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## **Seasonal Abundance of *Protogonyaulax* sp. Causing Paralytic Shellfish Poisoning in Funka Bay, Hokkaido, Japan, 1978-1980**

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For the last ten to 15 years extensive endeavors have been made to culture scallop in Japan with the most promising results shown in the northern coastal areas. By artificial propagation, scallop production has reached more than 100,000 tons. The artificial propagation of scallop is usually carried out with the use of young shells which are collected by spat collector and cultured in cages for about three to six months. There are two culturing methods used: the sowing culture, and the hanging culture (Motoda, 1977).

Funka Bay (Volcano Bay) in Hokkaido is one of the most famous commercial scallop production areas where the hanging culture method is well established. This area alone produced about 60,000 tons in 1977. However, since 1977, mass mortality of scallops culture by the hanging method has occurred in Funka Bay. This mass mortality is similar to that which occurred in some areas of the northern coast of Honshu, the main island of Japan (Motoda, 1977).

In addition to the occurrence of a mass mortality, high levels of the paralytic shellfish toxin in scallop were discovered in 1978 in Funka Bay. Due to the high level of this toxin, the scallop fisheries in the bay suffered heavily; because the whole meat, including the digestive gland where most of the toxin is accumulated, was marketed and eaten in Japan.

Though Prakash, et al., (1971) reported that more than 400 persons died from paralytic shellfish poisoning in Japan, this number also included deaths caused by other kinds of shellfish poisoning. According to Noguchi and Hashimoto (1980), paralytic shellfish poisoning occurred five times and three persons have died as a result in Japan since 1948.

After a red tide of *Protogonyaulax* sp. occurred in Mie Prefecture (central part of Honshu), the presence of the paralytic shellfish poisoning was reported in 1975 (Hashimoto, et al., 1976). Monitoring of the toxin and survey on the causative organisms have been carried out in various areas in Japan, especially on the Sanriku coast, Mutsu Bay and Funka Bay, where scallop culturing is well established. In addition to paralytic shellfish toxin, the presence of a new type of shellfish toxin was recently reported from these areas by Yasumoto, et al., (1978), which has limited the marketing of scallops for several months each year.

Paralytic shellfish poisoning is caused by several species of the genus *Protogonyaulax* (Sommer, et al., 1937; and Prakash, et al., 1971). Investigations concerning the ecology of the causative organisms are not common, because of their infrequent occurrence. For the past three years, this writer has conducted investigations on the mechanism by which the scallop accumulates paralytic shellfish toxin in Funka Bay, Hokkaido. This paper describes the ecology of the causative organism and its relation to the toxification of scallops.

## Locality and the Ocean Conditions

Funka Bay is located at the southwestern part of Hokkaido Island, and faces the Pacific Ocean. The diameter of this bay is about 60 km, and the depth is 100 m at the center. Scallop culturing areas extend along the coast. Periodic samplings of plankton and scallops were carried out at the station which is 1.5 km off Sawara and 68 m in depth. Supplemental surveys were carried out at the station off Shikabe and off Rebunge (Figure 1).

Oceanographic conditions of this bay were well investigated (Ohtani and Kido, 1980). The waters from the Tsugaru Warm Current (high salinity) begin to enter into the bay in late summer and remain through the winter. In early spring, the waters from the Chishima Cold Current (low salinity) begin to flow into the bay and remain until summer. Low water temperatures of about 2°C around February and a high water temperature of more than 20°C in summer were recorded in the surface layer (Figure 2). The thermocline is well developed in summer. Around the end of winter or early spring, nutrients of the water in the bay are consumed by a blooming of diatomaceous phytoplankton, which occurs around the end of winter or early spring (Nishihama, 1980), and remains at a low level in the euphotic zone through late autumn (Figure 3).

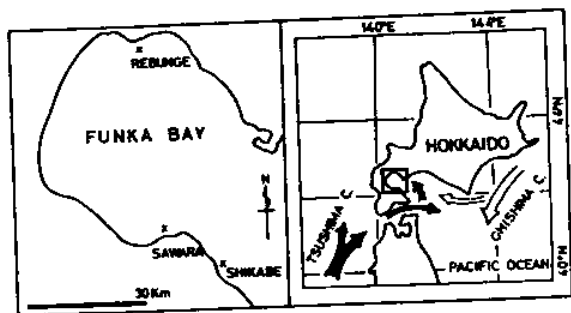


Figure 1. Maps showing sampling stations in Funka Bay (left side), and sea currents around Hokkaido Island (right side).

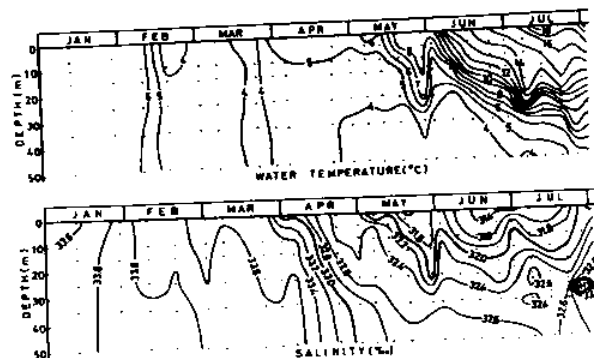


Figure 2. Seasonal change in water temperature and salinity off Shikabe, 1977-1979.

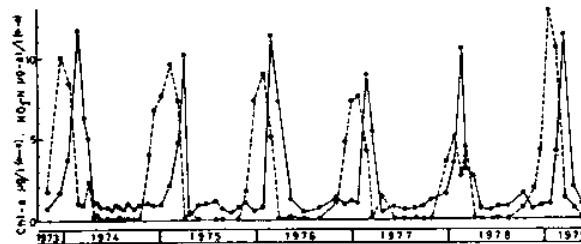


Figure 3. Seasonal change in levels of Nitrate-n and Chlorophyll-a in surface water (0-15 m depth) off Shikabe, 1973-1979. (Nishihama, 1980).

## Materials and Methods

Routine seawater samplings were repeated at the station off Sawara in order to measure water temperature, salinity and phytoplankters. Seawater samples (1 liter each) were collected from various depths and were fixed and concentrated to 2 ml by repeated precipitation using various volumes of cylinders. A 0.1 ml of concentrated samples were used for counting *Protogonyaulax* cells (for details see Uchida, et al., 1980).

In order to inspect the toxicity levels of shellfish, two or three year old scallops (*Patinopecten yezoensis*), cultured by the sowing method, were hung in cages at selected depths. The digestive glands of scallops were examined for toxin by the mouse test (Horwitz, 1975). The toxicity levels were recorded as MU per 1 gram of the digestive gland.

## Trend of Paralytic Shellfish Toxin at Funka Bay

Since 1976, monitoring the toxin of cultured scallops in Funka Bay has been continued periodically by members of the Hokkaido Institute of Public Health. The trend in paralytic shellfish toxin content of scallops at Funka Bay is shown in Figure 4. Low levels of toxin were observed in 1976 and 1977. In 1978, high levels of paralytic shellfish toxin were observed, and for the first time the marketing of cultured scallops from Funka Bay was limited to four months. In 1979, the highest concentration reached was 750 MU/g-digestive gland. Toxin levels increased around June in 1976, 1977 and 1978, and around April in 1979 and 1980.

In June 1978, the author started to conduct investigations on the causative organism.

## The Causative Organism: *Protogonyaulax* sp. (GCF)

In this paper, a species belonging to the genus *Protogonyaulax* (*Gonyaulax catenella*) observed at Funka Bay in 1978 to 1980 will be abbreviated to "GCF". The account of the organism was given in Nishimama, et al., (1979).

The causative organism of paralytic shellfish poisoning is due to several species of Dinophyceae known as "*Gonyaulax catenella*" or "*Gonyaulax tamarensis*". According to Taylor (1979), they are now classified under the genus *Protogonyaulax*.

The initial investigation was aimed at searching for cells of the genus *Protogonyaulax* in Funka Bay at the time when high levels of the toxin were observed. A *Protogonyaulax catenella*-like species was found. This organism resembles *P. catenella*, however it does not form a long chain. Cultured cells of the organism are round in shape, similar to those of *P. tamarensis*. The organisms in the sea show intermediate characteristics between *P. catenella*

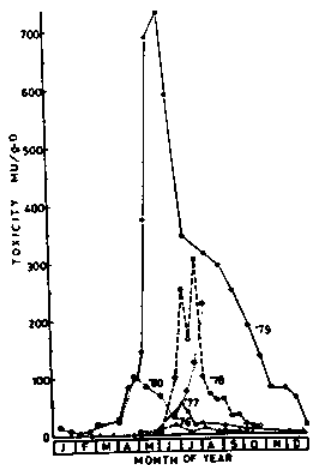


Figure 4. Trend in paralytic shellfish poison in the digestive gland of scallops at Funka Bay, 1976-1980.

and *P. tamarensis*. Therefore, they are considered as a *Protogonyaulax* sp. at the present time, and are referred to as "GCF" in this paper.

GCF presented a characteristic vertical distribution as shown in Figure 5. This data was obtained on 8 August 1978 at the station off Rebunge in Funka Bay (Uchida, et al., 1980). GCF cells were distributed only in the middle layer, where this organism was the dominant species. Though the temperature of the sea surface was 23°C, it suddenly went down with depth, because of a thermocline. At the 20 m depth it was 8°C, where GCF cells were aggregated.

Several taxa of the genus *Protogonyaulax* are toxic and others are non-toxic (Taylor, 1979). GCF was simply a member belonging to the genus *Protogonyaulax*. Therefore, the author tried to ascertain whether GCF made scallops toxic, using their characteristic vertical distribution.

Scallops with a toxicity score of 80 MU/g-digestive gland were hung at five different depths. At the beginning of the experiment, GCF cells were distributed in the middle layer as shown in Figure 6. After six days, they showed similar vertical distribution. Therefore, the scallops in the upper two cages did not take GCF cells. On the contrary, the scallops in the lower three cages took the cells as food. The toxicity was reduced in the upper two groups, but increased in those of the lower three, as shown in Figure 6.

Since the increase of the toxicity of scallops is associated with the presence or absence of GCF cells, it is reasonable to assume that the GCF is the causative organism.

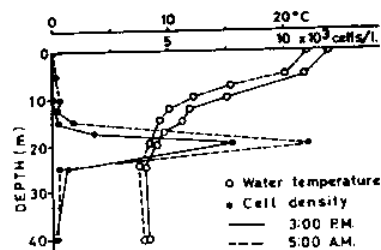


Figure 5. Vertical distribution of *Protogonyaulax* sp. off Rebunge in Funka Bay, August 1978. (Uchida, et al., 1980).

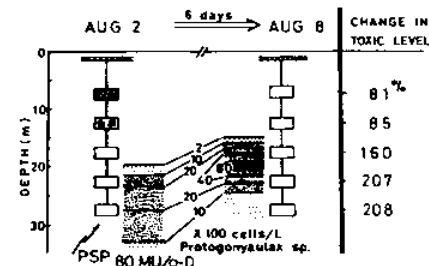


Figure 6. Experiment on the rate of toxicity change in scallop at various depths in Funka Bay, 1978. (Nishihama, et al., 1979).

In addition to this experiment in the sea, cultured cells of GCF showed that 7,000 cells contained 1 MU of the toxin. It is concluded that GCF (*Protogonyaulax* sp.) is the cause of the toxin in scallops in Funka Bay.

### Seasonal Abundance and Vertical Distribution of *Protogonyaulax* sp. and Its Relation to the Toxicification of Scallops

1978

The vertical distribution of GCF (*Protogonyaulax* sp.) and its relation to the water temperature condition from July to September 1978 were reported (Uchida, et al., 1980). In August, a thermocline was well developed, but it disappeared in September due to the inflow of warm water (Figure 7). At the same station, GCF appeared in the middle layer. The maximum density was 20,000 cells/liter. In September, GCF cells disappeared (Figure 8). The portion where the density is more than 1,000 cells/liter is presented by the dotted area. The depth where GCF cells aggregated fluctuated day by day. The three curves in the figure show water temperature. The high density zone of GCF was associated with the zone of 8° to 12°C. Water temperature condition for the growth of GCF was 8° to 12°C in its natural environment in the bay.

GCF cells were not observed in the surface layer in summer, where water temperature was more than 15°C. Since GCF need light for their growth, they ought to aggregate near the sea surface within the appropriate water temperature range. This is the reason why GCF cells occurred in the middle layer in summer. Therefore, a marked development of the thermocline near the surface in summer may be required for the propagation of GCF.

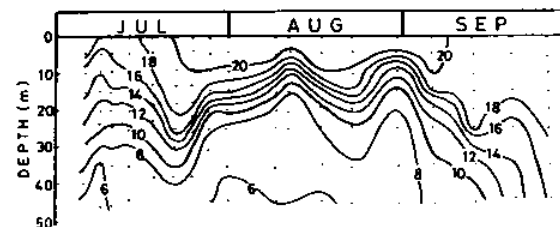


Figure 7. Changes in water temperature (°C) off Sawara in 1978. (Uchida, et al., 1980).

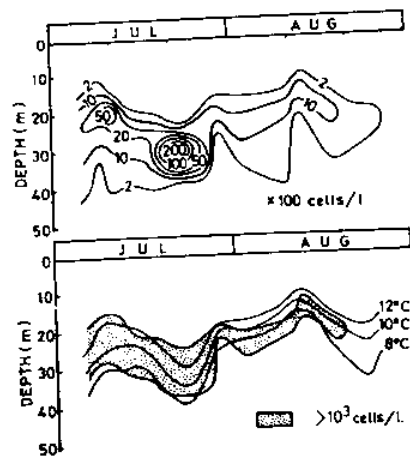


Figure 8. Vertical distribution of *Protogonyaulax* sp. and its relation to water temperature off Sawara in 1978. (Uchida, et al., 1980).

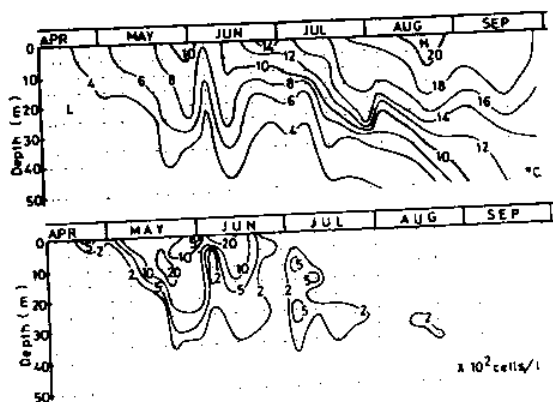


Figure 9. Change in water temperature (°C) and vertical distribution of *Protogonyaulax* sp. off Sawara between April and September, 1979. (Nishihama, et al., 1980).

1979

In the following year, 1979, the relation between the density of GCF cells and the toxicity of scallops was investigated (Nishihama, et al., 1980). A change in water temperature conditions from April to September is shown in Figure 9. The water temperature began to rise in May. From May to the middle of June, the surface water temperature was 8° to 12°C, and then it rose to more than 12°C. In July and August of 1979, thermocline was not formed as clearly as was observed in 1978.

In 1979, a very low density of the GCF cells was observed in March. Until the middle of April, however, they did not propagate actively because the water temperature was still very low. GCF began to propagate actively in the surface water in the middle of April with the

density of the cells reaching a maximum from the end of May to the beginning of June. After the surface water temperature rose to 14°C by the end of June, GCF disappeared in surface water, and appeared in the middle layer. Then, the density of the cells gradually reduced and disappeared by the end of August.

The displacement of GCF cells from the surface to the middle layer corresponded to the change of the depth where water temperature was about 10°C. The low density of the cells in the middle layer from July to August, 1979, is thought to be due to the less-developed thermocline.

In order to investigate the relation between the density of GCF cells and the toxin levels in scallops, scallops were suspended in cages at depths of 10 and 25 m. The toxicity of the scallops at 10 m depth suddenly increased at the end of May, and then began to decrease suddenly at the beginning of June. On the other hand, the toxicity of 25 m depth reached the maximum levels at the end of June (Figure 10). The change of toxicity at both depths corresponded very well with seasonal and vertical abundance of GCF cells. Toxicity levels of scallops at both depths continued to decrease gradually after September and almost disappeared by the end of January (Figure 11). It is interesting to note that due to the disappearance of the toxic plankton in the sea the toxicity of the digestive gland of scallops also suddenly decreased followed by a more gradual reduction.

1980

The toxicity level of scallops while low in April, 1979 (Figure 10), already exceeded the

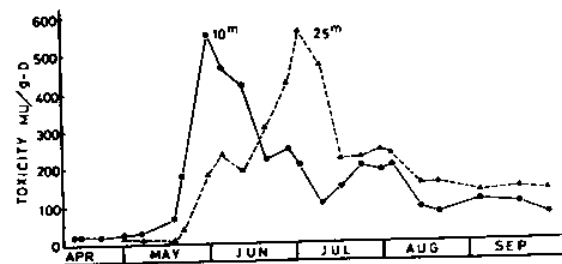


Figure 10. Change in the toxicity scores of scallops suspended at 10 and 25 m depth, off Sawara between April and September, 1979. (Nishihama, et al., 1980).

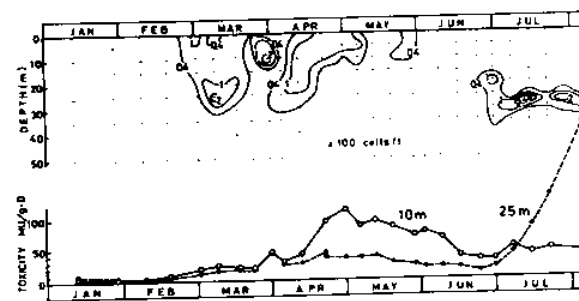


Figure 11. Vertical distribution of *Protogonyaulax* sp. and toxicity level of scallop off Sawara in 1980.

limit for marketing of scallops. Therefore, it was necessary to investigate the ecology of the causative organisms in early spring and to forecast the time when scallop toxicity would exceed the limit for marketing.

In 1980, a low density of GCF cells appeared in March. This was similar to the previous year's observation. In March, the toxicity of scallops increased gradually in spite of the very low density of GCF cells (Figure 11). An effective forecast based on plankton survey alone is very difficult, because accumulation of the toxin by scallops still goes on in spite of the very low density level of the causative organism, and because the increase of the toxicity is associated simultaneously with the increase of the toxic plankton.

GCF cells propagated to a density of several hundred cells/liter at the end of April. Then they disappeared for a short period in almost all depths of the water. At the beginning of July, they appeared again in the middle layer (the water temperature of the surface layer was already more than 15°C). The toxicity of scallops at 25 m depth rose again, too.

In 1979, GCF cells which appeared in low density in March and April, propagated quickly when water temperature rose to about 8° to 10°C at the end of May. But, in May and June 1980, GCF cells disappeared for a short period. In 1980, salinity dropped suddenly in April (Figure 12). This represents the inflow of the Chishima Cold Current which started to enter Funka Bay in April 1980. This exchange of seawater washed out the material for the propagation of GCF cells for a short period. Compared with 1979, the Chishima Cold Current started to enter the bay in February to March. This difference in the timing of the inflow of the Chishima Current explains why there were no GCF cells found in May and June 1980. Therefore, the stability of the seawater of the bay from spring to summer is thought to be a necessary condition for the propagation of GCF cells.

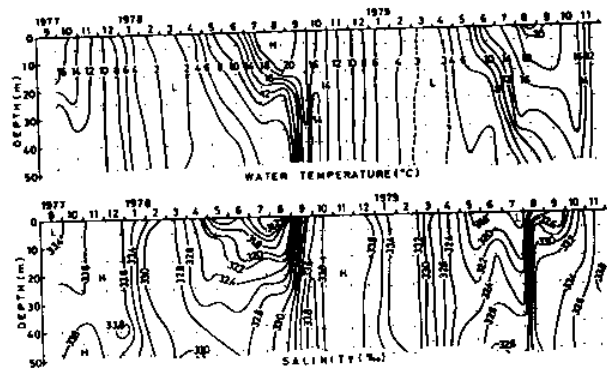


Figure 12. Change in water temperature and salinity off Sawara in 1980.

### Conclusion

*Protogonyaulax* sp. is the cause of the toxin found in scallops in Funka Bay. They showed three different aspects on the seasonal abundance and the vertical distribution each year from 1978 to 1980. These three different types of occurrence of the organisms are explained by the fact that the optimum water temperature condition for the propagation of the species is approximately 10°C in Funka Bay. However, *Protogonyaulax* cells were not found in late autumn, when water temperature was about 10°C. Seasonal abundance and density level of the species are thought to be dependent on the degree of the development of a thermocline in summer, and on the degree of the stability of seawater conditions from spring to summer.

The change of the toxicity of scallops corresponded very well with seasonal and vertical abundance of *Protogonyaulax* cells.

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