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A Contributory Dinoflagellate to Ciguatera, *Gambierdiscus toxicus*, in French Polynesia

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Introduction

Ciguatera is a general term implying an intoxication induced by the ingestion of many kinds of fishes mainly inhabiting the tropical and subtropical coral reef. Ciguatera is not usually fatal, but the number of the cases is so high that it is an urgent problem not only for fishing industry but also for the health of the inhabitants throughout the South Pacific. More than 80 % of those infected complains paralysis of the extremities, burning sensation when in contact with cold water, and arthralgia (BAGNIS et al, 1979). In some islands any coastal or coral fishes eaten would endanger the persons health for several years, because most fishes contained enough toxin to cause ciguatera when taken. In some occasions even migratory fishes such as tuna and skipjack were found to be toxic.

The annual rate of ciguatera infection was reported to be about 1 case per 1,000 people in the South Pacific regions. It was also suggested that reported cases do not represent the actual intoxication rate because the less serious cases did not visit doctors or that the symptoms were misdiagnosed. During the author's stay in Tahiti for a year, a worker in a medical institute had suffered from the poisoning about ten times by ingesting coral fishes that he caught at coral reefs around the island and none of these were recorded in the statistics. Thus it is estimated that several thousands of people suffer from the intoxication yearly. The diversity of symptoms might be explained by the different amount of toxin ingested or by the different natures or kinds of toxins included.

The causative organism for ciguatera was found in the Gambier Islands, French Polynesia (YASUMOTO et al, 1977) and was identified as a new species, *Gambierdiscus toxicus* (ADACHI and FUKUYO, 1979). The dinoflagellate inhabits the surface of benthic macro–algae growing at coral reef areas around several meters in depth. In this paper the author intends to discuss the survey results on the distribution of *G.toxicus* in the vicinity of Tahiti Island, with reference to some marine environmental factors. The poisoning by coral fishes was a serious problem in the middle of 1960s and still now

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some areas of Tahiti are known to be toxic. The population density of the dinoflagellate rather well reflects the ciguateric endemicity by fishes, but sometimes it does not reflect, because the toxin produced by the organism and taken in fishes could be stored for a long time without decreasing the toxicity and intoxicate humans at a later date. In another words the population density of *G.toxicus* indicates only the amount of toxin in the dinoflagellate cells in the area at the time while the toxicity in animals represents the accumulated effects up to the surveyed time. Therefore long term detailed surveys of the distribution is required to prevent or minimize the intoxication of the inhabitants.

Papeete is a capital of French Polynesia and is located at about 139.33 W and 17.33 S, on the northwestern end of Tahiti. On nearly the opposite side of Papeete, Hitiaa, had long been recognized as a toxic fish producing area, and the people wisely avoid ingesting the marine animals caught around there. The survey was initiated here and eventually covered the whole coastal area of the island.

Methods

1) Determination of the population density of *G.toxicus*

In preliminary surveys it was found that the dinoflagellate preferred inhabiting the thalli of *Turbinaria* sp., a tree–like brown alga, common in tropical and subtropical areas and attaining a height of about 15 cm. Considering the epibenthic nature of *G.toxicus*, it's population density on the *Turbinaria* was determined.

A. Vibrator method: Ten thalli were taken from ten individual *Turbinaria*. All of these thalli were transferred to a tapered tube containing 10 ml of filtered sea water and were shaken for a minute on an electric vibrator. The thalli were removed and the remains were centrifuged for 3 min. at around 1,000 rpm. The supernatants were removed and the contents were resuspended in an aliquot of sea water and the number of *G.toxicus* was determined microscopically.

B. Plastic bag method: Taking into account of the unavailability of *T. ornata* and of an electric vibrator in the sampling place, a more practical method was tested. About 300 g of macro–algae are put into a plastic bag containing about 1,000 ml of in situ sea water and shaken vigorously for about 3 min. by hand. The sea water in the plastic bag was then passed through two sieves having mesh sizes of about 300 and 40 μ successively. The residue on the smaller mesh sieve was washed with enough sea water to make a volume of 25 or 50 ml depending on the amount of residue and was observed microscopically. The results of these two methods are compared and they are shown in Table 1.

As indicated in this Table, Method A resulted in a higher number of *G.toxicus* than Method B. But both methods did not show a great disparity in the population density of the dinoflagellate found. The author adopted the simpler and more practical plastic bag

Table 1 Comparison of vibrator method with plastic bag method

Species of Algae	No.	Vibrator Method	Plastic Bag Method
	1	980*	765
Turbinaria ornata	2	100	70
	3	888	850
	1	404	440
Amphiroa sp.	2	1313	912
	3	12	0

*Cell number of *G. toxicus* found on 100 g of substrate algae

method thereafter.

2) Survey of vitamin B_{12} concentrations in sea water

Vitamin B_{12} concentrations in filtered sea water were analysed by the microbiological assay method using *L. leichmanii* as reported in a previous paper (Inoue et al, 1973).

3) Measurements of inorganic phosphur, nitrogen and silicate–Si

Sea water samples were analysed for their inoraganic–P and–N according to the method by Strickland and Parsons (1972).

Results and Discussions

The survey stations are shown in Fig. 1. Those stations covered most passages in the barrier reef of Tahiti. *G.toxicus* prefers the passages to the other localities (YASUMOTO et al, 1979). The survey was carried out at the cooler season of the year there in June to July of 1981. Average surface water temperature during the period was 27.1 C with a maximum difference of 0.8 C depending on the station. The algal layer is usually denser in summer than winter in the Great Barrier Reef (CRIBB, 1973), and the growth of algae in Tahiti was also more abundant in the hotter season of the year, November to March. At each sampling station, *T. ornata* was taken as a host benthic alga if possible. When this brown alga was not available, another species of alga was picked up instead. The distribution of three other epibenthic unicellular algae, *Ostreopsis renticularis, Ostreopsis ovata* and *Prorocentrum lima,* which were usually found in the prepared samples for microscopic observation of *G.toxicus* (FUKUYO, 1981) were also investigated. The results are shown in Table 2. The concentrations of inorganic P, N, silicate Si and salinity are also indicated in the same table. The highest population density of 5,100 *G.toxicus* cells per 100 g of benthic macro alga, *Jania* sp., was observed at Mahaena Passage, located on the northeastern part of the island. The muscle of surgeon fish, *Ctenochaetus striatus,*

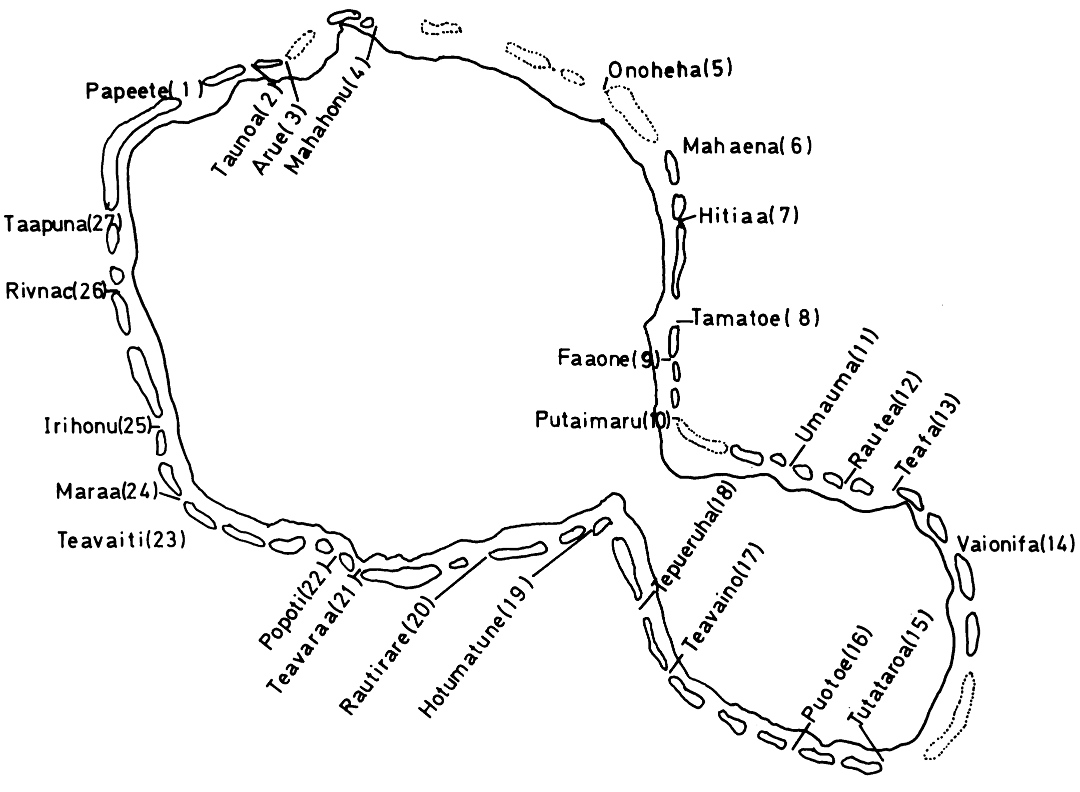

Fig. 1 Sampling stations in Tahiti Island in 1981.

caught in this area showed DLm value of 1.5 mg on mice. Among 27 samples tested, 9 were found to contain the dinoflagellate. The number of the dinoflagellate changes constantly, so these numbers should be interpreted to represent the situation at the sampled time. Fig. 2 shows the results of the same kind of survey in 1977. The difference of the results between these two surveys at each station is obvious. The numbers of three other dinoflagellates also changed from one place to another but at different rates from that of *G.toxicus*. In the micro–cosmos on macro–algal surface, there should be competition for growth among the attached epibenthic algae. Salinity varied from 33.65 to 35.96 % remaining almost constant except for the five stations where the effects of river water influx were felt. The laboratory culture of *G.toxicus* exhibited no remarkable ill effects when the salinity was above 33 % (INOUE, unpublished). There was little rain fall in the districts both before and during the surveying period. The salinity is known to decrease much after heavy precipitation.

Inorganic–P concentrations were at ordinary levels for coastal sea water of tropical regions (see, for example, YASUMOTO et al, 1979). The remotest sampling station from land was about 500 m offshore. Sea water samples were obtained just above the bottom where the substrate algae were taken. The depth of each station at sampling time, usual-

Table 2 Distribution of *G. toxicus* and some inorganic nutrients concentrations in Tahiti Island

St.	Passage	No. of Dinoflagellate* (1) *G. t*	(2) *O. l*	(3) *O. o*	(4) *P. l*	Salinity (‰)	NO_2-N (μg at/1)	NO_3-N	Inorg. P	Sil. -Si	Sp. of Alg.
1	Papeete	0	223,000	29,000	29,000	35.60	0.22	0.29	0.57	1.03	*T. ornata*
2	Taunoa	0	790	3,100	110	35.74	0.25	0.24	1.20	2.24	〃
3	Arue	49	3,000	99	0	34.49	0.22	0.68	1.16	8.93	〃
4	Mahahonu	0	4,100	2,700	180	35.52	0.28	0.20	1.01	2.36	〃
5	Onoheha	0	0	1,400	0	34.31	0.22	0.12	0.49	12.07	〃
6	Mahaena	5,100	80	0	80	35.96	0.16	0.11	1.01	1.11	*Jania* sp.
7	Hitiaa	0	56	3,400	0	35.43	0.16	—**	1.67	1.12	*T. ornata*
8	Tamatoe	0	120	9,200	0	35.78	0.16	0.12	0.60	1.35	〃
9	Faaone	75	750	9,600	70	35.86	0.28	0.29	0.48	1.64	〃
10	Putaimaru	0	720	8,800	0	35.46	0.24	—	0.73	2.06	〃
11	Umauma	27,270	1,300	2,200	160	35.74	0.40	—	0.48	1.36	〃
12	Rautea	0	750	3,800	0	35.78	0.19	0.14	0.56	2.04	〃
13	Teafa	49	250	2,300	1,100	35.66	0.14	0.46	0.73	2.42	〃
14	Vaionifa	66	660	56	0	35.63	0.33	0.46	—	4.16	*Jania* sp.
15	Tutataroa	120	1,000	2,400	0	35.78	0.33	—	0.64	1.88	*T. ornata*
16	Puotoe	0	600	15,000	0	35.06	0.14	0.11	0.58	—	〃
17	Teavaino	0	1,100	8,500	0	35.94	0.19	0.29	0.51	—	〃
18	Tepueruha	0	600	380	0	35.78	0.31	—	0.24	4.77	〃
19	Hotumatune	0	49	2,100	0	33.65	0.31	—	0.50	3.34	〃
20	Rautirare	0	1,500	1,500	0	35.81	0.27	0.22	0.59	1.18	〃
21	Teavaraa	0	1,400	420	0	34.85	0.40	0.62	0.49	3.62	〃
22	Popoti	0	48	1,200	48	35.87	0.39	0.18	0.49	1.38	〃
23	Teavaiti	62	370	1,000	50	35.88	0.27	0.13	0.64	1.76	〃
24	Maraa	0	7,700	460	0	35.46	0.33	0.23	0.28	2.51	〃
25	Irihonu	0	4,900	1,200	0	35.47	0.28	0.53	0.41	3.07	〃
26	Rivnac	0	130	1,700	0	35.22	0.18	0.48	0.56	7.94	〃
27	Taapuna	160	980	2,100	0	35.82	0.33	0.55	1.04	1.00	〃

(1) *Gambierdiscus toxicus* (2) *Ostreopsis lenticularis* (3) *Ostreopsis ovata*
(4) *Prorocentrum lima*

* Number of the dinoflagellate on 100 g of substrate algae

** Below detection limit

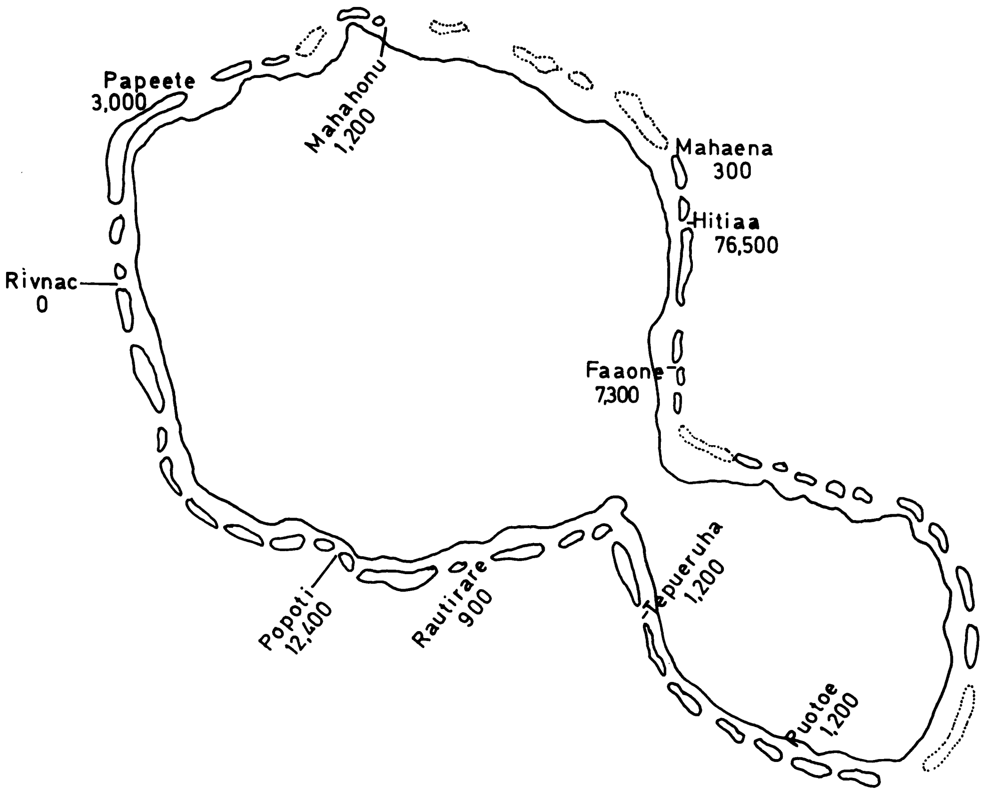

Fig. 2 Sampling stations in Tahiti Island in 1978 and number of *G.toxicus* on 100 g of macro -algae.

ly at low tide level, was 0.5–2.0 m. For two kinds of inorganic nitrogen, NO_2–N and NO_3–N, the former was within the ordinary range and the latter was far higher than other results reported.

The number of *G.toxicus* on the thalli of macro–algae changes with the total effects of the surrounding circumstances. For the prediction of ciguatera in some peculiar area, more detailed observations are required. Such a survey was carried out at three localities of Tahiti from May 15 to June 6 in 1978. *Turbinaria ornata* was taken at each station at about weekly intervals and the number of *G.toxicus* was counted. These results are indicated in Fig. 3. Even during such a short period the population density changed very greatly in Vairao, but in another place, Faaone, little fluctuation was recognized. The ciguatera endemicity did not agree with the number of *G.toxicus* found at individual locality or with its periodical change. The diurnal changes of some environmental factors at Faaone reef is shown in Fig. 4. Vitamin B_{12} concentrations were higher in the day time than at night, but dissolved oxygen concentrations decreased during the day.

Vitamin B_{12} concentrations were thought to be sufficient for the primary production there regardless of the time. The biggest tidal difference in the surveyed period was 70

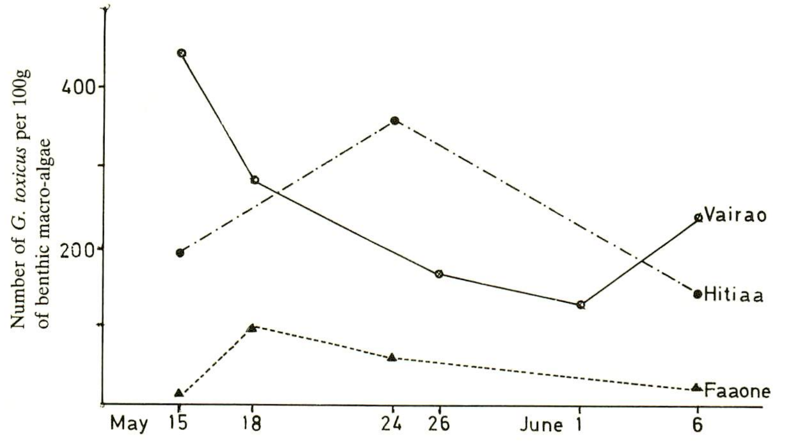

Fig. 3 Change of the population density of *G.toxicus* during a short period.

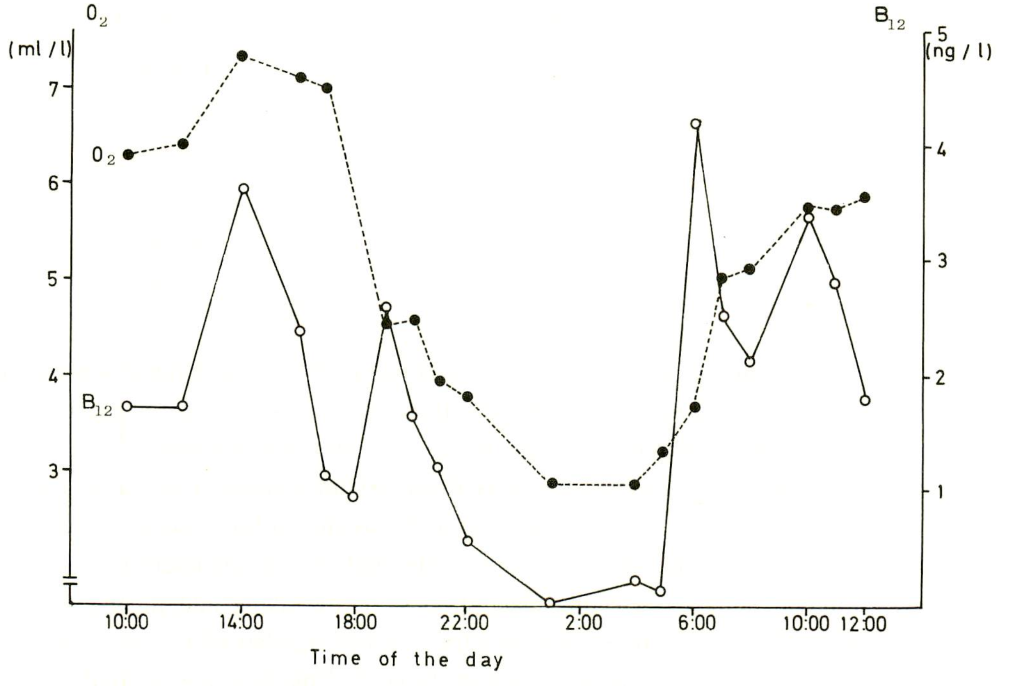

Fig. 4 Diurnal changes of vitamin B_{12} and dissolved oxygen at Hitiaa Reef.

cm reaching to the lowest sea level at midnight which governed the chlorinity changes. The growth of *G.toxicus* was usually observed on the surface of benthic macro–algae, *Turbinaria ornata*, a brown alga, and *Jania* sp., a calcareous red alga, among the many algae tested (Table 3 and 4). In Table 3 the results on six different algal species collected

Table 3 The number of *G. toxicus* on 1 g of several different algal species in the same area

Species of Algae	Number of *G. toxicus*
Ulva sp.	0
Chlorodesmis sp.	26
Halimenia sp.	34
Colpomenia sp.	68
Sargassum sp.	0
Turbinaria ornata	1088

Table 4 The number of *G. toxicus* found on 1 g of algae in Mangareva Island

Sampling Locality	Species of Algae	No. of *G. toxicus*
1	*Jania* sp.	470,000
	Halimeda sp.	16,000
2	*Jania* sp.	520,000
	Halimeda sp.	12,000
3	*Jania* sp.	360,000
	Halimeda sp.	10,000

at the same time within the area of about 1 m in Hitiaa is shown. *Turbinaria* was by far the best host species for *G.toxicus* growth, and three other species of green algae were the next showing little differences among them. The attached number of the dinoflagellate on the thalli in Mangareva Island was about 500 times higher than that in Tahiti. The number shown in Table 4 was the greatest as far as the author's survey concerned until now. The field survey for the estimation or the prediction of ciguatera intoxication should therefore be done by selecting algal species, or by taking and mixing several species of macro -algae. Even in a small area the attached number of *G.toxicus* on one algal species differs more or less as shown in Table 5. This test was carried out in Mangareva Island where seven fragments of dead corals were collected within an area of about 4 m^2 and *Jania* sp. growing on the coral was picked.

In case of both unialgal and axenic culture, *G.toxicus* grows in a free -living state sometimes adhering to the surface of the container when the surrounding circumstances become unfavorable or it comes to a period of senescence. The growth is commonly inhibited by fluorescent lights above 5,000 lux. In nature it usually inhabits the surface of substrate matters, preferentially benthic macro–algae in less than 10 m of water. The

Table5 Differences of population density of *G. toxicus* in small area

No. of Corals	Substrate Algal Species	No. of *G. toxicus**
1	*Jania* sp.	229, 500
	Jania sp.	218, 800
	Peyssonelia sp.	254, 400
2	*Jania* sp.	55, 100
3	*Jania* sp.	104, 000
4	*Jania* sp.	58, 500
5	*Jania* sp.	222, 400
6	*Jania* sp.	167, 000
7	*Jania* sp.	238, 700
Mean		172, 000

*No. of *G.toxicus* was expressed as the number per 1g of substrate algae

light intensity in the natural environment was measured in May of 1981. The results are indicated in Fig. 5. The solar radiation was very strong attaining to 120 Klux at the surface at noon and to 30 Klux at 10 m at the same time of the day. Therefore the light attainable to the individual dinoflagellate cells on the thalli should be more than 30 Klux at the strongest time of the day. These two contradictory premises might be caused by the excretion of some mucous substances surrounding the cells which diminish the intensity on the cells themselves or that the substrate algae could shade the undersirably stronger sunshine.

Ciguatera has occurred in all the areas of the South Pacific, sometimes inducing very serious problems both in the public health and fishing industry. When the varieties and complexities of ciguatera are considered, it can not be attributed to a single cause. The nature of the toxins is being investigated now and their chemical natures may be understood in near future. Fishes become toxic through their food chains, and thus all fishes, both herbivorous and carnivorous, can theoretically acquire the toxin. This is the reason why the toxicity of the fishes and preferably other marine animals must be tested as frequently as possible.

The distribution of *G.toxicus* has widely surveyed (SHIMIZU et al, 1980: INOUE, 1983: INOUE and RAJ, 1985: INOUE and GAWEL, 1986), but the population densities of *G.toxicus* fluctuates during a short time. Even in a small area, there are distinct regional variations. To our regrets we don't have the knowledge to reduce or exterminate the toxic dinoflagelate in the natural environment. Further laboratory experiments on its physiological nature are essential.

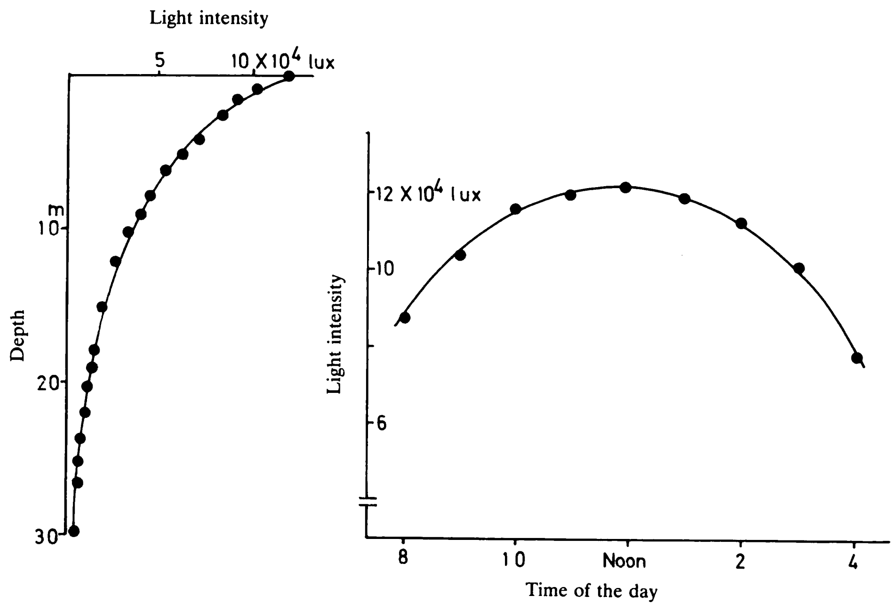

Fig. 5 Changes of light intensity by depth and time of the day.

The destruction of coral reefs in the tropical regions, such as in cases of construction of new airports and harbours, usually leads to ciguatera occurrences. People in the Mangareva Island believe that ciguatera there was triggered by the desertion of iron materials on the nearly coral reef during and after the building up an airport. Ciguatera is commonly reputed to exist around sunken iron ship, but it was not always true in the investigation in Micronesia. Concerning ciguatera in the South Pacific there are many questions to be answered and various problems to be solved yet.

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