each standard assay when positive results are obtained. The median time to death is entered in the Sommer table (Appendix Table III. 1) and the corresponding number of mouse units determined. If a shellfish extract contains large amounts of poison, it is diluted with acidified distilled water at *p*H 3.5 to produce a median time to death of between 5 and 7 min. The amount of poison per 100 g of shellfish tissue is calculated by multiplying the number of mouse units by the dilution factor (if any) and then by 200. Mouse units are then converted to micrograms of poison by multiplying by the CF 0.22.

## Factors Affecting Accuracy

Bioassays are inherently inaccurate because truly uniform test animals cannot be obtained. Even within a single standardized animal colony, individuals may respond very differently to identical dosage injections. Also, animal sensitivity may be influenced by the time of day at which the injections are made (Halberg 1960). Further, the response to a given dosage of poison varies with age, weight, and sex of the mice.

Correction factors are sometimes used to correct for deviations from the optimum (20-g) weight of test mice (Appendix Table III. 2). However, no correction is made for differences that may arise (Kao 1966) from differences in the age of mice.

Errors in technique of intraperitoneal injection also contribute to inaccuracy. Steward et al. (1968) injected a radiological contrast material intraperitoneally into 150 mice. In 21 (14%) they found all or part of the inoculum in a site other than the peritoneal cavity. They concluded that such errors are inherent in the technique, and cannot be minimized by simple modifications of procedure. This may explain the occasional failure of a mouse to show the usual reactions to an injection.

In line with these limitations, the accuracy of the standard test has been estimated as about ±20% (Schantz et al. 1958; McFarren 1959). But bioassays of shellfish with low toxicity (80 μg/100 g) may underestimate toxicity by as much as 60%. This can be attributed partly to high salt concentration in undiluted extracts injected into mice. As the toxicity increases, the estimates become pro-

gressively more reliable and approach 100% poison recovery at higher poison concentrations, where large dilutions of the extracts are used for the bioassay.

Reasonably consistent and accurate bioassay results are obtained when dilutions are adjusted to give median times to death of 4–8 min or, preferably, 5–7 min. This time range covers the portion of the curve for the relation between time to death and dosage where the dose is most accurately determined.

To further standardize the bioassay, only female mice are used by NHW because females are slightly more sensitive to the poison than male mice. The precision is also slightly improved by the use of six mice in determining the median time to death for each extract assayed; 10- and 20-mouse assays are used for specimens of special importance.

Median times to death are used in calculating the poison levels because one or more mice sometimes survive injections, and it is then impossible to calculate an average time. The problem of survivors is especially important concerning extracts with low levels of poison (median times of 9–15 min). In 199 preliminary assays with three mice each, with median times to death ranging from 1.0 to 1.5 min, 6 (1%) of the 597 mice (CM 0.16) survived; with a median time of 3.5–4.0 min 3% survived. In assays with 6 or 10 mice of the strains used before and after May 1966 (Table 15, Fig. 22), for extracts with median times of 9–15

TABLE 15. Percentages of mice of two strains surviving in assays (6 or 10 mice each) with median times to death ranging from 4 to 15 min.

| Median time to death (min:sec) | Webster strain (CF 0.16) | | | Charles River strain (CF 0.22) | | |
|---|---|---|---|---|---|---|
| | No. | | | No. | | |
| | Tests | Mice | % surviving | Tests | Mice | % surviving |
| 4:00–4:25 | 1120 | 7112 | 5.4 | 421 | 2622 | 2.8 |
| 4:30–4:55 | 1155 | 7398 | 6.6 | 452 | 2936 | 3.6 |
| 5:00–5:25 | 1009 | 6486 | 7.4 | 499 | 3382 | 4.1 |
| 5:30–5:55 | 761 | 4910 | 8.2 | 424 | 2968 | 4.5 |
| 6:00–6:25 | 570 | 3644 | 9.4 | 270 | 1824 | 5.1 |
| 6:30–6:55 | 455 | 2910 | 10.1 | 217 | 1462 | 6.2 |
| 7:00–7:25 | 308 | 1952 | 11.5 | 147 | 946 | 5.4 |
| 7:30–7:55 | 193 | 1250 | 12.6 | 142 | 892 | 5.8 |
| 8:00–8:25 | 158 | 1008 | 12.1 | 120 | 748 | 5.7 |
| 8:30–8:55 | 133 | 818 | 13.6 | 106 | 664 | 7.8 |
| 9:00–9:25 | 121 | 774 | 15.0 | 100 | 620 | 7.7 |
| 9:30–9:55 | 93 | 586 | 17.2 | 71 | 454 | 11.0 |
| 10:00–10:25 | 87 | 546 | 19.0 | 67 | 414 | 12.1 |
| 10:30–10:55 | 67 | 414 | 19.6 | 64 | 404 | 13.9 |
| 11:00–11:55 | 95 | 606 | 21.1 | 83 | 530 | 17.2 |
| 12:00–12:55 | 52 | 340 | 26.5 | 75 | 462 | 23.2 |
| 13:00–15:00 | 63 | 418 | 31.3 | 86 | 556 | 32.4 |
| Total or avg | 6440 | 41172 | 9.1 | 3344 | 21884 | 6.5 |

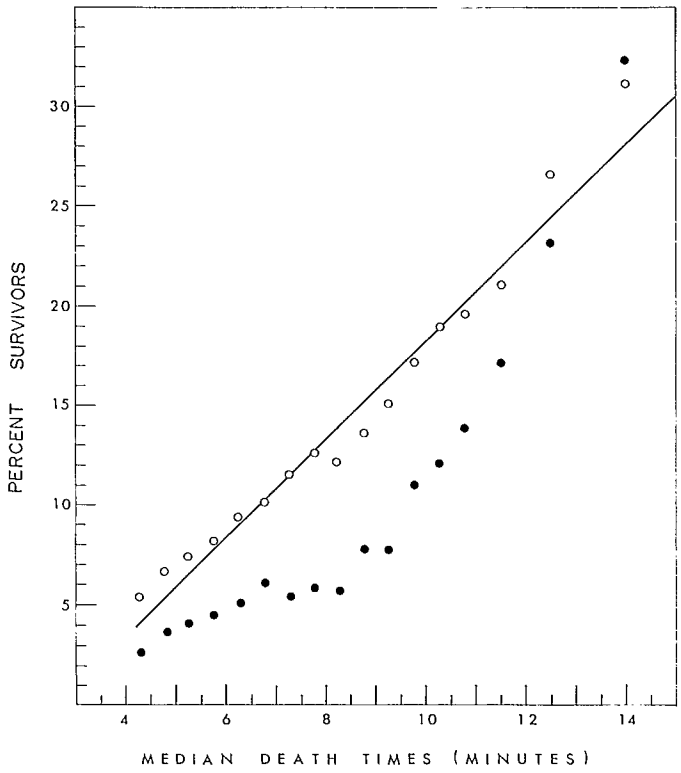


FIG. 22. Percentages of mice of two strains (Swiss Webster, O, CF value 0.16; Charles River, ●, CF value 0.22) of mice surviving in assays (6 or 10 mice each) with median times to death ranging from 4 to 15 min.

min the percentages of survivors ranged from 15 to 31 and from 8 to 32, respectively. Even for extracts with median times in the standard range (5–7 min) the percentages were as high as 10 and 6, respectively. Survivors were found much more frequently with the mice in use during the 1959–66 period (Swiss Webster) although these mice are more sensitive to the poison than the strain now used (Charles River).

## VARIABILITY OF BIOASSAY RESULTS

The precision of results is affected by the long times to death occasionally encountered in routine bioassays. To evaluate the variation, we conducted 120-mouse tests on 18 shellfish extracts which gave median times in the 4.0- to 6.5-min range. The data obtained were subjected to statistical analysis by Dr D. F. Bray of the Food and Drug Directorate, NHW, Ottawa.

As expected, the distribution of times to death was skewed in the direction of long times. The standard bioassay procedure allows for excessively long times, and deaths occurring after 20 min are therefore not recorded. The skewed distribution was the main factor prompting use of median (not average) times, because these are better measures of central tendency for skewed distributions.

The distributions become essentially symmetrical when the longer times (and survivors) are not considered and either the mean or the median times are used. Our data indicate that the test cut-off time could be shortened to 9 min with very little loss in information from assays with median times to death from 5 to 6.5 min. Truncation at 9 min produced distributions that appear nearly normal. Standard deviations were computed from each set of data, and these indicate a relation between the median (or the mean) and the variance (standard deviation). Reasonable approximations are

| | | | | |
|---|---|---|---|---|
| Median (or mean) time (min) | 5 | 5.5 | 6 | 6.5 |
| Standard deviation (sec) | 50 | 55 | 60 | 65 |

A conservative approach would be to use 65 sec. as the standard deviation regardless of the median obtained.

The same data permitted construction of 95% confidence intervals (CI). Table 16 shows how the length of the 95% CI is affected by varying probability levels and the number of mice used in tests. Increasing the number shortens the interval; for example, increasing it from 3 to 12 approximately halves the interval. Decreasing the probability ($P$) that the interval does not include the true mean lengthens the interval; for example, using five mice with $P = 0.01$ and using three mice with $P = 0.05$ give approximately the same length of confidence interval.

TABLE 16. Value of $\frac{t^a}{\sqrt{n}}$ for varying sample size and varying probability of noninclusion of the true mean.

| $n$ | $\sqrt{n}$ | $t_{.05} = 1.960$ | $t_{.02} = 2.326$ | $t_{.01} = 2.576$ |
|---|---|---|---|---|
| 1 | 1 | 1.960 | 2.326 | 2.576 |
| 2 | 1.41 | 1.39 | 1.65 | 1.83 |
| 3 | 1.73 | 1.13 | 1.34 | 1.49 |
| 4 | 2.0 | .98 | 1.16 | 1.29 |
| 5 | 2.24 | .88 | 1.04 | 1.15 |
| 6 | 2.45 | .80 | .95 | 1.05 |
| 7 | 2.65 | .74 | .88 | .97 |
| 8 | 2.83 | .69 | .82 | .91 |
| 9 | 3.00 | .65 | .78 | .86 |
| 10 | 3.16 | .62 | .74 | .82 |
| 12 | 3.46 | .57 | .67 | .74 |
| 16 | 4.0 | .49 | .58 | .64 |
| 20 | 4.47 | .44 | .52 | .58 |

[a] $n$ = sample size (number of mice on test); $t$ = $t$ statistic (at $P = .05$, $P = .02$, and $P = .01$).

Table 16 can be used to determine the CI for routine test data derived in this way. For example, six 20–g mice are used to test an extract which kills five mice before 9 min while one mouse survives, and the mean time to death of the five mice that died is 5:30 min:sec. With a conservative standard deviation of 65 sec, and $t_{.05} = .88$ (Table 16), a conservative CI is

$$\begin{aligned} \text{CI } (95\%) &= 5{:}30 \text{ min:sec} \pm (.88)(65) \text{ sec} \\ &= 5{:}30 \pm 57 \text{ sec } (4{:}33\text{–}6{:}27 \text{ min:sec}). \end{aligned}$$

Toxicity estimates corresponding to times to death of 4:33, 5:30, and 6:27 min:sec may be obtained (as mouse units) from Appendix Table III.1, and converted to micrograms (CF 0.22). Thus, the 95% CI for the observed bioassay result of 77 μg toxin/100 g shellfish is from 65 to 94 μg.

## Chemical and Serological Assay Methods

A chemical method for quantitative estimation of paralytic shellfish toxin was developed by McFarren et al. (1958). This involves selective adsorption of the poison in an acid aqueous extract on XE-64 ion exchange resin. The poison is then eluted and measured colorimetrically. It gives the characteristic Jaffe colour with trinitrophenol (picric acid) in alkaline solution. Later the same authors (1959) modified the chemical assay by introducing extraction of the unreacted picric acid from the reaction products after colour developments with a mixture of 25% pyridine in ethyl acetate.

In either form the chemical test is complex and time-consuming, and is generally less sensitive than the bioassay. Its accuracy depends on purity of samples. Interfering substances such as glucosamine cause increasing difficulties as the storage time of specimens increases. The method, however, can be used when mice are not available, and is more precise than the bioassay at higher poison levels.

Serological tests for detecting and measuring poison were studied by Johnson and Mulberry (1966). A chemically derived antigen for shellfish toxin showed haptenic properties as demonstrated by passive agglutination and mouse protection tests with the antisera produced, and could be adsorbed to tanned sheep blood cells for use in haemagglutination tests. The test was more sensitive than the bioassay, but the blood cell preparation was very unstable. Shellfish toxin antigen was also adsorbed to bentonite particles. The preparation was stable, but the sensitivity of the test was of the same order as that of the bioassay. Reagent preparation and test procedures are complex and time-consuming.

In conclusion, there is nothing to indicate that a chemical or serological test will replace the mouse bioassay for the detection and measurement of toxicity in the immediate future. In spite of its inherent variability and lack of precision, the bioassay has proved a practical and reliable test for the routine estimation of poison in toxicity monitoring programs.

## Effects of Processing on Toxicity

### Home Cooking

Home cooking may protect consumers from PSP, but the protection is far from complete. Nearly all the recorded poisonings have resulted from eating cooked shellfish.

Tests were made to show the effects of the three common home cooking methods, steaming, boiling, and pan-frying, on toxicities of soft-shell clams. The steaming and frying tests followed usual home procedures (Table 17). The boiling tests were simplifications. The meats were merely cooked in equal volumes of boiling water with none of the other ingredients (vegetables, milk, and condiments) that are added in making a chowder.

Table 17. Effects of home cooking on toxicity (μg) of three lots of soft-shell clams taken from Lepreau Basin, N.B., on (1) Aug. 20; (2) Aug. 12; and (3) Sept. 4, 1945 (after Medcof et al. 1947).

| Method of cooking | Lot no. | Meats (raw) | Cooking time (*min*) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 5 | 10 | 15 | 20 | 30 | 40 |
| Chowder making[a] | 1 | 4230 | 2160 | 1060 | – | – | 803 | – |
| | 2 | 2320 | 830 | – | – | 1180 | – | 510[d] |
| Pan frying[b] | 2 | 2320 | – | – | 672 | – | – | – |
| | 3 | 545 | – | 115 | – | 141 | – | 35 |
| Steaming[c] | 1 | 4230 | – | 1680 | 1350 | – | 800 | – |
| | 2 | 2320 | 1380 | – | 674 | – | – | 208 |
| | 3 | 545 | – | 45 | – | 333 | – | 48 |
| Bouillon | 3 | – | – | 99 | – | 96 | – | 35 |

[a]Boiling shucked meats with water, milk, vegetables, and a variety of condiments.

[b]Pan frying of raw or cooked shucked meats coated with bread crumbs or a batter.

[c]Steaming whole aminals in their shells in a covered pot with just enough boiling water to cover the bottom.

[d]Boiled 60 min.

In all tests some clams were cooked for regular periods (15–20 min) and some for longer or shorter periods to determine the possible effects of under- and over-cooking. Steaming time was measured from the time steam began to escape from under the lid. Extracts for bioassay were prepared from the cooked meats. In one of the steaming tests, samples of "bouillon" (liquid left in the cooking pot after steaming) were removed at intervals and assayed for toxicity.

Cooking the clams, even for brief periods, reduced their toxicity, and cooking them for the usual home cooking times reduced the scores by as much as 70% (Table 17). Other results show that there is a 30–40% reduction in meat weight when clams are cooked for 15 min (Medcof et al. 1947). For this reason, more cooked meats than raw meats are required to make up a sample for bio-

assay. This means that cooking soft-shell clams actually reduces toxicity by more than 70%.

Steaming and frying reduced toxicity more than boiling (Table 17), and frying produced no poison-bearing fluids such as bouillon or chowder broth. For these reasons frying may be the safest way to home-cook shellfish that may be toxic, because only the meats are eaten.

Soft-shell clam bouillon scores decreased with steaming time and, weight-for-weight, bouillon contained more than twice as much poison as the steamed meats (Table 17). Steaming tests with blue mussels (Ingham et al. 1968) and with British Columbia butter clams (Quayle 1969) indicate that over 50% of the poison present in raw shellfish survives in the bouillon. Thus, the common practice of discarding all or part of bouillon from home-steamed toxic shellfish affords consumers some protection from PSP.

There is no step in chowder making that is equivalent to discarding part or all of the bouillon from steamed clams. So eating a chowder is much like eating steamed clams and drinking all their bouillon. A hearty eater may eat several servings of a chowder and, if it is toxic, he may take in large amounts of poison even if its toxicity is low. For example, we have records (Bond 1958) of poisonings from a chowder scoring only 352 that was made from clams scoring 1040.

Our estimate that 70% of the poison in raw toxic shellfish meats is removed from the meats or destroyed by cooking is close to the 68% reported from similar tests with blue mussels (Ingham et al. 1968). We have used 70% as a working figure in calculating poison intakes (like those described in Fig. 5) by persons who have eaten specified numbers of cooked shellfish of any species whose raw scores are known and whose meat yields are known.

## Commercial Processing

Most commercial catches of shellfish are processed before marketing and, depending on the process and species involved, toxicities may be decreased or increased. The two most common commercial processing treatments for shellfish are shucking and canning.

### Shucking

This involves removing meats from shells and trimming away unwanted parts of meats and is regularly employed for scallops, clams, mussels, and whelks.

In *Scallops,* commercial shucking eliminates risk of PSP. The adductor muscle, which is poison-free (Table 7), is the only part marketed. Poison in toxic scallops is located in the discarded parts (rims), which constitute 75% by weight of the body.

In *Soft-shell clams,* shucking does not reduce toxicity and may in fact increase the hazard of poisoning. These clams are shucked raw for the fried clam

trade or shucked after cooking for canning. The membranous cover of the siphons and the siphon ends, constituting 25% of the total weight of untrimmed shucked clam meats (Thurber MS 1949), are trimmed away. The discarded parts contain no poison (Table 7), so although the consumer eats only 75% by weight of the clam meat, he takes in all the poison if the clam is toxic.

A comparison of scores of trimmed and untrimmed commercially shucked raw clams from Lepreau Basin, N.B., indicated that shucking and trimming increases scores by 35–40% (Medcof et al. 1947). This is in sharp contrast with results for British Columbia butter clams, which carry nearly half their poison in the siphons and shucking and trimming off the siphons reduces risks of PSP (Quayle 1969).

In home shucking, raw whole clams are frequently given a quick scalding that facilitates opening, and before it was prohibited, scalding was also practiced industrially. A test in a shucking plant in 1945 indicated that scalding reduces scores by 50%. More data are needed to confirm this effect.

The effect of shucking on the toxicity of steamed clams has not been studied. However, it has been shown that steaming toxic mussels and whelks (Medcof et al. MS 1966) does not alter the relative toxicities of their various organs. From this it is reasonable to assume that shucking and trimming cooked clams raises their scores just as it raises scores of raw clams.

*Mussels* are not eaten raw on our coast and shucking always involves preliminary steaming but none of the meat is trimmed away. Only the holdfasts (beards) are removed and these are poison-free in both the raw and the cooked state (Table 7). Holdfasts generally constitute less than 1% of the total meat weight, so shucking and trimming can have no important effect on toxicity of steamed mussels.

*Whelks* are always steamed to facilitate shucking. All commercial and most home shucking also involves trimming away the digestive gland that comprises the soft, dark-coloured, upper body whorls. The digestive gland contains most of the poison in toxic whelks and may show high scores even after steaming (Table 7). Removal of digestive glands from whelks therefore reduces risks of PSP.

### Canning

Canning greatly reduces the toxicity of shellfish but soft-shell clams are the only shellfish that are commercially canned on a large scale in eastern Canada. Before reaching consumers, the canned product undergoes five factory treatments that affect the scores: (1) steaming in loosely covered containers for 20 min at atmospheric pressure, at temperatures about 212 F; (2) shucking and trimming of steamed meat; (3) washing in fresh water, packing in cans, adding bouillon, and sealing; (4) retorting at 250 F for 45 min; and (5) storing in warehouse, usually about 2 weeks but sometimes extended to several months.

Results of commercial canning tests on toxic soft-shell clams (Medcof et al. 1947) demonstrate the effects of standard and modified commercial canning

procedures (Table 18). This shows that:

1. Regular canning reduces toxicity by more than 90%;
2. Steaming is responsible for 70–90% of this reduction;
3. Halving or doubling the standard 20-min steaming period has no significant effect on steamed scores;
4. Retorting produces a further small drop in scores (equivalent to 5–6% of raw scores);
5. Halving the retorting time increases final scores by 10% and doubling it decreases them by 30%;
6. Retorting at 220 F instead of 250 F raises final scores by 25–50%.

TABLE 18. Effects of successive treatments in the canning process on toxicity of soft-shell clams from two areas.

| Material assayed | Period of treatment (*min*) | Toxicity ($\mu g/100\,g$) Pocologan stock | Lepreau stock |
|---|---|---|---|
| Raw meats (duplicate samples) | | 112 | 800 |
| | | 138 | 930 |
| Steamed meats | | | |
| After steaming | 10 | 42 | 102 |
| | 20[a] | 38 | 99 |
| | 40 | 38 | 102 |
| Canned meats (steamed 20 min) | | | |
| After retorting at 220 F | 20 | 32 | 81 |
| | 45 | 32 | 75 |
| | 90 | 32 | 62 |
| After retorting at 250 F | 20 | 34 | 64 |
| | 45[a] | <32 | 50 |
| | 90 | <32 | 45 |

[a]Standard commercial treatment.

Results not reported in Table 18 indicate that a second 250-degree retorting of packs that are still toxic after several months of storage does not reduce scores significantly (Bond and Corbeil MS 1958b) and that retorting at higher temperatures reduces toxicity more than 250-degree retorting.

Raw scores of shellfish affect their canned scores (Medcof et al. 1947) and tests with soft-shell clams show that poison-free packs are regularly obtained when raw scores are below 200 and that packs scoring less than 80 are regularly obtained when raw scores are below 1000 (Table 19). Tests with red mussels give similar results (Bond MS 1957).

Management officers (see Control Programs) have found that low-scoring packs of Bay of Fundy soft-shell clams become poison-free during storage. This phenomenon was explored in 1948 and 1950 with canned whole rims (five or six per can) and with canned samples of a large mass of homogenized digestive glands ("livers") of Bay of Fundy scallops that were retorted for 45 min at 250 F

(Medcof MS 1949; MS 1952). The rims were assayed 7 days after retorting and the livers 4 days after. The rest of the packs were stored at room temperature and assayed for shellfish poison at prescribed intervals.

TABLE 19. Effects of the regular commercial canning process on scores (μg/100 g) of 15 lots of soft-shell clams of various toxicities (after Medcof et al. 1947).

| No. lots tested | Toxicity range of raw stock | Toxicity of canned product |
|---|---|---|
| 1 | 800–960 | 50 |
| 1 | 210 | 32 |
| 4 | 160–175 | <32 |
| 6 | 80–160 | <32 |
| 1 | 40–80 | <32 |
| 2 | 32–40 | <32 |

Scores for rims show wide scattering, which is attributed to variations in toxicity of individual rims, and scores for homogenized livers show little scattering (Fig. 23). But results for both rims and livers indicate that the toxicity does decrease during storage and that the decrease is an orderly process. The decrease is rapid during the first few days after canning, then slows down progressively

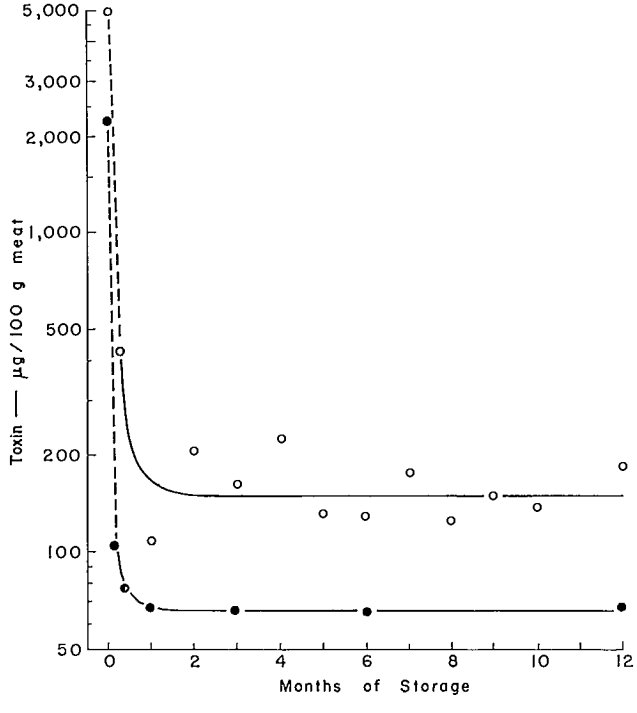


FIG. 23. Effect of canning (broken lines) and storage after canning (solid lines), on toxicity of scallop rims (open circles), and scallop livers (solid circles).

and almost stops after 2 months, the residual score being equal to about 3% of the raw score (Table 20). Quayle (1969) reported on toxin content of canned butter clams stored up to 8 months. The data as he has presented them support our conclusions in showing an inverse correlation between toxicity and storage time (correlation coefficient, 0.51 at close to the 90% probability level).

Because the change is so rapid immediately after canning and because the first assays were made 4–7 days after retorting, the scores immediately after canning are not known. However, it is safe to say that values listed for "initial scores after canning" in Table 20 are lower than the true initial scores after canning and that Table 20 and Fig. 23 overestimate effects of canning and underestimate effects of storage in reducing scores.

TABLE 20. Effects of canning and storage on toxicities (μg/100 g) of scallop rims and livers.

| | Rims 1948 | Livers 1950 |
|---|---|---|
| Score when raw | 4940 | 2290 |
| Initial score after canning | 424 | 104 |
| Decrease during canning (%) | 91 | 95 |
| Score after 6 months' storage | 152 | 66 |
| Decrease in score during storage | 272 | 38 |
| Decrease during canned storage (%) | 65 | 36 |
| Decrease during canning and storage (%) | 97 | 97 |

The conclusions from these tests are that canning is more effective than other types of commercial processing and more effective than home cooking in reducing toxicity of shellfish.

## DETOXIFICATION OF SHELLFISH

In spite of many attempts in Canada and the United States, no commercially practical method of eliminating or reducing shellfish toxicity has been developed. The method most often tried is transplanting toxic shellfish to nontoxic or low toxicity areas (Chambers et al. MS 1955; Neal MS 1967; Medcof et al. 1947). The decreases obtained are too slow to be economic.

Substantial reductions in shellfish toxicities have been achieved by modifying commercial processing methods. This has involved removal of toxic parts of

shellfish prior to packing, increasing the steaming period, raising or lowering the *p*H, and subjecting toxic meats to ionizing radiations. Although successful in reducing toxicities, such procedures may also produce extreme changes in the quality of products and reduce their market acceptability.

In recent years, it has been found that the toxin absorbed by the shellfish, does not undergo any change in its chemical structure (Schantz 1970). This fact prompted a search for ways of inducing shellfish to "spit out" their toxin. In 1966, tests were made with toxic soft-shell clams and blue mussels by subjecting them to various physiological stresses. Some of the shellfish (controls) were placed in tanks of filtered sea water at the temperatures and salinities to which they were accustomed. Others were exposed to higher or lower temperatures and salinities.

In all the salinity experiments, water temperatures were maintained at 12 C (54 F) and both clams and mussels responded to changes in salinity by reducing more than half of their toxicity within a week (Table 21). In both cases the controls showed relatively little reduction in toxicity. At salinities higher than 34‰, the shellfish gaped and died within a few days.

TABLE 21. Toxicities (μg/100 g) of soft-shell clams and blue mussels after exposure to different salinities for various times.

| Exposure time (*hr*) | Salinity (‰) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Soft-shell clams[a] | | | | | Blue mussels[a] | | | | |
| | 28 | 30.6[b] | 32 | 33 | 34.6 | 28 | 30.6[b] | 32 | 33 | 34.6 |
| 17 | 97 | 88 | 88 | 95 | 92 | 286 | 286 | 264 | 242 | 286 |
| 88 | 55 | 84 | 59 | 62 | 68 | 264 | 264 | 213 | 220 | 117 |
| 136 | 47 | 77 | 52 | <44 | 55 | 156 | 242 | 130 | 169 | Dead |
| 184 | 44 | 62 | 44 | <44 | Dead | 108 | 209 | 110 | 121 | Dead |

[a]The initial toxicity (in air) was 101 for clams and 242 for mussels.
[b]Filtered sea water from St. Andrews Station supply (control).

Increasing water temperature seemed more effective in detoxifying mussels than increasing or decreasing the salinities (Table 22). However, the data in Table 22 should be interpreted with caution because, in the tanks held at 17 and 21 C, the salinity increased from 30.6 to 32.2‰ three days after the start of the experiment, possibly due to evaporation.

It appears that quickest detoxification would be achieved by sudden exposures of shellfish to combined elevations of both temperature and salinity. More tests are needed and other toxic shellfish species need to be tested for firm conclusions.

There is a tendency for recently fished shellfish to increase in toxicity when placed in seawater tanks (Table 21, 22, Fig. 24). This initial increase is followed by a slow decrease. Medcof et al. (1947) observed similar increases and decreases in clams and mussels held in flowing sea water and in air. We are unable to explain this phenomenon, but it seems to be a characteristic response. It may be linked with maintenance of oxygen equilibrium because Newell (1964) has shown that both mussels and soft-shell clams build up oxygen debts when exposed to air and that this is offset by increased rates of pumping when they are resubmerged. Since neutralization of shellfish toxin occurs in the presence of a strong oxidizing agent (Chin 1970), it is probably that its reverse may happen when the shellfish pay off their oxygen debt. Thus, the initial increase in toxicity following submergence may be more apparent than real.

TABLE 22. Toxicities (μg/100 g) of blue mussels[a] after being held in sea water at three temperatures for various times.

| Exposure time (*hr*) | Water temp (*C*) | | |
|---|---|---|---|
| | 12 | 17 | 21 |
| 24 | 88 | 81 | 88 |
| 96 | 62 | 54 | 59 |
| 120 | 70 | 51 | 48 |
| 144 | 59 | 59 | 53 |
| 168 | 59 | 47 | 47 |
| 192 | 59 | 47 | <44 |

[a]Initial toxicity (in air) = 75.


FIG. 24. Percentage change in toxicity of shellfish held in seawater tanks (12.0 C; 30.8‰) for varying lengths of time. The base line at zero indicates the initial toxicity on the first day.

## Control Programs

### Objectives and Activities

The objectives of control programs are to reduce risks and incidence of PSP and at the same time make the fullest possible use of shellfish resources. The programs are based on results of monitoring toxicities of shellfish from affected shellfish-producing areas in the Bay of Fundy and the St. Lawrence regions. All these areas have been mapped and most have been classified according to levels of toxicity. Shellfish samples for monitoring toxicity are taken regularly from fixed sampling stations, selected after experience has proved them to be representative of the worst conditions in their areas.

Commercial packs of canned and shucked shellfish are also monitored.

Monitoring programs and appropriate actions e.g., imposing and lifting quarantines, are based on toxicity levels. A control scheme has been in effect since 1943 in the Fundy region, and 1949 in the St. Lawrence region and is modified from time to time by control agencies at annual meetings of the Interdepartmental Shellfish Committee. The latest modifications were made in 1969 (Boyd and Lachance MS 1970). The control agencies have agreed that it is safe to market shellfish provided their scores are less than 80.

### Roles of Various Agencies

Pursuit of the above objectives requires close cooperation among different agencies. Protection officers and fish inspection officers of the Department of Fisheries and Forestry of Canada collect samples for monitoring from area sampling stations and from processing plants in New Brunswick and Nova Scotia. Extracts of most of these samples are prepared at federal fish inspection laboratories at Blacks Harbour, N.B., and at Halifax, N.S. In the province of Quebec, sampling is done by fishery officers of the Quebec Department of Industry and Commerce, and extraction by its Technical Services Laboratory. Extracts of all samples of canned shellfish are prepared at the federal fish inspection laboratory in Montreal.

All extracts of samples from the Atlantic provinces and from the Pacific coast (British Columbia) are sent by air to NHW and bioassayed at its Environmental Health Centre, Ottawa. Thus there is a nationwide standardization of bioassays. Only one mouse strain is used and one group of technicians using one carefully controlled procedure carries out all tests in one laboratory. The NHW laboratory conducts 2000–3000 bioassays annually and communicates results for the Atlantic provinces, by mail or by telegram, to the agents of the federal and provincial departments concerned. It also serves as a consultant and recommends necessary action such as imposing and lifting quarantines.

The Department of Fisheries and Forestry of Canada and the Quebec Department of Industry and Commerce are responsible for imposing and lifting quarantines; formally advising commercial packers of these changes; posting

warning signs in affected areas; maintaining liaison with district medical health officers; releasing press, radio, and television publicity on PSP hazards; and policing quarantined areas.

The Fisheries Research Board of Canada is responsible for most of the biological and epidemiological research programs, with help from other federal and provincial agencies. The latter agencies occasionally carry out investigations they are best able to conduct because of their geographic location or special competence.

All agencies are responsible for checking and reporting real or suspected cases of PSP as soon as they are encountered.

## Classification and Monitoring of Shellfish Areas for Toxicity

### Bay of Fundy region

*Key stations.* Shellfish in certain Bay of Fundy areas, called key areas, begin accumulating poison each summer a week to 10 days earlier than those in other areas. By sampling key stations within key areas, it is usually possible to forecast imminent increases in toxicity beyond the danger level (80 μg/100 g) in other shellfish areas. Three key stations supply the information needed for initiation of sampling throughout the Fundy region (Table 23).

Table 23. Summary of toxicity sampling programs and harvesting controls for the three classes of areas in the Bay of Fundy region (< = less than, > = greater than; all scores are μg/100 g meat).

| Classification of areas | Sampling program for monitoring toxicity | Purpose for which commercial harvesting is permitted |
|---|---|---|
| Key stations (all in Class III areas) | Every 2 weeks Nov. 1–May 1: every week for balance of year | |
| Class I | Every week while key station score exceeds 80 | For all purposes while scores in all Class I areas are <80. For canning only, while scores in the worst affected Class I area vary from 80 to 160 |
| | | For no purpose if score in any Class I area exceeds 160 |
| Class II | Every week from June 1 until Oct. 1 or June 1 until closure or until need dictates | For all purposes while scores in all Class II areas are <80. For canning only while scores in the worst affected Class II area vary from 80 to <160 |
| | | For no purpose if scores in any Class II area >160 |
| Class III | Every week from June 1 until Oct. 1 or until closure or as needed for surveillance or for opening | For canning only and only while scores of all Class III areas are <160 and key station scores indicate no imminent rises |

From the early 1940's to the mid-1950's, Head Harbour on Campobello Island (Fig. 6) was a dependable key station for the New Brunswick side of the Bay of Fundy. For obscure reasons, this is no longer true and Lepreau Basin and Crow Harbour (Cove) (Fig. 6) are now used jointly as key stations (Boyd and Lachance MS 1970). Centreville on Digby Neck (Fig. 6) has always been a reliable key station for the Nova Scotia side of the Bay of Fundy and is still used.

All the shellfish areas, including the key areas, are grouped into three classes on the basis of their general toxicity conditions. Every area has a sampling station, and information from monitoring toxicity at these stations and the key stations is used in regulating the harvesting of shellfish. All the areas in each class are treated as a group in the control scheme (Table 23).

In some areas, called *Class I areas,* shellfish are never or seldom toxic, and when they do become toxic their periods of toxicity are short and their scores always below 80. Class I areas are almost never quarantined.

The scores of all *Class II areas* are consistently less than 80 for long periods each year but they may be high for part of the summer. In most years, Class II areas are quarantined for a brief period.

Shellfish toxicities in *Class III areas* are often hazardously high for long periods and may remain above 80 the year round. In some years, however, they never reach 80. Class III areas are usually quarantined for a long period each year, but limited harvesting is sometimes permitted. All the key stations are in Class III areas.

### St. Lawrence Region

The St. Lawrence region shows greater area-to-area variation in seasonal toxicity patterns than the Fundy region. Control agencies are still assembling the considerable body of detailed information needed for complete mapping and classifying of areas, for selecting satisfactory key stations and area sampling stations. The affected coastline in this region is very long, so it may be several years before a control scheme comparable to that in the Fundy region can be worked out and the number of shellfish samples reduced (830 in 1969). This can be done only by grouping areas into classes as has been done in the Fundy region (404 samples in 1969).

## Harvesting Controls

Commercial harvesting of clams for marketing in the shell or for raw shucking is permitted only in Class I and Class II areas and only while their shellfish scores are consistently less than 80 (Table 23). When their scores exceed 80 these areas are posted with warning signs (Fig. 25) that forbid harvesting except for commercial canning and that explain the need for this regulation. Class III areas are permanently posted and it is illegal at all times to harvest shellfish from these areas except for commercial canning or, under a special permit, for use as fish bait.

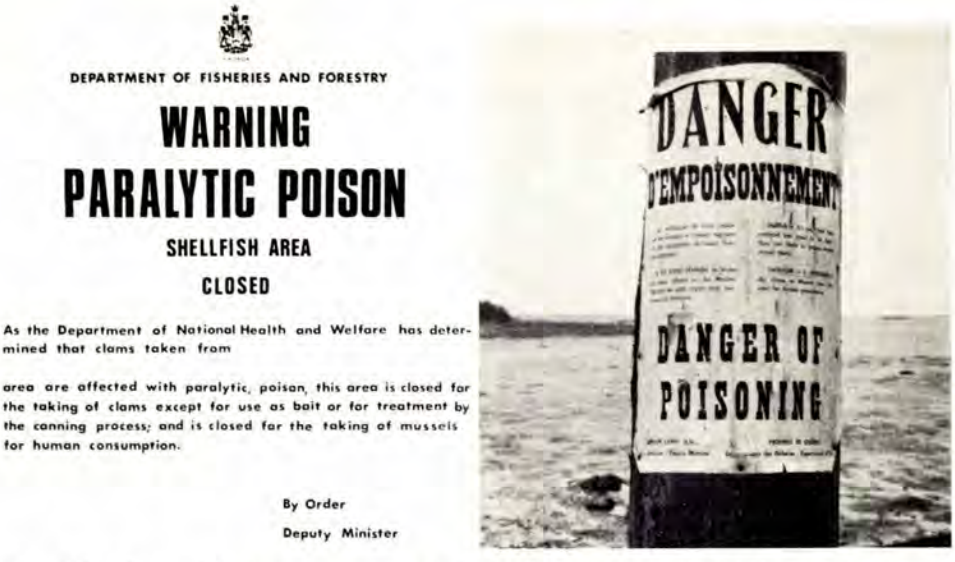


FIG. 25. PSP warning signs currently used in the Bay of Fundy and St. Lawrence regions.

When scores in any area exceed 160, all areas in its class are closed to harvesting for all purposes (Table 23). This provides consumers a wide margin of safety. Possibly the regulation could be relaxed somewhat, because there is evidence that raw stocks with scores higher than 160 produce canned packs scoring less than 80. Poison-free packs of canned shellfish are regularly obtained from stocks with raw scores of 160 or less (Table 19). This matter has not been sufficiently studied.

## PROCESSING CONTROLS

In the current control program, each day's pack of canned clams is treated as a separate lot and no lot is released for marketing until bioassay shows that its score is less than 80. Until 1969, the regulations were more stringent: all packs had to test poison-free and some packs put up in the Fundy region (Bond and Corbeil 1958b) and in the St. Lawrence region (Environmental Health Centre MS 1968) had to be confiscated.

Toxicity of canned shellfish decreases during storage, rapidly at first, then more and more slowly. Final scores may be above or below 80. Packs of canned clams that score above 80 on first bioassay are placed in bond by fish inspection officers and resampled after reasonable periods of storage. If scores have decreased to required levels, the packs are released for marketing, but if scores still exceed 80, the packs must be destroyed. Slowness of score reduction during storage (Fig. 2) and high storage costs tend to offset industrial advantages of storage as a

means of reducing scores. Destruction of packs that fail to meet market standards is even more costly. For these reasons clam canners are careful to observe PSP control regulations.

Raw shucking presents fewer control problems than canning because shuckers handle only clams that have zero or very low scores. Class II areas would be expected to supply the highest-scoring stocks but these areas are closed to harvesting for shucking as soon as key station scores or any one Class II area sampling station score starts to rise. Thus chances of high-scoring clams reaching shucking plants are few. Shucking increases scores slightly but consumers are well protected for the reasons mentioned, and because raw-shucked shellfish are almost always cooked and cooking reduces low scores to zero or near-zero levels.

Because of their perishability, commercial packs of raw shucked clams are cleared for market (if otherwise acceptable) before results of bioassays of plant samples are known. This might seem risky but fish inspection officers visit shucking plants almost daily and sample the packs when key station scores are above 44. So there is little likelihood of undetected evasions of regulations. Detection of high-scoring shucked shellfish results in seizure and destruction of packs. Shuckers are careful to observe regulations and specially careful if they are catering to trade with United States markets. They know their packs will be checked by both Canadian and United States public health officials and that detections made in the United States will result in suspension of export licences, as well as seizure and destruction of packs. In practice, the control system works well. It is 12 years since the last seizure of a pack of toxic shucked Atlantic coast clams on our domestic market (Bond and Corbeil MS 1958b) and more than 20 years since such a seizure was made on the United States market.

## Critique of Control Scheme

As just stated, the control scheme is effective but has its shortcomings. A rough estimate of annual costs of monitoring risks of PSP and of regulating harvesting and marketing is over $50,000. This seems high compared with the annual landed value of the soft-shell clam (the most hazardous species) on the Canadian Atlantic coast (e.g., $257,000 in 1967).

It has been proposed that sampling should be abandoned and that shellfish harvesting in affected areas should be made illegal at all seasons. This might save governments some money but it would reduce many fishermen's and processors' incomes and would take away the livelihood of many others. Furthermore, such a prohibition would result in buildups of dense stocks of shellfish in affected areas. Without question, this would increase poisonings among picnickers, who often disregard warnings and are the most common sufferers from PSP. Even now, when clams are not abundant in many affected areas, picnickers persist in harvesting and eating them in spite of prohibitions, warning signs, and policing of the hundreds of miles of coast that are quarantined from time to time.

Blanket, year-round closures of affected areas would also introduce new risks from professional clam diggers, who now respect quarantines. They appreciate the temporary nature of area closures and are willing to defer harvesting until quarantines are lifted.

It is 12 years since a case of PSP has been recorded as due to consumption of shellfish processed commercially in eastern Canada, and the total number of cases from this source approximates only 1% of the total number of illnesses on record. But if there were no open seasons, the resulting dense stocks of toxic shellfish would tempt out-of-work commercial diggers to find "bootleg" markets, and thus increase the PSP risks and create needs for additional policing. The extra costs could offset any savings this proposed scheme might provide.

Less drastic measures have also been proposed such as closing all shellfish areas from June to October. This would reduce sampling programs but not eliminate the need for them, especially in the St. Lawrence region where high off-season scores are common. Furthermore, this proposal is open to the same criticisms as the year-round closure scheme, because winter fishing is impossible in the many areas that are ice-bound, and because market demand and market prices are highest in summer months.

For these reasons, control agencies favour continuation of the present scheme of regulated harvesting because it permits full exploitation of shellfish resources, besides providing public health protection at what they consider to be reasonable costs.

## Problems in Control

### Ignorance and distrust

The fact that shellfish accumulate poison is the primary cause of PSP. Ignorance, distrust, and folklore are important secondary factors. The task of PSP investigators, public health administrators, and fisheries officers would be easier if they knew the answers to many of the still puzzling questions about PSP and knew more about how to communicate what they now know to the public. The public often seem apathetic to or distrustful of new information.

Of all groups, residents of shore communities in PSP-affected regions are the most distrustful of what they hear or read about PSP and even of illnesses that they have seen with their own eyes. Many refuse to admit that these are caused by shellfish and are sincere nonbelievers in PSP. This might not matter except that shore residents often tell visitors that PSP is a myth and that PSP warning signs may be safely disregarded. We have evidence that local fishermen sometimes destroy warning signs because they believe these are wrong. We attribute this distrust principally to the fact that many shore residents, without being aware of it, have acquired tolerance for shellfish poison (see "Features of poisoning"). They have eaten their local shellfish for years without ill effects and it is inconceivable to them that other people may be unable to do the same.

There are many traditional beliefs in our shore communities about toxic shellfish and PSP. Many are sound and useful generalizations, e.g.: mussels are the most poisonous shellfish on the beach; rims of Bay of Fundy scallops are poisonous. However there are many that might seem to be innocent bits of folklore but are, in fact, dangerously misleading. They are accepted and applied by uninitiated visitors to the seashore in gathering "safe" shellfish, in "testing" shellfish for poison or in "getting rid of the poison" in toxic shellfish. Reports of PSP victims show that this has happened many times. It is they, not the tellers of the tales, who suffer.

Ten dangerous folktales are listed below. Their reliability can be judged by reference to the information sources listed below.

| *Ten dangerous folktales* | *References* |
|---|---|
| 1. Shellfish are safe in months with an "R". | Fig. 4 |
| 2. Mussels are the only poisonous shellfish on the beach because they live at the surface and get "sun struck". | Table 5 |
| 3. All poisonous shellfish live high on the beach where they get "sun struck" in summer. | Page 19 |
| 4. Mussels growing on sand and mud are poisonous. Those on rocks are wholesome. | We find that all mussels are poisonous where and when *G. tamarensis* abounds. |
| 5. PSP is caused by only one or two sickly shellfish in any collection. | Page 18 |
| 6. Only large, old clams are poisonous. | Page 18 |
| 7. The beard (holdfast) of mussels contains all the poison. | Table 7 |
| 8. "It's all good" (referring to shucked but untrimmed meat of rough whelks). | Table 7 |
| 9. You can "tell" when shellfish are poisonous by opening them. | In our experience toxic shellfish look, taste, and smell the same as the nontoxic. |
| 10. A silver spoon in the cooking pot tarnishes if the clams are toxic and stays bright if they are nontoxic. | This has been tested in the laboratory. It is not true. |

Essentially, today's PSP control problem is not technological, although it has technological aspects. It is mainly a public education problem and requires new approaches.

*Difficulty in forecasting PSP maxima* is a technological control problem. The forecast method now used is short-range and depends on shellfish sampling as already described. Sometimes shellfish toxicities increase to hazardous levels within a few days and closing down shellfish processing plants and posting warning signs become panic operations. Better forecast methods are needed.

Study of plankton for *Gonyaulax tamarensis* might give better short-range forecasts because checking plankton should be quicker than bioassay. It could be done at the coast without preparing extracts but it would require special equipment and technical training of field staff. Judging from Table 12 and Fig. 19, it might provide earlier forecasts than shellfish sampling because there is a slight lag between the first appearance of *Gonyaulax* and the first rise in toxicity. But there is little knowledge of how *G. tamarensis* behaves in its natural habitat and of how to interpet plankton observations. Monitoring shellfish toxicities is judged to be less costly than monitoring plankton because much of the work can be done without specially trained staff, without special equipment including boats, and without costly research into behaviour of the causative organism. Even if plankton study were successful, it would still be necessary to continue some shellfish sampling.

Long-range forecasting for the Fundy region has been attempted (Prakash and Medcof 1962) with meteorological and hydrographic data, but these and other biological factors interact with such complexity that useful results are difficult to obtain. With increasing knowledge of factors affecting abundance of *Gonyaulax,* long-range predictions may still be possible.

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# Appendix I

CASE HISTORY PARALYTIC SHELLFISH POISONING

No.....

Name..... Date of Illness.....
Address..... Time of Onset.....o'clock
Sex.....Age.....Weight..... Duration of Illness.....
(hours or days)
General Health..... Date Illness Reported.....
To Whom Reported..... How? (interview etc.).....
Source of Shellfish..... Kind of Shellfish Eaten.....
Other Food or Drink Taken.....
Preparation of Shellfish (raw, steamed, etc.).....
How Much Shellfish Broth or Bouillon Taken?.....
Number of Shellfish Eaten..... Time..... Date.....
Toxicity of Raw Shellfish.....m.u./100g. Estimated Dosage.....m.u.

*Symptoms* (Underline symptoms observed and indicate by number 1, 2, 3, etc., the order in which they appeared.)

Numbness of { Lips / Face }

Difficulty with Speech

Numbness of { Fingers / Toes }

Dizziness or Giddiness

Numbness of { Arms / Legs }

Difficulty with Breathing

Difficulty with { Standing up / Sitting up }

Feeling of Stomach Sickness

Vomiting

Headache

Backache

Other Symptoms

*Any Other Information or Remarks*

# Appendix II

## COMPOSITION OF MARINE MEDIA USED FOR GROWING *GONYAULAX TAMARENSIS*

A. Marine synthetic medium — $ASP_7$ (Provasoli 1963)

| | | | |
|---|---|---|---|
| Distilled $H_2O$ | 100 ml | $Na_2$-glycerophosphate·$5H_2O$ | 2 mg |
| NaCl | 2.5 g | P II metals[a] | 3 ml |
| $MgSO_4 \cdot 7H_2O$ | 0.9 g | Vitamin $B_{12}$ | 0.1 µg |
| KCl | 0.07 g | Vitamin S3 mixture[b] | 1 ml |
| Ca(as $Cl^-$) | 30 mg | NTA (nitrilotriacetic acid) | 7 mg |
| $NaNO_3$ | 5 mg | tris(hydroxymethyl) amino methane | 0.1 g |

*p*H 7.8–8.0

B. Enriched seawater medium — Erd-Schreiber

| | |
|---|---|
| Filtered sea water (double pasteurized) | 1 liter |
| $NaNO_3$ | 0.2 g |
| $Na_2HPO_4 \cdot 12H_2O$ | 0.03 g |
| Soil extract | 50 ml |

[a]1 ml of P II metal mixture contains: B(as $H_3BO_3$), 0.2 mg; Fe(as $Cl^-$), 0.01 mg; Mn(as $Cl^-$), 0.04 mg; Zn(as $Cl^-$), 5 µg; Co (as $Cl^-$), 0.01 mg; $Na_2$-EDTA, 1 mg.

[b]1 ml of vitamin S3 mixture contains: thiamine HCl, 0.05 mg; nicotinic acid, 0.01 mg; Ca pantothenate, 0.01 mg; *p*-aminobenzoic acid, 1.0 µg; biotin, 0.1 µg; inositol, 0.5 mg; oleic acid, 0.2 µg; thymine, 0.3 mg.

# Appendix III

## A.O.A.C. PARALYTIC SHELLFISH POISON BIOASSAY

*(Excerpted from A.O.A.C. Official Methods of Analysis, Tenth Edition, Biological Method 18, 282–284, 1965)*

1.1 *Materials*

1.11 Paralytic shellfish poison standard solution (100 μg/ml). Available from Public Health Service, Washington, D.C. 20201, as acidified 20% alcoholic solution. Standard is stable indefinitely in a cool place.

1.12 Paralytic shellfish poison reference solution (1 μg/ml). Dilute 1 ml-standard solution to 100 ml with $H_2O$. Solution is stable several weeks at 3–4 C.

1.13 Mice. Healthy mice, 19–21 g, from stock colony used for routine assays. If <19 or >21 g, apply correction factor to obtain true death time (see Appendix Table III. 2). Do not use mice weighing >23 g, and do not reuse mice.

1.2 *Standardization of bioassay*

1.21 Dilute 10-ml aliquots of 1 μg/ml reference solution with 10, 15, 20, 25, and 30 ml $H_2O$, respectively until intraperitoneal injection of 1-ml doses into a few test mice causes median death time of 5–7 min; *p*H of dilutions should be 2–4 and must not be >4.5. Test additional dilutions in 1-ml increments of $H_2O$, e.g., if 10 ml diluted with 25 ml $H_2O$ kills mice in 5–7 min, test solutions diluted 10 with 24 and 10 with 26.

1.22 Inject group of 10 mice with each of 2, or preferably 3, dilutions that fall within median death time of 5–7 min. Give one milliliter dose to each mouse by intraperitoneal injection and determine death time as time elapsed from completion of injection to the last gasping breath of mouse.

1.23 Repeat assay 1 or 2 days later, using dilutions prepared above which differed by 1 ml increments of $H_2O$. Then repeat entire test, starting with testing of dilutions prepared from newly prepared reference solution.

1.24 Calculate median death time for each group of 10 mice used on each dilution. If all groups of 10 mice injected with any one dilution gave median death times of <5 or >7 min, disregard results from this dilution in subsequent calculations. On the other hand, if any of the groups of 10 mice injected with one dilution gave a median death time falling between 5 and 7 min, include all groups of 10 mice used on that dilution, even though some of the median death times may be <5 or >7 min. From the median death time for each group of 10 mice in each of the selected dilutions, determine the number of mouse units per ml from Sommer's table. Divide the calculated μg poison/1 ml by the mouse units per ml to obtain conversion factor (CF value) expressing μg poison equivalent to 1 mouse unit. Calculate the average of the individual CF values, and use this average value as a reference point to check routine assays. Individual CF values may vary significantly within a single laboratory if techniques and mice are not rigidly controlled. This situation will require continued use of reference standard or secondary standard, depending on the volume of assay work performed.

1.3 *Use of standard with routine assay of shellfish*

1.31 Check CF value periodically. If shellfish products are assayed less than once a week, determine CF value on each day assays are performed by injecting five mice with an appropriate dilution of the reference standard. If assays are made on several days during each week, only one check need be made each week on a dilution of the standard such that the median death time falls within 5–7 min. The CF value thus determined should check with the average CF value within ± 20%. If it does not check within this range, complete group of 10 mice by adding 5 mice to the 5 mice already injected, and inject a second group of 10 mice with the same dilution of standard. Average the CF value determined for the second group with that of the first group. Take the resulting value as the new CF value. A variation of >20% represents a significant change in the response of mice to poison, or in the technique of assay. Changes of this type require a change in the CF value.

1.32 Repeated checks of CF value ordinarily produce consistent results within ±20%. If wider variations are found frequently, the possibility of uncontrolled or unrecognized variables in the method should be investigated before proceeding with routine assays.

1.4 *Preparation of sample*

1.41 Clams, oysters, and mussels. Thoroughly clean outside of shellfish with fresh water. Open by cutting adductor muscles. Rinse inside with fresh water to remove sand or other foreign material. Remove meat from shell by separating adductor muscles and tissue connecting at hinge. Do not use heat or anesthetics before opening shell, and do not cut or damage body of mollusk at this stage. Collect about 100–150 g of meats in a glazed dish. As soon as

possible, transfer meats to a No. 10 sieve without layering, and let drain for 5 min. Pick out pieces of shell, and discard drainings. Grind in household-type grinder with $\frac{1}{8}$–$\frac{1}{4}$-inch holes, or macerate in blender until homogeneous.

1.42 Scallops. Separate edible portion (adductor muscle) and apply test to this portion alone. Drain and grind as in 1.41.

1.43 Canned shellfish. Place entire contents of can (meat and liquid) in blender, and macerate until homogeneous. For large cans, drain meat in large buchner or sieve and collect all liquid. Determine weight of meat and volume of liquid. Recombine portion of each in proportionate quantities. Macerate recombined portions in blender until homogeneous.

1.5 *Extraction*

1.51 Weigh 100 g of well-mixed material into a tared beaker. Add 100 ml 0.1 N HCl, stir thoroughly, and check *p*H. (*p*H should be <4.0, preferably about 3.0. If necessary, adjust *p*H as indicated below.) Heat the mixture, boil gently 5 min, and let cool to room temperature. Adjust cooled mixture to *p*H 2.0–4.0 (never >4.5) as determined by BDH Universal Indicator, phenol blue, Congo red paper, or *p*H meter. To lower *p*H, add 5 N HCl dropwise with stirring; to raise *p*H, add 0.1 N NaOH dropwise with constant stirring to prevent local alkalinization and consequent destruction of poison. Transfer mixture to graduated cylinder and dilute to 200 ml.

1.52 Return mixture to beaker, stir to homogeneity, and let settle until portion of supernatant is translucent and can be decanted free of solid particles large enough to block a 26-gauge hypodermic needle. If necessary, centrifuge mixture or supernatant for 5 min at 3000 rpm or filter through paper. Only enough liquid to perform bioassay is necessary.

1.6 *Mouse test*

1.61 Inoculate each test mouse intraperitoneally with 1 ml of the acid extract. Note the time of inoculation and observe mice carefully for time of death as indicated by the last gasping breath. Record death time from stopwatch or clock with a sweep second hand. One mouse may be used for the initial determination, but two or three are preferred. If the death time, or the median death time of several mice is <5 min, make a dilution to obtain death times of 5–7 min. If the death time of one or two mice injected with undiluted sample is >7 min, a total of at least three mice must be inoculated to establish the toxicity of the sample. If large dilutions are necessary, adjust the *p*H of the dilution by the dropwise addition of dilute HCl (0.1 or 0.01 N) to *p*H 2.0–4.0 (never >4.5). Inoculate three mice with a dilution that gives death times of 5–7 min.

1.7 *Calculation of toxicity*

1.71 Determine median death times of mice, including survivors, and from Sommer's table determine corresponding number of mouse units. If test animals weigh <19 or >21 g, make correction for each mouse by multiplying mouse units corresponding to death time for that mouse by the weight correction factor for that mouse from Sommer's table; then determine the median mouse unit for the group. (Consider the death time of survivors as >60 min or equivalent to <0.875 mouse unit in calculating the median.) Convert mouse units to μg poison per ml by multiplying by the CF value.

1.72 μg poison/100 g meat = (μg/ml × dilution factor) × 200.

1.73 Consider any value >80 μg/100 g as hazardous and unsafe for human consumption.

APPENDIX TABLE III. 1. Death time:mouse unit relations for paralytic shellfish poison (acid) (Sommer's table).

| Death[a] time | Mouse units | Death[a] time | Mouse units | Death[a] time | Mouse units |
|---|---|---|---|---|---|
| 1:00 | 100 | 4:00 | 2.50 | 9:00 | 1.16 |
| 10 | 66.2 | 05 | 2.44 | 30 | 1.13 |
| 15 | 38.3 | 10 | 2.38 | | |
| 20 | 26.4 | 15 | 2.32 | 10:00 | 1.11 |
| 25 | 20.7 | 20 | 2.26 | 30 | 1.09 |
| 30 | 16.5 | 25 | 2.21 | | |
| 35 | 13.9 | 30 | 2.16 | 11:00 | 1.075 |
| 40 | 11.9 | 35 | 2.12 | 30 | 1.06 |
| 45 | 10.4 | 40 | 2.08 | | |
| 50 | 9.33 | 45 | 2.04 | 12:00 | 1.05 |
| 55 | 8.42 | 50 | 2.00 | 13:00 | 1.03 |
| | | 55 | 1.96 | 14:00 | 1.015 |
| 2:00 | 7.67 | | | 15:00 | 1.000 |
| 05 | 7.04 | 5:00 | 1.92 | 16:00 | 0.99 |
| 10 | 6.52 | 05 | 1.89 | 17:00 | 0.98 |
| 15 | 6.06 | 10 | 1.86 | 18:00 | 0.972 |
| 20 | 5.66 | 15 | 1.83 | 19:00 | 0.965 |
| 25 | 5.32 | 20 | 1.80 | 20:00 | 0.96 |
| 30 | 5.00 | 30 | 1.74 | 21:00 | 0.954 |
| 35 | 4.73 | 40 | 1.69 | 22:00 | 0.948 |
| 40 | 4.48 | 45 | 1.67 | 23:00 | 0.942 |
| 45 | 4.26 | 50 | 1.64 | 24:00 | 0.937 |
| 50 | 4.06 | | | 25:00 | 0.934 |
| 55 | 3.88 | 6:00 | 1.60 | | |
| | | 15 | 1.54 | 30:00 | 0.917 |
| 3:00 | 3.70 | 30 | 1.48 | 40:00 | 0.898 |
| 05 | 3.57 | 45 | 1.43 | 60:00 | 0.875 |
| 10 | 3.43 | | | | |
| 15 | 3.31 | 7:00 | 1.39 | | |
| 20 | 3.19 | 15 | 1.35 | | |
| 25 | 3.08 | 30 | 1.31 | | |
| 30 | 2.98 | 45 | 1.28 | | |
| 35 | 2.88 | | | | |
| 40 | 2.79 | 8:00 | 1.25 | | |
| 45 | 2.71 | 15 | 1.22 | | |
| 50 | 2.63 | 30 | 1.20 | | |
| 55 | 2.56 | 45 | 1.18 | | |

[a]Minutes:seconds.

APPENDIX TABLE III. 2. Correction table for weight of mice.

| Wt of mice (*g*) | Mouse units | Wt of mice (*g*) | Mouse units |
|---|---|---|---|
| 10 | 0.50 | 17 | 0.88 |
| 10.5 | 0.53 | 17.5 | 0.905 |
| 11 | 0.56 | 18 | 0.93 |
| 11.5 | 0.59 | 18.5 | 0.95 |
| 12 | 0.62 | 19 | 0.97 |
| 12.5 | 0.65 | 19.5 | 0.985 |
| 13 | 0.675 | 20 | 1.000 |
| 13.5 | 0.70 | 20.5 | 1.015 |
| 14 | 0.73 | 21 | 1.03 |
| 14.5 | 0.76 | 21.5 | 1.04 |
| 15 | 0.785 | 22 | 1.05 |
| 15.5 | 0.81 | 22.5 | 1.06 |
| 16 | 0.84 | 23 | 1.07 |
| 16.5 | 0.86 | | |

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