

# FISHERIES RESEARCH BOARD OF CANADA

## MANUSCRIPT REPORT SERIES

No. 1163

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Winter variability in paralytic shellfish poison  
scores for Crow Harbour, New Brunswick

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### Establishment

Biological Station  
St. Andrews, N.B.

**Dated** December 1971

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## Abstract

Shellfish from Crow Harbour are monitored for paralytic shellfish poison content because toxicity increases usually occur there a week or ten days before they begin in other parts of the Bay of Fundy. Crow Harbour is an early-warning station of approaching hazards of shellfish poisoning. However, between January and May 1971, the monitoring scores varied so much that they seemed unlikely to serve this purpose. The poison source organism (Gonyaulax tamarensis) was not found in the May plankton and it was concluded that the poison in the shellfish was a carry-over of unexcreted poison accumulated during the summer of 1970. The scores of shellfish from the monitoring station should therefore have been stable because poison excretion at low temperatures is slow.

It is known that shellfish toxicity varies from place to place in any area and investigation indicated that 1971 winter and spring samples of Crow Harbour shellfish were not always taken from the same place because the monitoring station had no permanent marker. This and the error inherent in the bioassay method seemed to be the likely source of most, if not all, the observed score variation.

A step-by-step study of the sampling procedure was made to discover other sources of variation. When summer samples of toxic shellfish are stored their scores rise by as much as 100% within four days, then decrease. For strict comparability of samples, it is therefore necessary to standardize their storage periods (times between fishing and extraction for bioassay). Records show that storage periods of Crow Harbour winter samples varied and variations in periods of storage may have contributed to their score variations. However, there is reason to expect that storage might have less effect on scores of winter samples than on scores of summer samples. The winter records for Crow Harbour seem to fulfil this expectation.

After the sampling station was permanently marked in May 1971, there were fewer erratic changes in monitoring scores and the Crow Harbour program served its purpose as an early-warning station for 1971 summer rises in shellfish toxicity in the Bay of Fundy. Precautions like those taken in Crow Harbour are needed wherever shellfish toxicity is to be monitored.

WINTER VARIABILITY IN PARALYTIC SHELLFISH POISON  
SCORES FOR CROW HARBOUR, NEW BRUNSWICK

THE CROW HARBOUR PROBLEM

Crow Harbour, sometimes called Crow Cove (N.Lat. 45°06'; W.Long.66°37') is an inlet on the Bay of Fundy coast of Charlotte County and approximately two miles west of Pocologan Harbour. It is used by the Fisheries Service of Environment Canada as a Key Area (Medcof in Prakash, Medcof and Tennant, in press) in its efforts to limit risks of paralytic shellfish poisoning. Fisheries Officers take monitoring samples of soft-shell clams (*Mya arenaria*) there because annual rises in their toxicity usually begin about a week or ten days earlier than in other parts of the Bay of Fundy. This early warning allows the Fisheries Service time to quarantine some fisheries and alert the public to hazards of paralytic shellfish poisoning.

Records (Boyd and Lachance, 1970; Boyd and Turgeon, 1971) show that Crow Harbour shellfish from the key station in West Creek are often toxic the year-round. Scores<sup>1</sup> may be high in summer but from November to June they are usually low and so near to being stable that their variations can be reasonably attributed to error inherent in the bioassay method (Quayle, 1969). However, the monitoring sample taken by the Fisheries Service on 20 April 1971 indicated a large and unexpected increase in toxicity. On 23 April and 5 May Fisheries Service took spot samples from neighbouring sites and detected even greater variations. They feared that this was an abnormal situation and that if the variation continued, they would find it difficult or impossible to detect the first small 1971 summer rises in shellfish toxicity.

Detecting the first small rises is basic to the region's molluscan shellfish fisheries management scheme. Under

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<sup>1</sup> Scores are the numerical results of bioassays of shellfish for paralytic poison content and indicate the number of micrograms present in 100 g of shellfish meat. With our bioassay method, the minimum detectable score is 44. Scores for samples that test negative in bioassays are reported as <44 which, for practical purposes, are taken as zero.

this scheme, quarantines are imposed on fishing areas during periods when their shellfish scores exceed prescribed limits. So unusual variations in the 1971 monitoring scores for Crow Harbour posed a real public health and fisheries management problem.

The Fish Inspection Branch of the Fisheries Service requested cooperation from the Fisheries Research Board's St. Andrews Biological Station in studying the Crow Harbour situation in the hope of explaining it and of possibly finding ways of reducing the score variations.

The Crow Harbour situation is not unique. Similar cases have arisen in other areas. They will probably arise again and the Fisheries Service will have to deal with them. Some of the principles involved in this study (for example, changes in toxicity of samples during live storage) are neither generally recognized nor understood. These are our reasons for circulating this report on the Crow Harbour investigation.

#### WINTER VARIABILITY, 1971

Table 1 is compiled from the Fish Inspection Laboratory's records and lists 1971 Crow Harbour toxicity scores for soft-shell clam samples, most of which were made by the Conservation and Protection Branch of the Fisheries Service. The samples were dug in different areas as indicated (Table 1 and Figure 1). The same data are displayed graphically in Figure 2. Between 6 January and 5 May, the scores varied more than 500% (48 to 350) if all samples are considered, and by more than 400% if only samples from the key station are considered (66 to 290). It is known that the bioassay "error" in estimating low scores is greater than that in estimating high scores (Quayle, 1969). However, even when this was allowed for, the 1971 Crow Harbour variations seemed high and were specially high in April compared with winter samples in 1969 and 1970 (Fig. 2).

The dinoflagellate, Gonyaulax tamarensis, is the only known source of shellfish poison in eastern Canada. It occurs in the summer plankton of parts of the Bay of Fundy and parts of the estuary of the St. Lawrence but it is not a component of our winter plankton and inshore shellfish are usually poison-free in winter (Needler, 1949; Prakash, in Prakash, Medcof and Tennant, in press). We have many records of onset of cold weather before shellfish have excreted all the poison they accumulated during the preceding summer-autumn season. In

these instances, they remain toxic throughout the winter and show little or no variation in toxicity scores during winter. It is deduced from this that winter toxicities are stable because low temperatures retard poison excretion by shellfish (Medcof, in Prakash, Medcof, and Tennant, in press). In a few cases like Crow Harbour, shellfish are toxic in winter regardless of whether cold weather sets in early or late. To date, this has not been explained.

The suggestion has been made (Medcof et al., 1947; Bourne, 1965) that shellfish may feed on benthic spores of G. tamarensis which may carry poison and that this accounts for winter toxicity and winter variations in toxicity scores for shellfish. However, there are no firm data to support these speculations and we usually assume that winter-spring variations are not caused by true changes in toxicity.

Two possible explanations of the 1971 Crow Harbour variations were examined. The first was examined on special request from the Fisheries Service.

1. The causative organism possibly did occur in Crow Harbour during the winter and spring of 1971, even though this behaviour would be inconsistent with what has been previously observed. Possibly its abundance varied and possibly the toxicity of the clams varied with the changing abundance of this plankter.

2. There were no true changes in toxicity of Crow Harbour clams and the apparent changes in toxicity resulted from inappropriate application of procedures in monitoring toxicity scores. This explanation is based on earlier demonstrations that methods of collecting field samples, methods of treatment of samples after collection and methods of laboratory processing of samples all affect bioassay results.

#### BIOLOGICAL AND HYDROGRAPHIC CONDITIONS 1971

A plankton collection with a number 18 bolting cloth net was taken on 5 May in West Creek near the regular sampling station (Fig. 1). Microscopic study of the collection showed the presence of green algae (sections of Ulva and Enteromorpha and a filamentous variety we could not identify); Diatoms (Navicula, Melosira and Coscinodiscus) and Dinoflagellates (Ceratium only)). We looked hard for Gonyaulax but found none of any species.

The surface water temperature at the plankton station on 5 May was 4.7°C. From her study of G. tamarensis, Needler (1949) concluded that its cysts do not sporulate to give rise to populations of the flagellated form of this species until water temperatures reach 10°C.

The salinity of the water at the plankton station was 22‰. We lack Crow Harbour salinity data for other years but the 5 May 1971 record seems normal for a Bay of Fundy inlet of its type at this season. Surface salinity in St. Croix River at the Biological Station, St. Andrews, was 21‰ on 14 May 1971 and this salinity is favourable to growth of G. tamarensis (Prakash, in Prakash, Medcof and Tennant, in press).

Our conclusions are that G. tamarensis was not present and would not be expected to be present in Crow Harbour under the conditions observed 5 May 1971.

#### LIKELY CAUSES OF VARIATION

The above findings indicate that the first possible explanation of score variation is unlikely and that the true explanation should be sought elsewhere. We therefore examined each step in the monitoring procedure (from field collection of shellfish samples to the preparation of their extracts), looking for likely causes of variation.

#### Site of key area sampling station

Discussions with the local Conservation and Protection Officer showed that on 1 April 1971 there was a change in officers responsible for gathering the Crow Harbour shellfish samples for monitoring toxicity. It also appeared that all samples fished after 1 April may not have been taken from exactly the same site because of tidal variations and because there was no permanent marker for the sampling station. This is likely to produce variability in bioassay results for three reasons.

1. Beach transect sampling (Medcof et al., 1947) shows that toxicity of intertidal shellfish is low near high-water mark (HWM) in the Fundy region and increases by as much as 40% toward low-water mark (LWM).

2. Toxicities of clams taken from beaches near the heads of harbours are low and those of clams from beaches near the mouths are high--up to ten times as high as those near the heads. Table 1 refers to the station near the head of Crow Harbour as the "high flat" and to the station near the mouth as the "low flat". These terms do not refer to the level in the intertidal zone.
3. In many Bay of Fundy areas the mean size of clams varies from place to place and there is evidence that toxicity of small clams is up to 15% higher than that of large clams taken at any given site (Medcof et al., 1947). We were unable to determine whether there were differences in the average sizes of shellfish sampled in 1971 but this seems possible if not likely.

In the hope of reducing variability, our Mr. D. E. Graham, who was responsible for FRB field work in this study, assisted the fisheries warden on 5 May in marking the key sampling station (Fig. 1) by driving a steel pipe into the ground. The site is readily accessible at half-tide and it has an adequate stock of medium-sized clams.

#### Changes in scores after fishing

Summer samples. Toxicity scores of summer samples of living shellfish change after they are fished (Medcof et al., 1947). At first the scores rise suddenly then decrease more slowly (Fig. 3). As well as I can recall, extracts whose scores are plotted in Figure 3 were prepared from samples within 24 hours of collection.

Changes in toxicity of living shellfish during storage seem to have been observed only on our Atlantic coast where shellfish derive their poison from G. tamarensis. Apparently this poison has two interacting components that differ in their potency and other characteristics. Evidence of this emerged from canning tests in 1945 (Medcof et al., 1947) and from recent work in the U.K. (Evans, 1970). Interactions of these components may explain the peculiar changes shown in Figure 3.

The rise in toxicity scores during live storage may exceed 100% and occurs regardless of whether the shellfish are held in flowing sea water or air-stored at room temperatures, ^  
at temperatures

slightly above freezing or at temperatures well below freezing (-15 to -20°C). Figure 3 suggests that this cycle is completed more quickly when shellfish are held in air at 15°C than in water at slightly lower temperatures. For shellfish that are held in water, the rising scores reach their maximum about 96 hours after fishing.

These phenomena are not understood but should be allowed for whenever there is need for detecting small, true changes in toxicity - for example, when monitoring key areas. Tennant (in Prakash, Medcof and Tennant, in press) was aware of this and advocated standardizing the treatment of samples from the moment they are collected in the field until they are extracted. This should include standardizing air-storage temperatures and time intervals between collection and extraction. This regularization could help reduce variability of bioassay scores like those observed at Crow Harbour in 1971.

Winter samples. We say "could" above because to date it has not been shown that scores of winter samples change after fishing like those of summer samples. We searched the Crow Harbour data for evidence of score changes during storage. Table 1 shows that the interval between collection and extraction varied from 0 to 3 days but was usually one day. Shellfish toxicity scores for the period 6 January to 27 July 1971 were plotted against the lengths of the intervals (days) between digging and extraction of the corresponding samples (Fig. 4).

The scattering of points for samples taken from the regular sampling station (Fig. 4) suggests that scores were about the same throughout the period and that score levels were about the same regardless of whether samples were stored 0, 1, 2 or 3 days before extraction.

Actually, the situation cannot be fully assessed from this simple analysis because measurements of time intervals between collections and extractions do not fully describe the treatments of samples. The storage intervals were composite, consisting of a period of air storage in the warden's automobile and a period of air storage in the Fish Inspection Laboratory. The lengths of both these periods varied (Table 1).

#### Holding by the Fisheries Warden

We cannot say how any particular sample was handled while it was in custody of the fisheries warden. Sample collection times varied with tidal cycles, and the warden's other duties sometimes interfered with his delivery of samples

to the laboratory. He usually delivered them on the collection date but sometimes on the first or second day following collection (Table 1) and conditions under which he held them probably varied. He may have carried them in the body of his automobile (warm during the day and cool at night) or in the trunk (cool at all times).

Figure 3 suggests that the cycle of changing scores of summer samples is completed more rapidly at high than at low temperatures even though the amplitude of changes may be about the same. So, some variation in winter-spring sample scores might be expected from the variety in ways of handling and in the lengths of the periods of holding by the warden.

#### Laboratory Holding

Staff at the Black's Harbour Fish Inspection Laboratory, where extractions for bioassay are prepared, stated that after shellfish samples reach the laboratory their conditions of holding are uniform: they are stored in plastic bags in a refrigerator at 5°C (40°F). However, there are differences in the length of the storage period (Table 1). Thus, laboratory storage may also be a source of score variation where summer samples are involved.

#### Extraction Procedure

Table 1 shows that the "pH" of extracts prepared from Crow Harbour samples were well within the range (3.2-3.8) that assures stability of their toxicity. And the staff of the laboratory assured us that all extraction procedures were standard (Tennant, in Prakash, Medcof and Tennant, in press). This suggests that variations in scores are not attributable to irregularities in extracting procedures.

#### Possible Differential Effects of Handling on Scores of Summer and Winter Samples

The failure to find a relationship between variations in scores of Crow Harbour samples and the lengths of their periods of live storage in air suggests that scores of winter and summer samples respond differently to storage treatments.

In summer the scores of toxic soft-shell clams are high and the poison is concentrated in the digestive gland and gills. In winter the scores are low and the poison is concentrated in the gills (Medcof, in Prakash, Medcof and Tennant, in press). Apparently poison stored in the digestive gland is mobile and

readily excreted and poison stored in the gills is immobile and hard to excrete at low temperatures. If true this would account for the stability of winter scores of most stocks of toxic shellfish. It would also account for our failure to find support in Figure 4, for the idea that winter score variations are affected by the length of the period of storage and by methods of handling of samples between collection and extraction.

Figure 3 demonstrates the need for standardizing holding treatment of summer samples. Ideally they should all be extracted when they are in the same phase of their cycle of toxicity changes. This should assure strict comparability of results. More information is needed on characteristics of these cycles for various species. But it seems clear that conditions of holding (e.g. the medium (air or water) and the temperature of the medium) should be fixed and that the time intervals between digging and extraction should also be fixed.

Where winter samples are concerned it may be less important to take these precautions. The results suggest that irregular treatments of January-May 1971 Crow Harbour samples, by the warden and by the Fish Inspection Laboratory, did not affect toxicity scores. However, this has not been demonstrated in a controlled experiment. Until this is done, it seems wise to standardize the handling of both winter and summer samples.

#### SUMMARY AND CONCLUSIONS

1. Samples of soft-shell clams are regularly taken from the key area sampling station in Crow Harbour for monitoring changes in their paralytic shellfish poison content. Ordinarily the Crow Harbour scores are low and stable during winter and spring but on 20 April 1971 they rose alarmingly. A study of a 5 May sample of Crow Harbour plankton showed no G. tamarensis and temperature observations made on the same date showed that conditions were unfavourable to its presence. It was concluded that no true changes in toxicity had taken place in April and that score variations were artifacts possibly attributable to inconsistencies in sampling procedures.
2. The Fisheries Service officers sampled the harbour extensively after 20 April and found place-to-place variations in toxicity scores of Crow Harbour shellfish. These variations were less extreme than have been observed in some harbours but greater than those reported for monitoring samples taken from the Crow Harbour key area sampling station before 20 April.

The site of this station was not precise until a marker was set up there on 5 May. Since then the monitoring scores have shown no startling variability. This indicates that the 20 April rise is attributable to a change in the sampling site. It also supports the conclusion that there was no true change in winter toxicities of the shellfish.

3. Fixing the sampling station overcame the problem that prompted this study. After 5 May 1971 the variations in scores of monitoring samples were so small that they did not obscure the late-July jump in Crow Harbour scores that heralded the onset of high summer toxicities in the middle and lower reaches of the Bay of Fundy. This result is a strong recommendation for fixing and marking all shellfish toxicity monitoring stations.
4. Fluctuations in monitoring scores after 5 May were small enough to be attributed entirely to the error inherent in the bioassay method. However, other steps in sampling procedures were examined to see if there were also inconsistencies in them that might have contributed to variations.  

There was irregularity in the interval between digging and processing (extraction) of samples. The fisheries warden and the Fish Inspection Laboratory sometimes held samples for shorter or longer periods than usual. These differences were great enough to affect bioassay results of summer samples because toxicities of summer samples rise suddenly by as much as 200% during storage, then decrease more slowly. If such changes took place in the Crow Harbour winter-spring samples, they should have been conspicuous and might be expected to show up in analysis but none was apparent. This suggests that toxicity scores of winter-spring samples do not react to storage treatments like scores of summer samples.

5. Three other recommendations are implied in various sections of this report. First, where it is important to have strictly comparable scores for several samples, precautions should be taken to extract them when they are at the same phase in their post-fishing cycle of changes in toxicity. Little is known about this cycle but what is known seems to justify the need for standardizing all treatments of all samples after digging and before extraction. The medium of storage (air or water), the temperature of the medium and the length of time between digging and extraction

should all be the same. This is important for summer samples but may be less important for winter samples. Second, there is need for studying variations in toxicity scores of both summer and winter samples of shellfish while they are stored alive after fishing and before extraction. The fact that there are changes in summer samples is not generally appreciated and their importance in toxicity monitoring for fisheries management is seldom taken into account. Third, there is a need for studies of the chemistry of paralytic shellfish poison along lines indicated by Evans, so as to provide basic understanding of the factors involved in phenomena described here.

#### ACKNOWLEDGMENTS

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Table 1. Data on 1971 toxicity samples of soft-shell clams from the Crow Harbour area. Except where otherwise indicated, samples came from the "regular" station. The four numbered stations, sampled only once or twice (see Fig. 1) were: (1) Ward's Creek, (2) Paddy's Trap, (3) Middle flat and (4) Upper flat.

DUG	RECEIVED	EXTRACTED	DAYS STORED	pH of EXTRACT	SCORE
6 Jan.	6 Jan.	7 Jan.	1	3.2	160
21 "	21 "	22 "	1	3.4	66
10 Feb.	10 Feb.	11 Feb.	1	3.5	90
3 Mar.	3 Mar.	5 Mar.	2	3.3	100
24 "	24 "	25 "	1	3.2	120
6 Apr.	6 Apr.	8 Apr.	2	3.4	100
20 "	21 "	22 "	2	3.3	290
23 "	23 "	24 "	1	3.4	130(1)
23 "	23 "	24 "	1	3.4	75(2)
23 "	23 "	24 "	1	3.5	120
23 "	23 "	24 "	1	3.2	350(3)
23 "	23 "	24 "	1	3.3	180(4)
29 "	29 "	29 "	0	3.4	120
3 May	4 May	6 May	3	3.6	140
5 "	5 "	6 "	1	3.5	150(1)
5 "	5 "	6 "	1	3.6	48(2)
5 "	5 "	6 "	1	3.2	150
11 "	12 "	13 "	2	3.5	120
18 "	19 "	20 "	2	3.4	150
26 "	26 "	27 "	1	3.5	150
1 June	2 June	3 June	2	3.3	190
8 "	8 "	10 "	2	3.6	130
15 "	15 "	17 "	2	3.4	110
21 "	21 "	24 "	3	3.3	110
29 "	29 "	30 "	1	3.4	200
8 July	8 July	9 July	1	3.2	130
14 "	14 "	15 "	1	3.5	120
21 "	21 "	22 "	1	3.4	100
27 "	27 "	29 "	2	3.6	140
4 Aug.	4 Aug.	5 Aug.	1	3.7	500
11 "	11 "	12 "	1	3.6	510
16 "	17 "	19 "	3	3.6	450
23 "	24 "	26 "	3	3.5	240

Continued

Table 1 Continued

DUG	RECEIVED	EXTRACTED	DAYS STORED	pH of EXTRACT	SCORE
1 Sept.	1 Sept.	2 Sept.	1	3.7	170
7 "	7 "	9 "	2	3.3	100
14 "	14 "	16 "	2	3.5	85
22 "	22 "	23 "	1	3.2	64
29 "	29 "	30 "	1	3.8	86
6 Oct.	6 Oct.	7 Oct.	1	3.6	87
13 "	14 "	14 "	1	3.3	70
27 "	27 "	28 "	1	3.8	69
9 Nov.	9 Nov.	12 Nov.	3	3.3	140
24 "	24 "	25 "	1	3.6	74

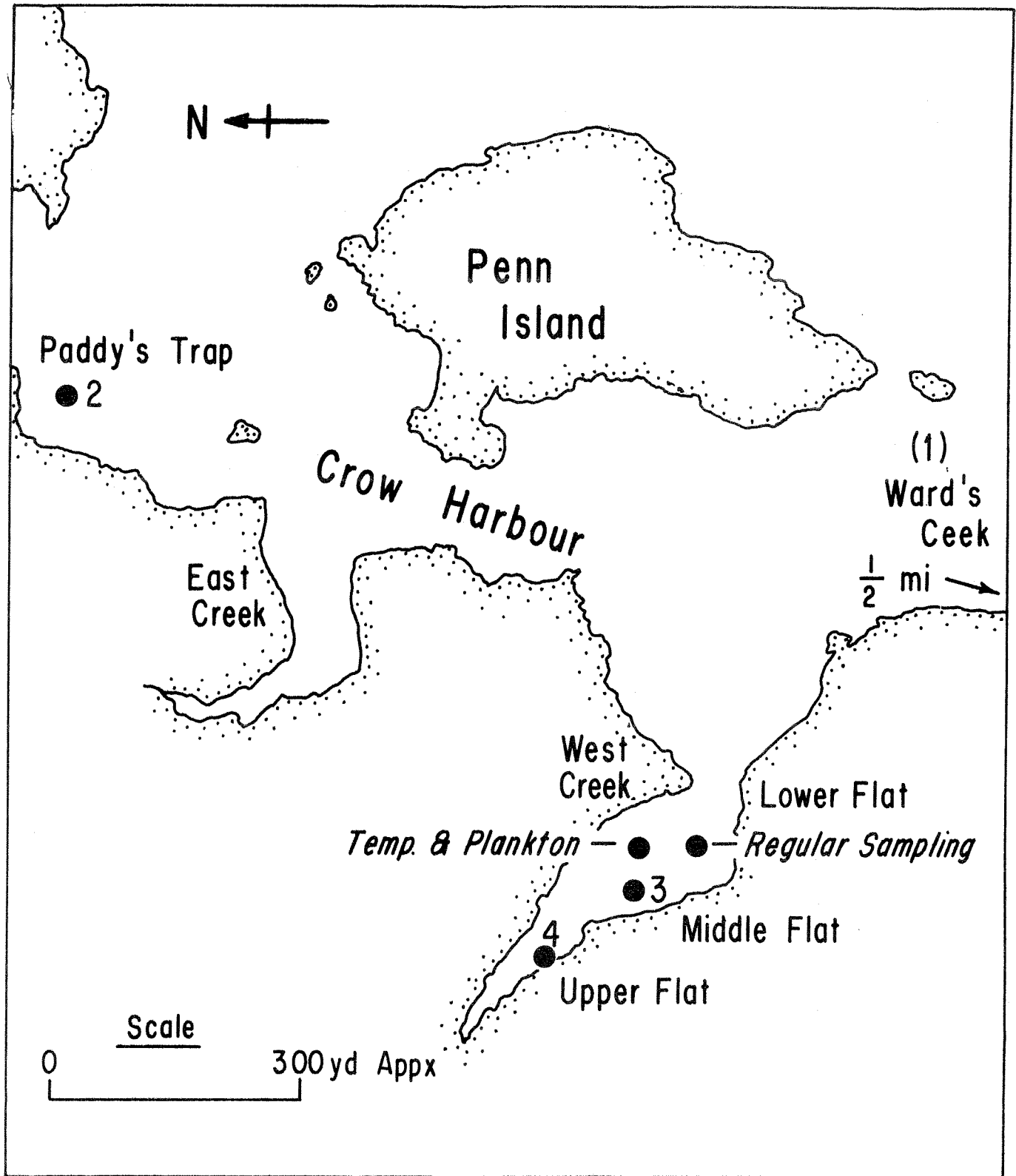


Figure 1. Shoreline of Crow Harbour, N.B., traced from aerial photograph 553-4, New Brunswick Department of Resources, and showing various sampling stations.

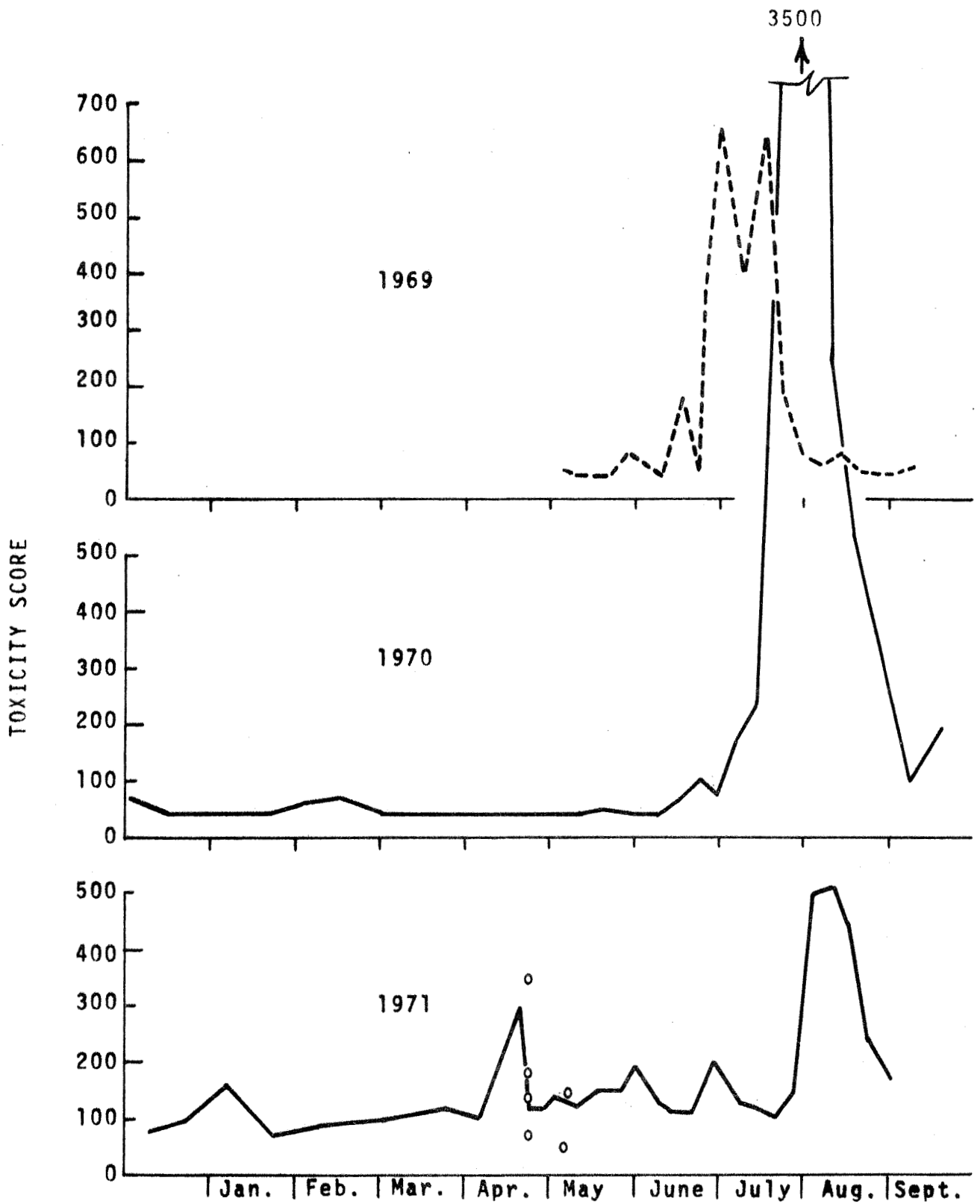
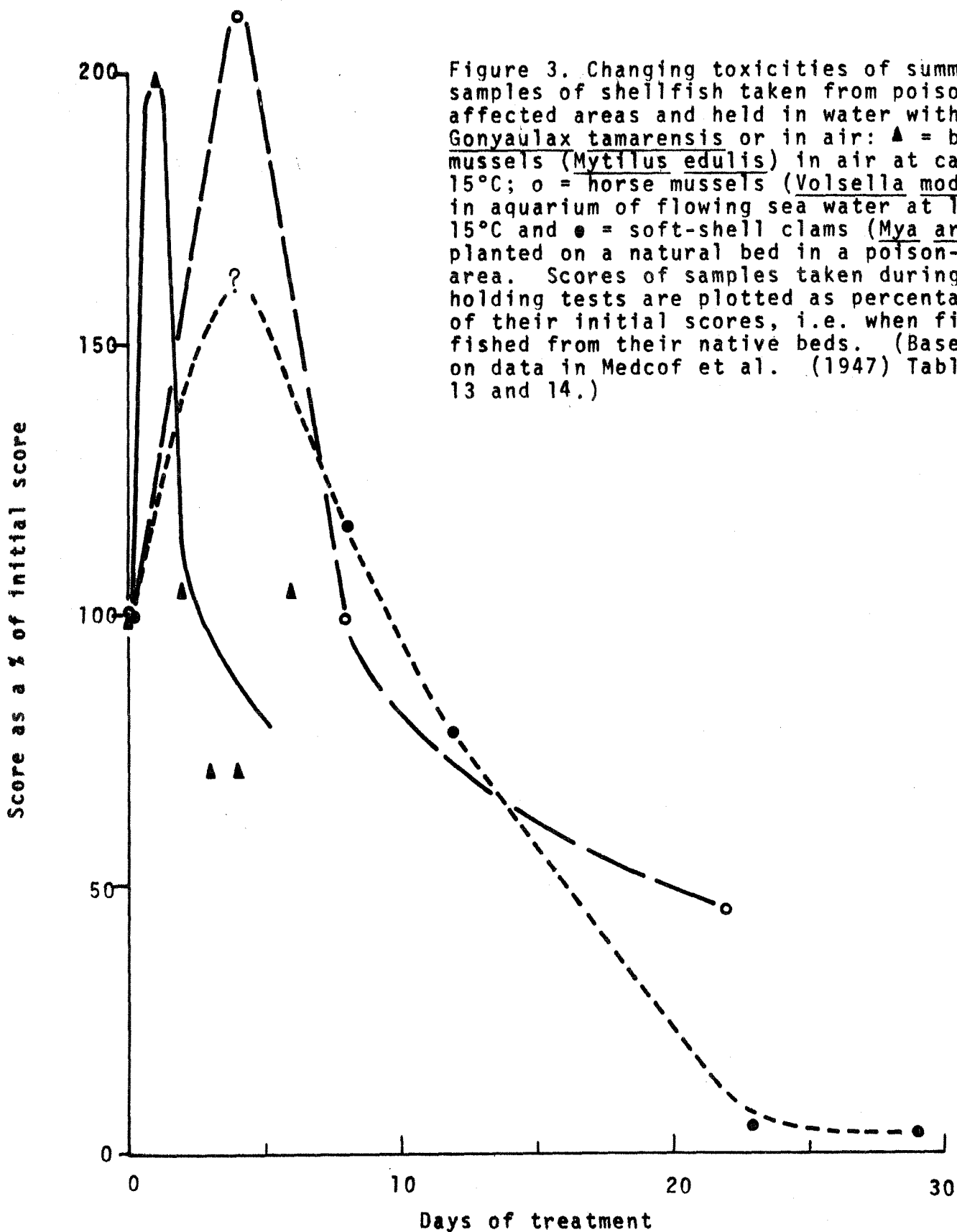


Figure 2. Toxicity scores for soft-shell clam samples taken from Crow Harbour, N.B., between January and September, 1969-1971. o = special samples (see Table 1).



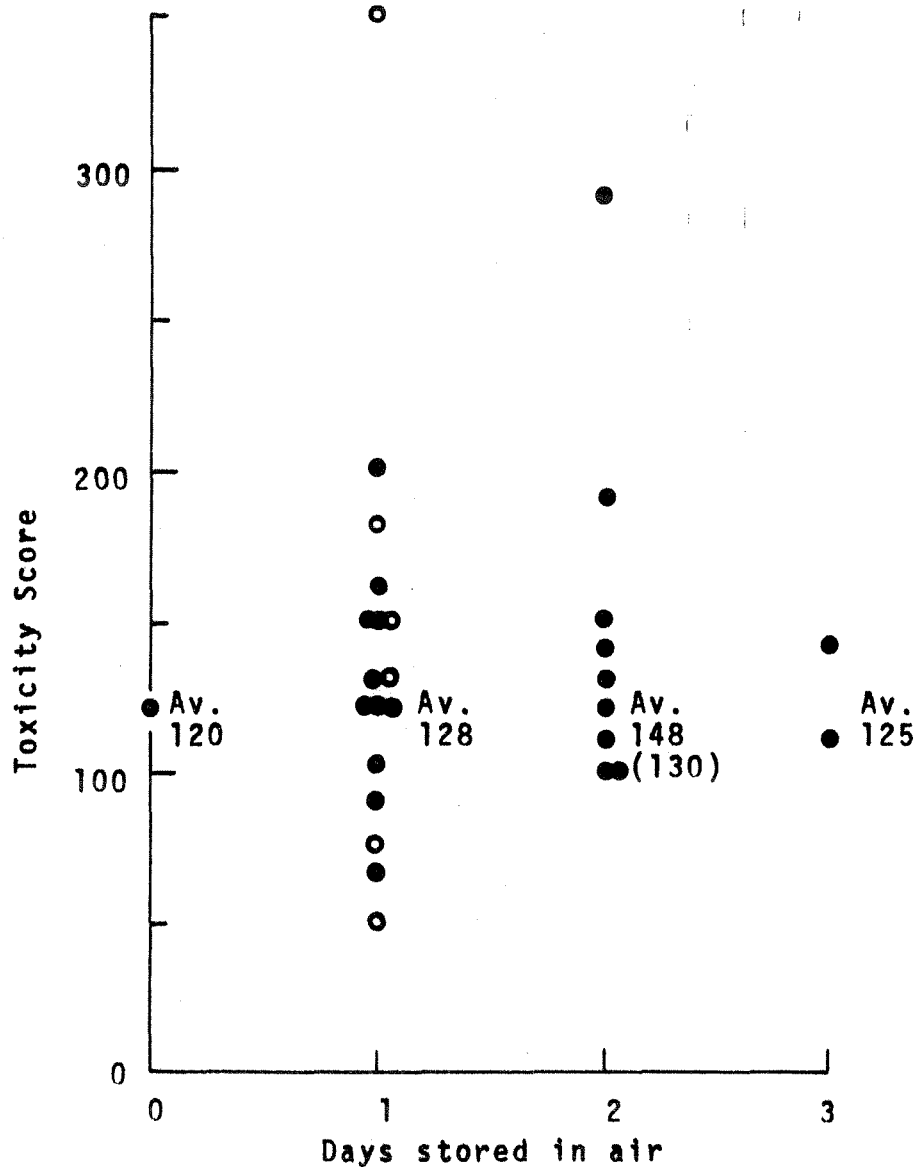


Figure 4. Toxicities of soft-shell clams from Crow Harbour district (6 January to 27 July 1971 grouped according to number of days samples were held alive in air before being extracted. ● = clams from regular sampling station. ○ = samples from other sites (see Table 1). Averages are for samples from regular station only. Bracketed value = average when the 20 April score of 290 is excluded from the calculation.