GEOGRAPHICAL AND SEASONAL PATTERNS OF PARALYTIC SHELLFISH TOXICITY IN WASHINGTON [1]

By

Albert K. Sparks, Arporna Sribhibhadh and Kenneth K. Chew [2]

Introduction:

The occurrence of paralytic shellfish poisoning on the West Coast has been well documented. McFarren, et al. (1960) summarized the occurrence and distribution of toxic shellfish in this area and discussed the ecology of both the causative organism and the shellfish. Davies, et al. (1958) discussed shellfish toxicity in cultivated oysters and Anderson (1960) described the 1957 outbreak of toxicity in British Columbia, discussing the toxicity levels among various species of shellfish and the symptomatology and scope of human cases. Both McFarren, et al. and Anderson pointed out the need for additional field research on the ecology of paralytic shellfish poisoning in the hope of developing a means of predicting outbreaks of toxicity in shellfish.

In October, 1960, a grant was obtained from the National Institutes of Health and later transferred to the Public Health Service to study the ecology of paralytic shellfish poisoning in Washington. This grant has been continued for three additional years to September 30, 1965.

Materials and Methods

In the present study, four floating stations were set up, three along the Strait of Juan de Fuca and one at Stackpole in Willapa Bay. For the three Strait locations, stations were placed in Clallam Bay, Crescent Bay, and Sequim Bay (Fig. 1).

1/ This work was supported by a grant from the Public Health Service, National Institutes of Health, Division of Research Grants.

2/ Contribution No. ___ from the College of Fisheries, University of Washington.

Four species of non-toxic shellfish were placed in each of eight woven-wire baskets at each site (Figure 2). These consisted of Pacific oysters (Crassostrea gigas), soft-shell clams (Mya arenaria), California mussels (Mytilus californianus), and butter clams (Saxidomus giganteus). Although the study was initiated October 1, 1960, field investigations did not begin until February 22, 1961. At each station one specimen of each species was collected from each basket at two week intervals during the period of low or undetectable toxicity, and each week during the summer when the toxicity may be high. Toxicity levels of each species were determined by standard mouse bioassay (McFarren, et al., 1960), and reported in micrograms (mmg) of toxin per 100 grams of shellfish meat. The level of 80 mmg. is considered unsuitable for commercial distribution by the public health authorities. Additionally, to ascertain the validity of data from the floating stations, samples of eight specimens of each available species from the natural environment were assayed. These regularly included butter clams and soft clams from Sequim Bay, California mussels from Agate Beach (near Crescent Bay) and from Slip Point, Clallam Bay, and oysters from commercial beds at Stackpole Harbor, Willapa Bay.

A supply of purified toxin was obtained from Dr. Edward J. Schantz and using the method outlined by Schantz, et al., (1958) determination of the correction factor (CF value) for the mice used in this study was made. (See Table 1.) Plankton samples, consisting of one liter grab samples, were taken at each visit. These were immediately fixed by adding neutralized formalin to give a 5 percent solution. The samples were then allowed to settle for one week and the dinoflagellates isolated and counted in a Palmer nannoplankton counting cell.

Recording thermographs are maintained at each station so that a constant record of water temperature is available. Dissolved oxygen, salinity, pH, nitrates, phosphates and silicates are determined from water samples from each site at each station check.

Table 1. DETERMINATION OF CF VALUE FOR MOUSE-BIOASSAY

Dilution	ml of stock solution 1/	ml of dist. water	Amt. of poison mmg/ml	Median m.u. m.u./ml	CF value mmg/m.u.
A	10	30	0.2500	1.574	0.1588
B	10	30	0.2500	1.415	0.1766
C	10	30	0.2500	1.615	0.1548
D	10	31	0.2439	1.592	0.1532
E	10	31	0.2439	1.234	0.1975
F	10	31	0.2439	1.455	0.1676
G	10	32	0.2381	1.450	0.1641
H	10	32	0.2381	1.321	0.1801
I	10	32	0.2381	1.388	0.1715

Mean CF value = 0.1694
Standard deviation = 0.0140 (or 8.3%)
Standard error of the mean = 0.0047

1/ The stock standard solution contained one microgram of purified crystalline poison per ml.

Mouse-units per ml for each mouse in the groups (10 mice per dilution) are as follows:

A	B	C	D	E	F	G	H	I
1.935	1.661	1.985	1.455	0.944	1.601	1.290	survived	1.211
1.000	1.484	1.678	1.373	1.302	1.709	1.208	1.451	1.149
1.612	1.732	1.744	survived	1.401	1.549	1.564	1.287	1.234
1.057	0.983	1.324	1.678	1.431	1.196	1.490	1.537	1.255
1.746	1.080	1.248	1.409	1.233	1.487	1.180	1.936	1.381
2.056	1.183	1.448	1.581	1.236	1.424	1.461	1.354	1.394
1.623	1.347	1.329	1.731	1.027	0.945	2.009	survived	1.930
1.536	1.605	1.579	1.603	1.184	1.361	1.262	1.264	1.415
1.426	1.501	1.650	1.749	1.377	1.648	1.714	1.422	1.657
1.454	1.252	1.751	2.080	1.150	1.167	1.440	1.109	1.753
Mean= 1.544	1.383	1.574	1.629	1.228	1.409	1.462	1.421	1.438
S.D.= 0.336	0.254	0.233	0.217	0.159	0.243	0.254	0.244	0.259

Sunshine records are obtained from the weather stations at Tatoosh Island, Washington, and Vancouver Island, British Columbia.

From the above data it is hoped that the following information can be obtained; under identical field conditions the species of shellfish that first attains high levels of toxicity; the species that becomes the most toxic; the retention rates of the four species; the geographic and seasonal pattern of toxicity and the planktonic and environmental features associated with developing toxicities.

Results

Toxicities in Willapa Bay:

The site selected for a station in Willapa Bay was Stackpole Harbor, the location in which toxic oysters were first found by Sommer in 1941 (McFarren, et al., 1960). Toxicities to date (July 1962) at the Willapa station have never reached levels higher than 35 mmg. per 100 grams of meat. From the latter part of June to the middle of July, 1961, a low peak developed in the California mussel, but no detectable toxin has occurred in mussels since that time. Low level of toxicity has occurred in a single instance (26 mmg. on June 29, 1961) in oysters from the float; but detectable levels have never been found in neither the oysters from the commercial beds, nor the soft-shell and butter clams from the float.

Preliminary examinations of the plankton samples reveal the presence of the dinoflagellates of the genus Gonyaulax in the waters as early as the first week of May, which coincides with the first occurrence of toxicity of detectable level in the California mussels from the float. Although no detectable toxin was found after the last week of July, the dinoflagellates were present in the plankton samples through the middle of August. These organisms have not yet been observed in the 1962 plankton samples. These forms are very similar, if not identical, to those observed in plankton samples from the Straits. Their chain formations are closely

comparable to those of G. catenella as described by Kofoid (1936). Other dinoflagellates found in isolated samples are Dinophysis spp., Ceratium spp., and Noctiluca scintillans as well as Peridinium and Gonyaulax spp. It was noted that the toxicities occurred during the period of maximum water temperatures (ranging from 64° to 69° F).

Toxicities in the Strait of Juan de Fuca:

Toxicity levels in California mussels at all stations are presented in Figure 3.

Levels of toxicity remained low in the Strait of Juan de Fuca stations from late February until the middle of June of 1961, at which time the toxicity in California mussels in the Clallam Bay station become elevated from 32 mmg on June 2, to 424 mmg. on June 14, while toxicity levels in mussels in other stations remained below 50 mmg. Two weeks later levels reached 161 mmg. in mussels at the Crescent Bay station (up from 50 mmg the week before), while the level in mussels in the Sequim Bay float was still below 50 mmg. However, on July 28, the toxicity in Sequim Bay rose suddenly from 39 mmg. the week before to 534 mmg. These data seem to indicate that toxicities developed first in the most seaward station and moved in a progressive manner up the Strait (Fig. 3). With very few exceptions the other species in the floats followed the same pattern, though lower peaks were attained, as the mussels. The same approximate situation was also shown in samples from the natural beds, with the exception of mussels from Agate Beach (adjacent to Crescent Bay) which did not attain levels of 50 mmg. when Crescent Bay mussels reached almost 165 mmg. in late June.

The data indicate that a second peak of high toxicity levels occurred during 1961 throughout the Strait of Juan de Fuca, but with progressiveness of pattern not quite so marked as the first peak (Fig. 3). The high toxicity levels of mid-June in Clallam Bay mussels dropped off in late June and early July, but became elevated to approximately 500 mmg. in late July and reached a peak of 768 mmg. on August 11, with a small peak of 358 mmg. in the interval during the middle of July. This small peak

was probably reflected by the sudden elevation from 94 mmg. to 353 mmg. in Crescent Bay on July 28. If the third peak (July 13 in Clallam Bay) hit Crescent Bay, it was apparently masked by the preceding peak just mentioned. More likely, the major peak which developed July 13 in Clallam Bay was largely responsible for the continued elevation above 150 mmg. at Crescent Bay which lasted until August 23. The first peak of high levels in mussels in Sequim Bay, which began July 28, gradually dropped off to a low of 50 mmg. by September 9, but then became elevated to 270 mmg. by September 25. This, one can surmise, resulted from the July 13 peak in Clallam Bay, again occurring several weeks after the peaking in Clallam Bay and further indicating the progressive nature of developing toxicity peaks. Although the 1961 data indicate a progression of toxicity from the outer coast inland, the alternative possibility exists that conditions became suitable for the development of toxicity only in the Clallam Bay region in June, simultaneously in all three areas in August and only in Sequim Bay in September.

For the 1962 season, determination of toxicity level is not up to date due to reduced supply of mice for bioassay during the summer. Therefore, it is yet to be seen whether the progressive pattern has prevailed in the development of toxicity peaks this year. However, detectable level of toxicity in mussels in any stations did not occur until late June 1962. In Clallam Bay, toxicity level in California mussel rose from 50 mmg. on June 20 to nearly 700 mmg. on August 1, followed by a rapid decline to about 300 mmg. a week later (August 8). If this represents the first peak in the Clallam Bay seasonal pattern, it would appear to be of higher magnitude but almost two months later than that of last year. These late developments of toxicity along the Strait seem to coincide with the below-normal temperature during May and June as recorded by the weather stations.

Results of analysis of the plankton samples indicate that several species of the dinoflagellate genus Gonyaulax have been collected at each Strait station, with

the greatest numbers apparently occurring prior to or during the peak of toxicity. The predominating species is tentatively identified as G. catenella or G. scrippsae. Other genera include Peridinium, Dinophysis, Gymnodinium, Oxytoxum, and Ceratium. Positive identification of these forms are forthcoming. In Sequim Bay, Gonyaulax spp. appeared in the plankton samples in early May and continued to be present through the month of September with its peak coinciding with that of the toxicity (see Fig. 4). From September, Gonyaulax were found periodically in Sequim Bay waters until the end of January, 1962. A similar situation occurred in Clallam Bay, whereas no Gonyaulax or other dinoflagellates were found in Crescent Bay waters after September. These may partly explain the longer presence of detectable toxin in California mussels from Clallam Bay and Sequim Bay stations than from Crescent Bay. The toxicity pattern in California mussels from Agate Beach (near Crescent Bay) natural beds is almost identical to Crescent Bay pattern.

Preliminary examinations of various environmental data show no apparent correlation with the developing toxicities with the exception of the maxima of surface water temperature. Further analysis of the hydrographic data is in order.

Comparison of Toxicities Between Species in the Same Location:

Due to the difficulty of maintaining butter clams and soft shell clams on the floats, our data are not complete on the uptake and retention among species. Further work is necessary in the problem of maintaining adequate populations of butter clams and soft shell clams. Of considerable value in this regard are the data on butter clams and soft shell clams from the natural beds in Sequim Bay.

We find that in all stations the California mussel attained high toxicity levels much more quickly than other species, although the Pacific oyster occasionally showed a rise at the same time as the mussel (see Fig. 5). In addition to becoming highly toxic more rapidly than the other species studied, the mussel, with the

exception of butter clams from the natural bed in Sequim Bay late in the season, attained higher levels of toxicity than any species in the study.

Following the mussel in degree of toxicity, we find the butter clam reaching the next highest levels, followed by the soft shell clam and finally the oyster.

The oyster had the lowest toxicity peaks and was found to lose its toxicity more rapidly than other species in most instances; although the soft shell clam possibly declines in level at about the same rate. Somewhat surprisingly, the mussel which is generally considered to become toxic rapidly, but also to decline rapidly, appeared to decline in toxicity more slowly than the oyster and the soft shell clam. The data indicates that the toxicity in the mussel in Sequim Bay was detectable as late as the middle of March, 1962 while detectable levels in the oyster and soft shell clam terminated before the end of October 1961. As previous workers have noted, the butter clam acquires high toxicity levels no more rapidly than the other species, and retains the high levels for a longer period. While the toxicity in other species remained at the level of less than 35 mmg. or non-detectable during the fall, winter and spring the toxicity in the butter clam fluctuated at high levels from 60 mmg. to over 200 mmg. Further data in the second season will possibly produce more understanding of this fluctuation.

The above discussion on the relationship between certain planktonic and environmental features, and the developing toxicities is based on the important assumption that photosynthetic marine dinoflagellates of the genus Gonyaulax are the source of shellfish toxicity. Although the validity of this assumption has been substantially supported by various indirect evidence and experimental works for many years, further studies to identify the source of toxin in Washington waters are in progress.

Literature Cited

Anderson, L. S. 1960. Toxic Shellfish in British Columbia.
American Journal of Public Health, 50 (1): 71-83.

Davies, F. R. I., H. I. Edwards, W. L. H. Kichen and H. O. Tomlinson. 1958
Shellfish Toxin in Cultivated Oysters.
Canadian Journal of Public Health, 49: 286.

McKarren, E. F., M. L. Schafer, J. E. Campbell, K. H. Lewis, E. T. Jensen and E. J. Schantz. 1960
Public Health Significance of Paralytic Shellfish Poisoning.
Advances in Food Research, Vol. 10, Academic Press.

Schantz, E. J., E. F. McFarren, M. L. Schafer, and K. H. Lewis 1958.
Purified Shellfish Poison for Bioassay Standardization.
Journal of Association of Official Agricultural Chemists.
41 (1): 160-168.

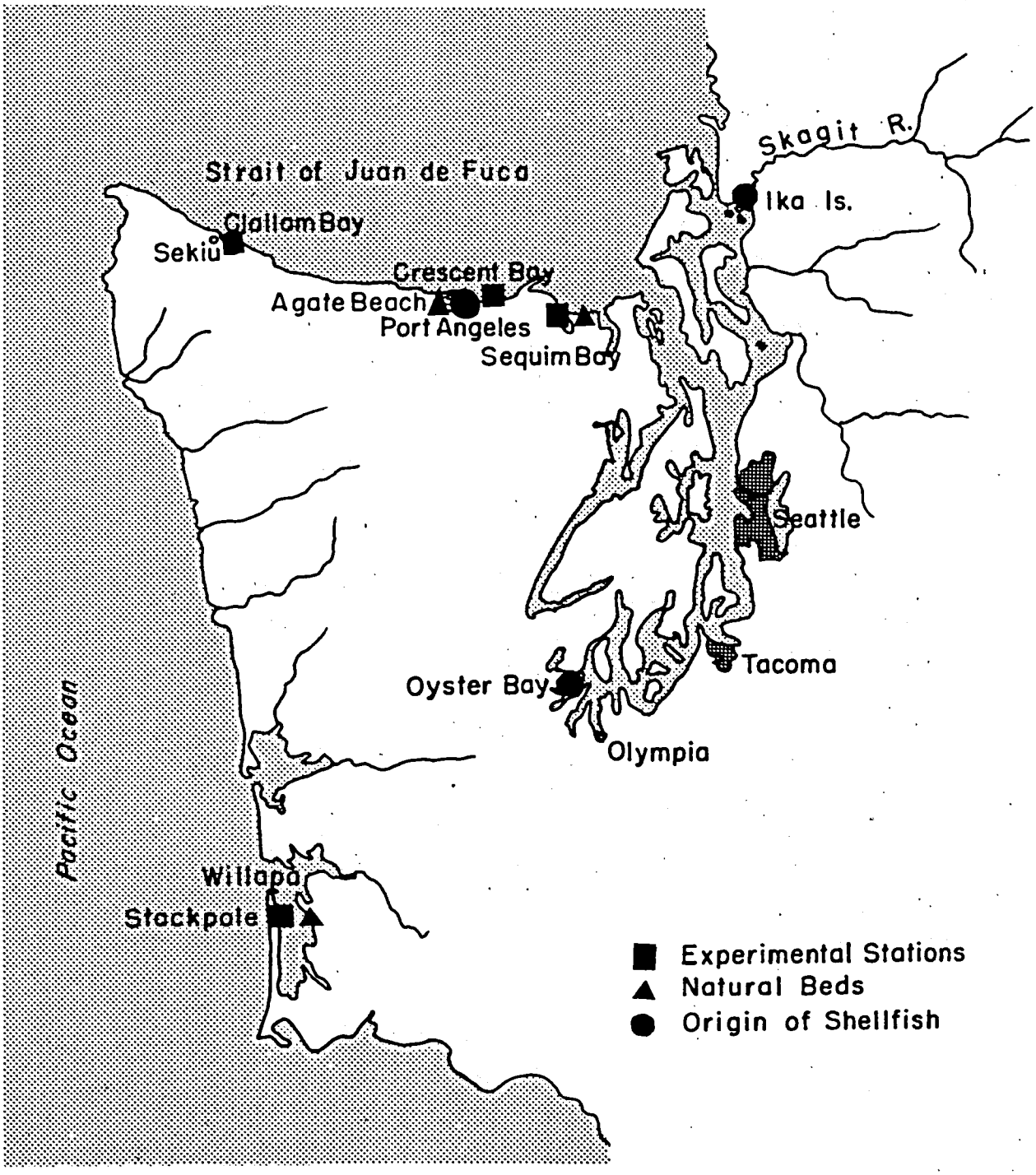

FIGURE I : SITE OF THE EXPERIMENTAL STATIONS FOR PARALYTIC SHELLFISH TOXICITY STUDY

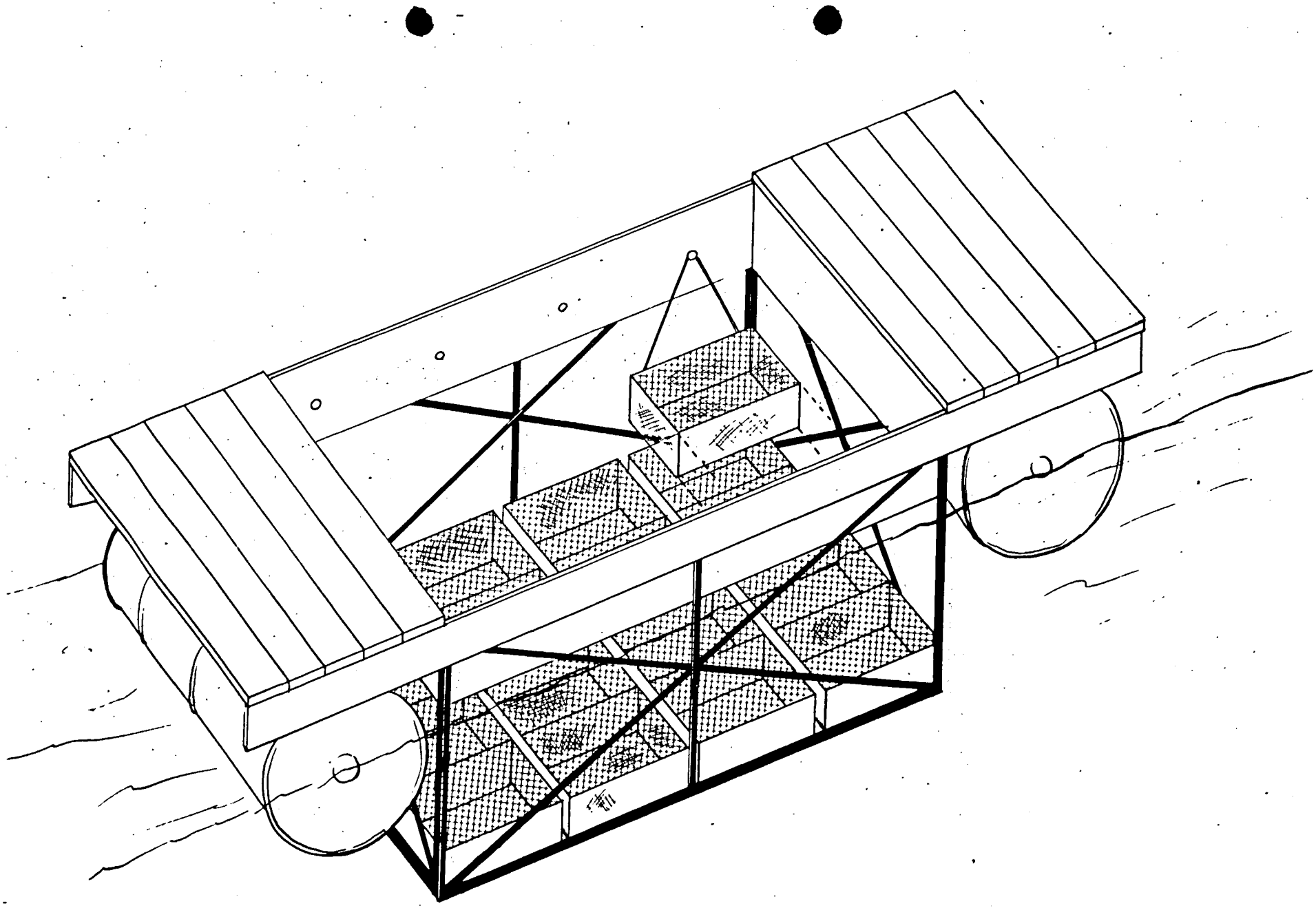

FIGURE 2: EXPERIMENTAL FLOAT

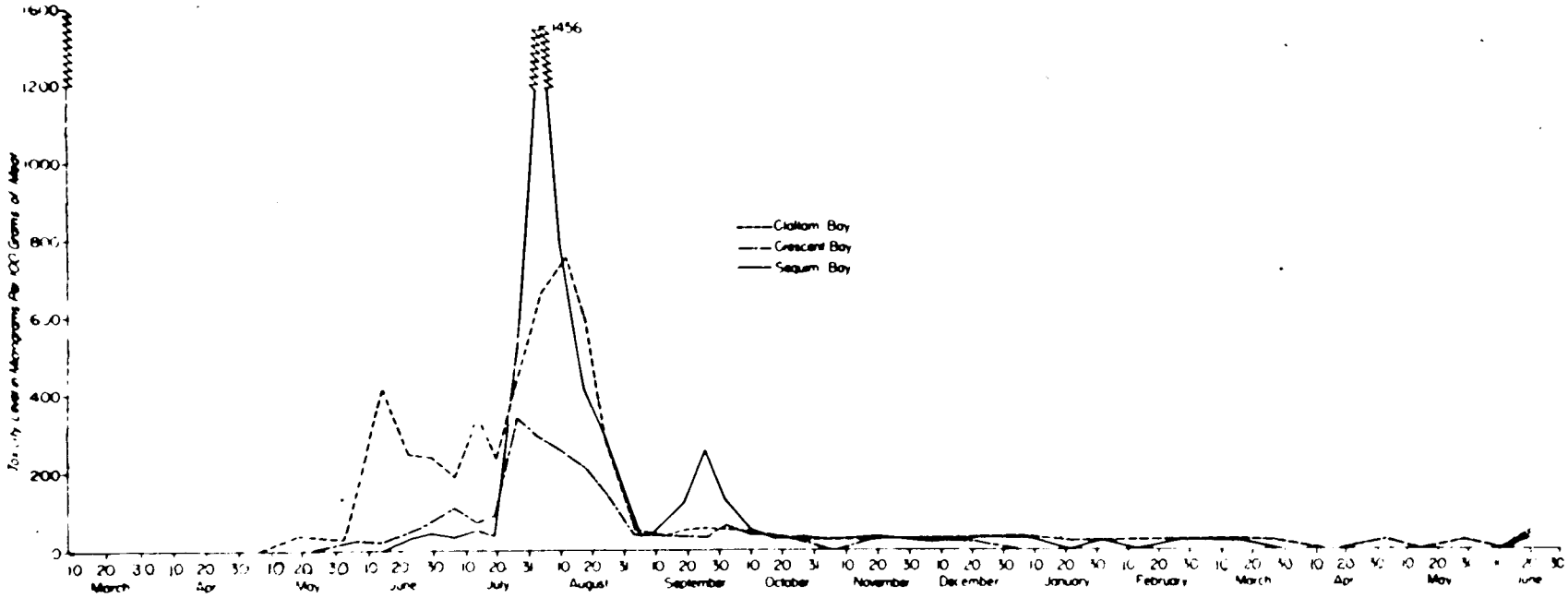

Fig. 3. TOXICITY LEVELS IN CALIFORNIA MUSSELS IN EXPERIMENTAL FLOATS ALONG THE STRAIT OF JUAN DE FUCA FROM MARCH 1961 TO JUNE 1962

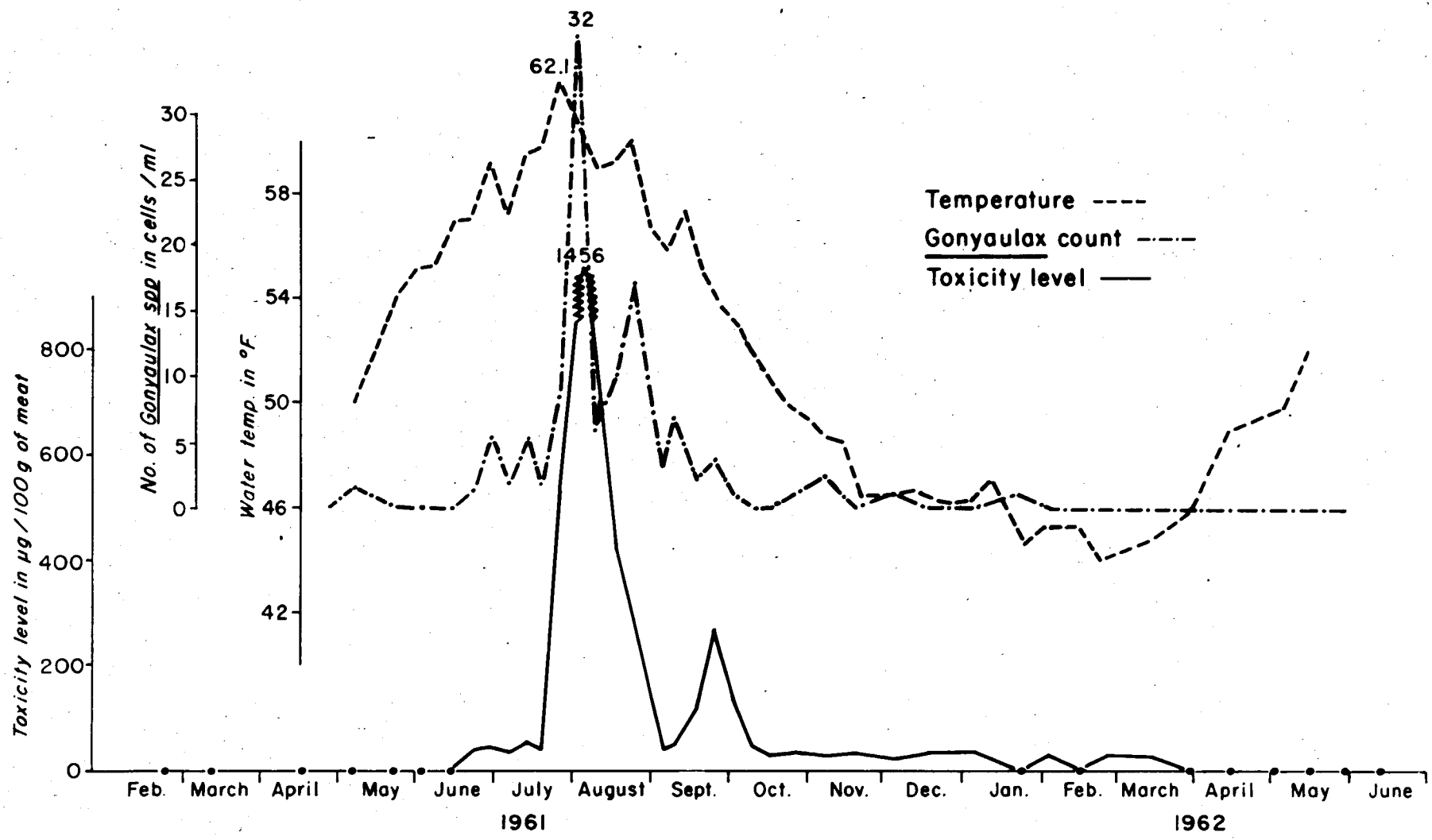

FIGURE 4 : TOXICITY LEVELS IN CALIFORNIA MUSSEL, NUMBER OF GONYAULAX, AND WATER TEMPERATURE AT THE EXPERIMENTAL FLOAT IN SEQUIM BAY

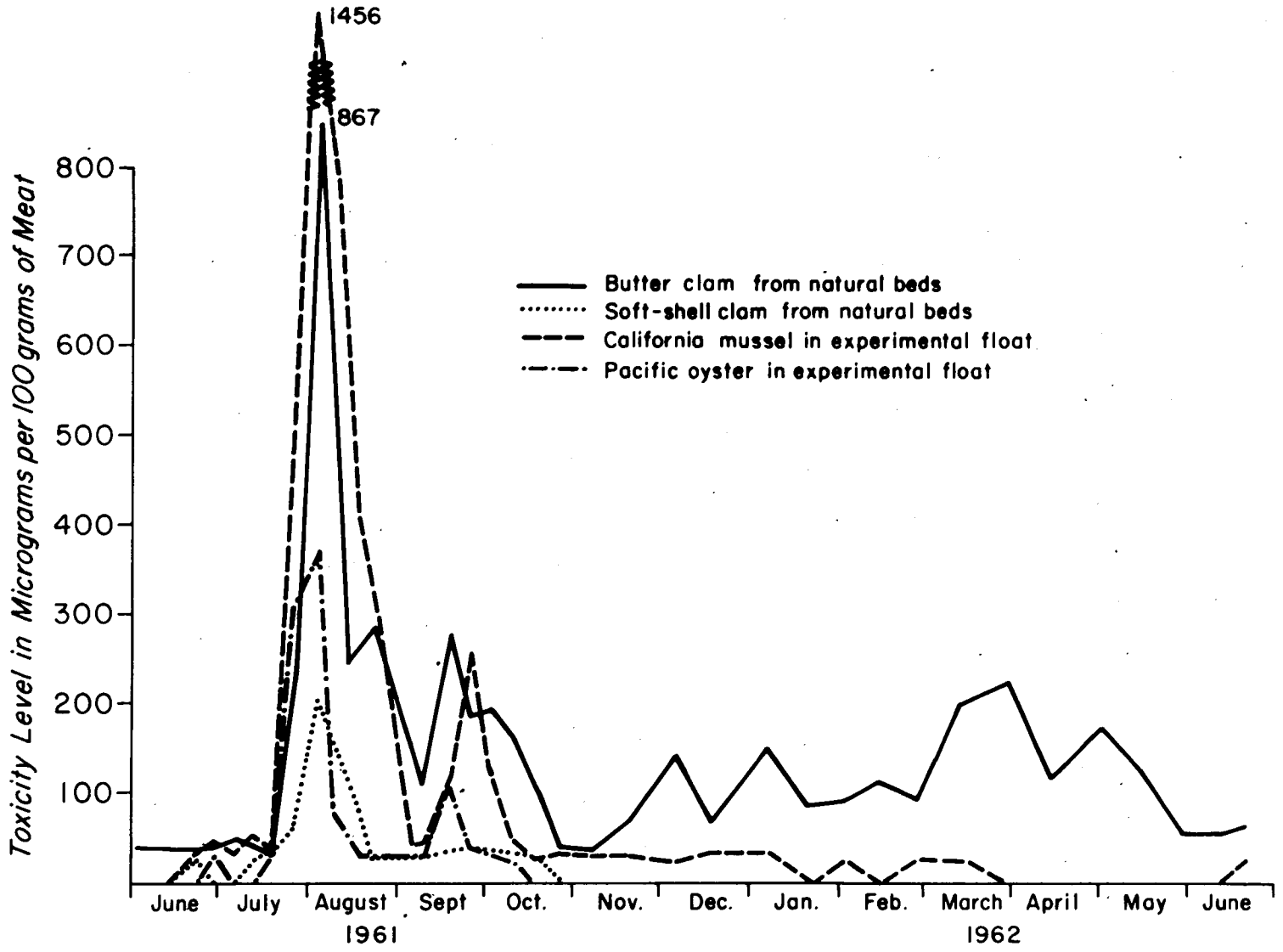

FIGURE 5: TOXICITY LEVELS IN FOUR SPECIES OF SHELLFISH IN EXPERIMENTAL FLOAT AND NATURAL BED AT SEQUIM BAY