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Vertical distribution of paralytic toxin-producing species, *Protogonyaulax* sp. in Funka Bay, Hokkaido

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The vertical distribution of the dinoflagellate *Protogonyaulax* sp., an organism causing paralytic scallop poisoning, was investigated from July to September, 1978, in Funka Bay, Hokkaido. During the period of abrupt thermocline, a relatively high cell density was only found in the middle layer. At the surface, water temperature was near 20°C, while at the bottom it was maximum 10°C. Cell density measurements show that *Protogonyaulax* sp. aggregated at the 8-14°C layer. This is the reason of aggregation in the middle water layer. A similar vertical distribution pattern is also considered to be maintained at night since distribution under dim light at daybreak was almost the same as that recorded during daytime. It is concluded that, unlike most other dinoflagellates studied, the vertical distribution pattern of *Protogonyaulax* sp. is significantly affected by temperature, but not by light. The application of the results obtained is briefly discussed for practical use in scallop cultivation.

Key Index Words: *Dinoflagellate*; Gonyaulax; *paralytic toxin*; Protogonyaulax; *scallop cultivation*; *temperature*; *thermocline*; *vertical distribution*.

Gonyaulax catenella and related species are linked with paralytic shellfish poisoning which can cause human illiness and sometimes death (BURKE *et al.* 1960, PRAKASH 1963, PRAKASH and TAYLOR 1966, MACLEAN 1977). Since 1976, members of Hokkaido Institute of Public Health have found paralytic toxins in scallops from Funka Bay (Official data). In June 1978, a higher level of toxicity than that measured before was observed. Investigations were made to determine causative phytoplankton species which appeared parallel with the scallop poisoning. A *G. catenella* like species[1] (*Protogonyaulax* sp.) was revealed to have close correlation with shellfish toxicity, and this dinoflagellate was found to be present in the middle water layer (NISHIHAMA *et al.* 1979).

NISHIHAMA *et al.* (1979) have cultured this species and assayed the toxicity of laboratory grown individuals. Their findings show that *Protogonyaulax* sp. contains a potent paralytic toxin. Ecological studies on such a toxic dinoflagellate are of immediate practical importance for all those interested in the mariculture of filter-feeding shellfishes. Therefore, we studied the vertical distribution of this species in Funka Bay to correlate its occurrence with

1) This alga was quoted as *Gonyaulax catenella* like species in the previous report (NISHIHAMA *et al.* 1979). The genus *Protogonyaulax* is established by TAYLOR (1978).

temperature.

Methods

From July to September 1978, routine seawater samplings were carried out to determine water temperature, salinity and phytoplankters at both Sawara and Toyoura stations (Fig. 1). At each station seawater was collected from different depths with a VAN DORN plastic sampler. Immediately after pouring the seawater sample into a plastic bucket its temperature was measured. From samples brought back to the laboratory, salinity was measured with a salinometer (MC-5, Electronic Switchgear (London) LTD). For identification and counting of *Protogonyaulax* cells, each one

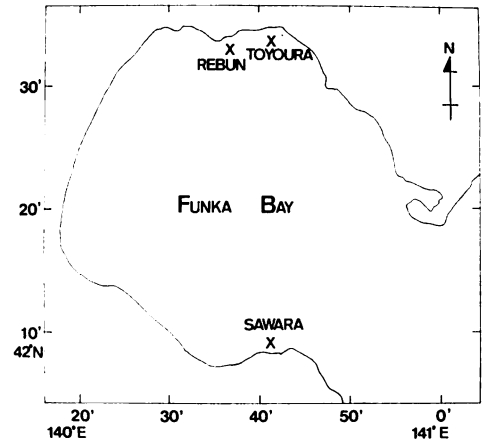

Fig. 1. Location of sampling stations.
Water depth: Toyoura: 38 m, Sawara: 45 m, Rebun: 45 m.

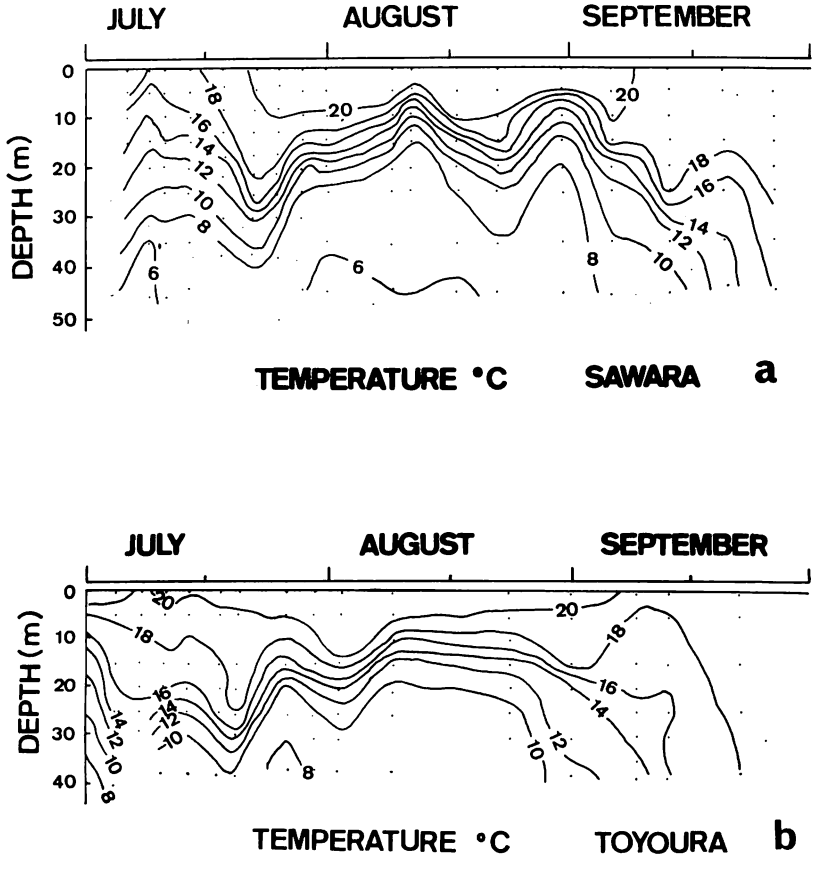

Fig. 2. Vertical seasonal distribution of water temperature at Sawara (a) and Toyoura (b).

liter sample from different depths was fixed with 2% ammonia-neutralized formalin. After concentration to 2 m*l* by allowing the phytoplankters to settle, the contents of a 0.1 m*l* sample was counted on a ruled slide.

Results

Temperature and salinity stratification is clearly observed during the period of investigation as shown in Figs. 2 and 3. These results show vertical stability of water in this season.

At both stations, *Protogonyaulax* cells were relatively abundant during July to August (Fig. 4). From the end of August until September, the number of dinoflagellate cells decreased and remained below 200 cells/*l*. At the end of September they could hardly be detected as phytoplankters. During the whole period of investigation, maximum cell density of *Protogonyaulax* sp. was 24.9×10^3 cells/*l* at Sawara, 35 m depth, July, 21; and 45.9×10^3 cells/*l* at Toyoura, 20 m, August, 8. In both cases, the cells aggregated in the middle layer. At Sawara, the layer containing more than 1,000 cells/*l* was narrow (5–14 m), although its depth varied considerably day by day. At Toyoura, temperature stratification was less pronounced and the layer containing more than 1,000 cells/*l* was wider (5–20 m) than at Sawara. During this period, a thermocline developed; the maximum temperature of bottom seawater was 10°C, while the surface temperature was approximately 20°C (Fig. 2). At both stations, the vertical distribution of *Protogonyaulax* sp. cells seems to be affected by temperature (Fig. 5). The upper thermal limit of the layer containing more than 1,000 cells/*l*

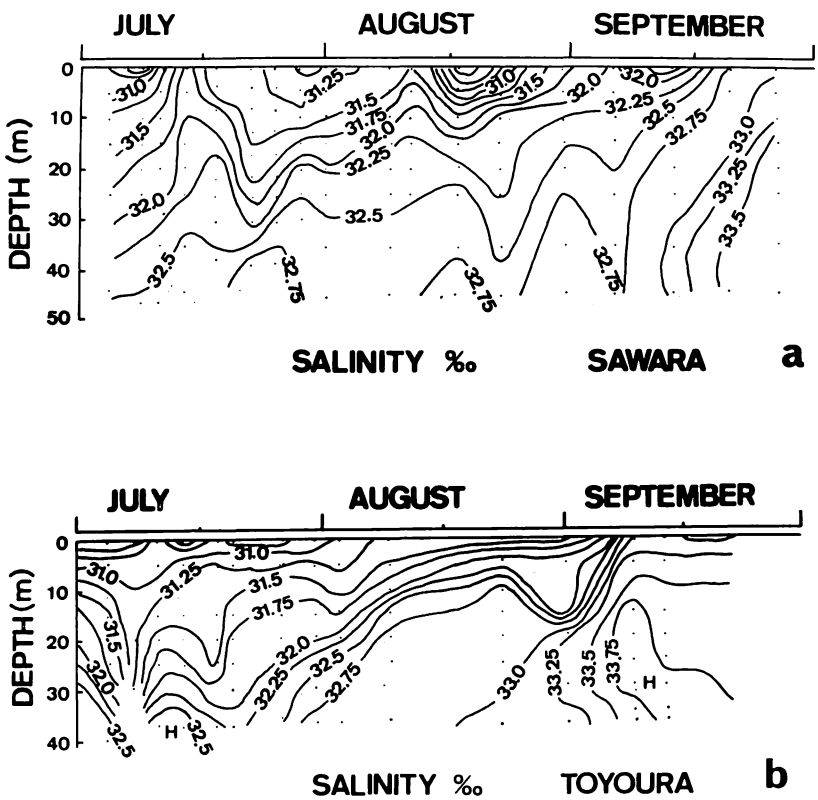

Fig. 3. Vertical seasonal distribution of salinity at Sawara (a) and Toyoura (b).

varied between 10 and 14°C. Furthermore, the maximum density layer of the cells was between the isothermal lines of 10 and 12°C. At Sawara, this relationship between temperature and dinoflagellate density was more clearly expressed (Fig. 5 a).

Since the above mentioned phenomena were based on daytime sampling, the observation on the distribution of *Protogonyaulax* sp. was carried out under dim light (5 : 00, August, 9) as well as during daylight (15 : 00, August, 8) at Rebun (Fig. 6). It took about one hour to complete sampling process. The sampling time described shows the time when the sampling had been finished. Since the sunrise was at 4 : 35, August, 9, 1979, the dim light sampling was conducted during before to after sunrise. As a result, similar distribution pattern was obtained in each case (Fig. 6). A relatively high cell density was recorded at 20 m depth, while only a few cells were observed in other water layers.

Starting from the end of August in Toyoura, high salinity (above 33‰), warm waters gradually intruded (Fig. 3). At Sawara, this intrusion was delayed for about 20 days. As soon as this water mass occupied all the layers, the *Protogonyaulax* sp. cells completely disappeared from the seawater layer.

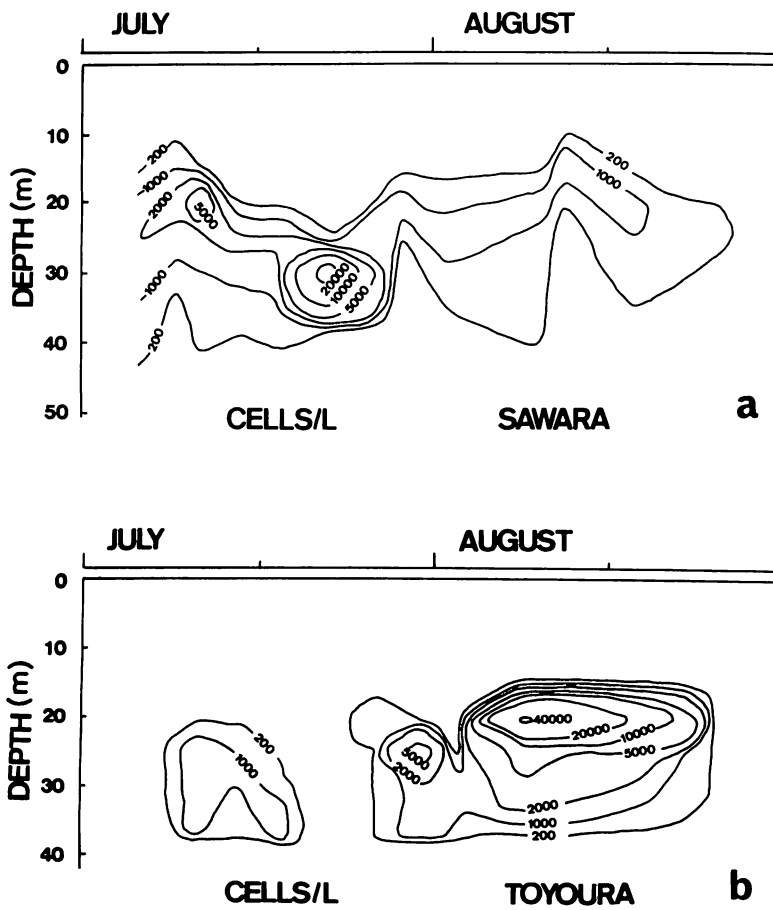

Fig. 4. *Protogonyaulax* sp. vertical seasonal distribution at Sawara (a) and Toyoura (b).

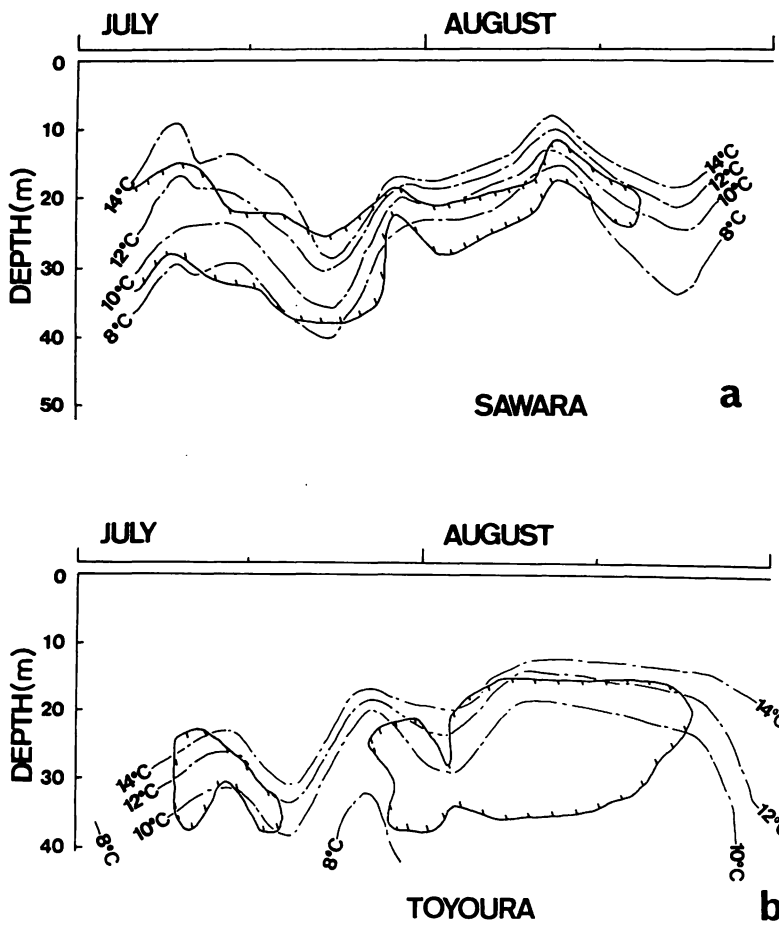

Fig. 5. Relationship between *Protogonyaulax* sp. distribution and water temperature at Sawara (a) and Toyoura (b). Area surrounded by slanted lines contains more than 1,000 cells/*l*. (Quoted by NISHIHAMA 1980)

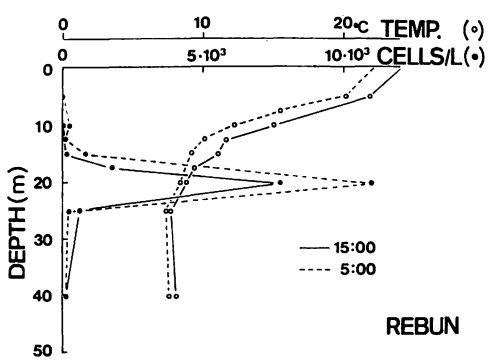

Fig. 6. *Protogonyaulax* sp. vertical distribution under daylight (15 : 00) and dim light at daybreak (5 : 00).

Discussion

Light is thought to be an important factor for dinoflagellates vertical distribution because of the existence of positive or negative phototaxis (HASLE 1950, 1954; NORDLI 1957; WHEELER 1964; EPPLEY *et al.* 1968; SELIGAR *et al.* 1970; TILZER 1973; MACLEAN 1977). The response to light resulted in a diurnal vertical migration of some dinoflagellates in response to light-dark cycles (HASLE 1950, 1954; WHEELER 1964; EPPLEY *et al.* 1968; TILZER 1973). Other than light, some factors are known to influence the migrative behavior of some dinoflagellate species. In *Pyrodinium*

bahamense, phototactic behavior is not an obligatory species pattern but a facultative response to nutrient availability (MACLEAN 1977). In addition to phototaxis, endogenous rhythm is a possible factor causing diurnal migration in *Gonyaulax polyedra* and *Cachonina niei* (EPPLEY *et al.* 1968). In Funka Bay, *Protogonyaulax* sp. was shown to respond differently from other related organisms, and no significant diurnal migration was shown. The pattern of vertical distribution was similar under daylight (15 : 00) and under dim light (5 : 00). Therefore, the distribution pattern obtained here from midday samplings is most likely maintained also during night.

The vertical distribution of *Protogonyaulax* sp. seems to be determined by temperature : a large number of the cells were found only in the water layer with temperatures of 8–14°C. At Toyoura, water temperature was above 8°C in all water layers examined from July to August, while at Sawara an 8°C isotherm prevailed in the middle layer. This may be the reason why at Toyoura *Protogonyaulax* sp. is distributed in deeper layer as compared to the situation at Sawara. Since this species shows active growth in culture at 20°C (UCHIDA, unpublished), the temperature dependent distribution cannot be explained in the present case merely by a growth phenomenon. These findings can be of importance to investigators involved in scallop cultivation. It is possible to eliminate the accumulation of toxin in cultivated scallops by avoiding the layer containing a dense population of *Protogonyaulax* sp. cells. The layer containing high cell densities can easily be detected by measuring water temperature.

A mid-layer distribution pattern of phytoplankters, as shown in the present study, is not limited to *Protogonyaulax* sp. According to NISHIHAMA (unpublished), in Funka Bay, dinoflagellate *Exuviaella* sp. also aggregates in the middle layer. The density of *Gonyaulax* sp. in Ofunato Bay was also higher in the middle layer (MURANO 1975). However, it is still unclear whether the vertical distributions of these two species are affected by temperature or other environmental factors. In Funka Bay, *Protogonyaulax* sp. was found in quite low numbers (45.9×10^3 cells/l), even in the highest density layer. The population did not develop a bloom perphaps because the nutrient levels are low during summer (NISHIHAMA *et al.* 1976).

By the end of September, warm water with a high salinity occupied all layers. This is due to the inflow of Tsugaru Warm Current (OHTANI and AKIBA 1970). When the intrusion had completed, *Protogonyaulax* sp. completely disappeared from water sample. This may be partly due to that the low-salinity and cold water mass containing the species was replaced by Tsugaru Warm Current water mass.

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内田卓志*・川真田憲治・西浜雄二**: 北海道，噴火湾における麻痺性貝毒原因種，*Protogonyaulax* sp. の鉛直分布について**

噴火湾産ホタテガイ毒化の原因種である *Protogonyaulax* sp. の鉛直分布を1978年7月から9月迄の3カ月間，噴火湾沿岸の定点において調査した。その結果，本種は水温8～14°Cの中層に局在し，この水温層の上下に伴って出現層が変化した。また細胞密度の最も高い層は常に水温10～12°Cの層に存在した。一方，同一地点で日中および夜明け時の鉛直分布を比較したところ，ほぼ同じパターンが得られ本藻は顕著な日周移動をしないものと考えられた。以上の結果および考察から，光依存の鉛直分布を示す他の渦鞭毛藻とは異なり *Protogonyaulax* sp. は水温に依存した鉛直分布を示すと結論される。(*051 室蘭市母恋南町1-13 北海道大学理学部附属海藻研究施設，**041-14 茅部郡鹿部村字本別 北海道立栽培漁業総合センター)