# Red Tides Biology, Environmental Science, and Toxicology

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Editors Tomotoshi Okaichi Donald M. Anderson Takahisa Nemoto



Biology, Environmental Science



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and Toxicology

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Proceedings of the First International Symposium on Red Tides held November 10-14, 1987, in Takamatsu, Kagawa Prefecture, Japan

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#### **PREFACE**

Research on red tides and toxic dinoflagellates has expanded significantly over the past 15 years. During that time, a series of International Conferences on Toxic Dinoflagelletes was initiated, with meetings held in 1974, 1978 and 1985. Despite this growing research activity and scientific interest, some areas of research and some areas of the world were not adequately represented by these conferences since they were generally restricted to dinoflagellates and all were convened in North America. Although the toxic dinoflagellate conferences will continue, with the fourth in the series scheduled for June, 1989 in Sweden, there was a need to hold a more general meeting that would include all bloom-forming organisms - animal and plant, toxic and non-toxic. To this end, the First International Symposium on Red Tides was convened in Takamatsu, Kagawa Prefecture, Japan in November, 1987. Twenty-seven countries were represented at the Symposium which was a unique opportunity for individuals from the many disciplines that fall under the "red tide" umbrella to meet each other and interact scientifically and socially. Red tides are clearly interdisciplinary in nature, and the symposium was purposefully held in one large room (i.e. without concurrent sessions) so that interactions across disciplines could take place.

The program of the Symposium consisted of keynote lectures and both oral and poster contributed communications. The subjects covered were:

- Regional Problems case reports or reviews of red tides in specific areas;
- 2. Biology taxonomy, physiology, ecology, and biochemistry;
- Environmental Science The physical, chemical, and biological structure of the marine environment, remote sensing, and numerical simulations;
- Toxicology toxic species, assay methods, and chemistry of red tide toxins.

Despite the complications inherent in bringing such a diverse assemblage of scientists, regulators, and industry officials together, the Symposium proceeded smoothly and was a technical and, we feel, a social success. The global distribution of this particular scientific community then became painfully apparent when it came to collecting over 100 manuscripts and reviewing them for scientific content and English grammar, all within a few short months of the meeting, as required by our publisher.

The editors and publisher believe that one of the values of a Proceedings Volume is that it provides an up-to-date picture of the status of a field of research that is difficult to obtain from the general scientific literature. Due to the large number of manuscript submissions, strict page limits had to be imposed that in turn restricted the depth and detail of the contributions, but we believe that it is important for all of those involved in this diverse field to have their efforts read and understood by their colleagues. This is a special problem for those who do not typically publish their results in English, and we hope that our efforts will allow the international communication that was so visible during the Symposium to continue. Those reading this volume will recognize that not all manascripts have perfect word structure and usage; the final product is a compromise between time constraints and editorial propriety. Some authors will find their papers to have been changed significantly prior to publication since many were edited extensively and some were re-typed completely of an we received them in "final form".

This Symposium would not have been possible without the interest and dedication of the participants and of certain Japanese and International organizations. The Intergovernmental Oceanographic Commission (IOC). consulting with WESTPAC, was the primary force behind the initial plans, with the proposal being supported by the Ministry of Education, Science and Culture, Kagawa Prefecture, and Takamatsu City. The organizers and editors wish to acknowledge Dr. Peter Bjornsen, Dr. Koichi Okutani, Ms. Tomoko Watanabe and Ms. Akemi Nakai for assisting with the organization of the Symposium. We thank the numerous volunteers, student assistants and chairpersons who were so efficient and courteous throughout the meeting, Dr. Theodore Smayda should be acknowledged for his willingness to take the time to assimilate all of the scientific contributions and to write an Overview for this book. We are also grateful to the workshop lecturers, Drs. F.J.R. Taylor, Kazumi Matsuoka and Yasuwo Fukuyo on Biology and Taxonomy, and Drs. Takeshi Yasumoto, Yasukatsu Oshima, Sachio Nishio and Michio Murata on Toxicology. The Editors gratefully acknowledge the efforts of the Editorial Committee in revising manuscripts, sometimes on exceedingly short notice, and Mr. Allan Ross of Elsevier for his enthusiasm and willingness to publish the Proceedings. Finally, we would like to express our sincere gratitude to the Symposium sponsors for providing the financial support that made it all possible.

Tomotoshi Okaichi Kagawa University Donald M. Anderson Woods Hole Oceanographic Institution Takahisa Nemoto University of Tokyo Red Tides
Biology, Environmental Science,
and Toxicology

#### SYMPOSIUM LOGO

Mrs. Fusa Miyauchi (1883-1986) produced beautiful paper-mache toys in Takamatsu City. One of her dolls, known as the "Taimochi Ebisu" (the Japanese folk God of fishermen who always carries a lucky "Tai" or sea bream) was selected as the design for the 1959 New Year's commemorative stamp by the Japanese Post Office. Miss Akemi Nakai, Kagawa University, used this doll and the red tide dinoflagellate <a href="Gymnodinium">Gymnodinium</a> to make the Symposium logo. This logo represents our wish for the extinction of red tides and an improvement of fish catch throughout the world.

### I KEYNOTE ADDRESSES AND OVERVIEW



RECENT PROGRESS OF RED TIDE STUDIES IN JAPAN: AN OVERVIEW

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#### ABSTRACT

The red tide problem in Japan is introduced, followed by an overview of recent progress of red tide studies in the following areas: 1) Taxonomy of red tide flagellates, 2) initiation of blooms, 3) nutrients, growth promoters, and environment, 4) vertical migration of the causative organisms as nutrient support and a concentration mechanism, 5) population growth and interaction of organisms, and 6) prediction of red tide occurrence.

#### INTRODUCTION

In Japan, the definition of red tide is still vague. Historically, a seawater discoloration, irrespective of the causative organisms, has been called a red tide. Phytoplankton blooms, even diatom blooms have often been included in the definition of a red tide by some workers. So, the Japanese meaning of red tide seems to be different from the American's or the Westerner's. Since 1900, when the first published report occurred, red tide has often been observed mainly in the cultural grounds of pearl oyster (Pinctada martensii). In the Seto Inland Sea, red tide outbreaks accompanied by damage to fisheries increased conspicuously in the 1960's. Therefore, the primary concern was concentrated on the damage to fisheries, although the appearance of paralytic shellfish poisoning was reported in 1961. To clear up the causative mechanisms of red tides, a research group was organized under the sponsorship of Fisheries Agency in 1966. The program of study was intended to clarify (1) the relation between the formation of low dissolved oxygen water mass in the bottom layer (anoxic condition) and the occurrence of red tide, (2) the influence of water pollution or industrial wastes on red tide occurrence, and (3) physiological characteristics of the causative organisms. The results l contributed to the development of a profile of red tides, and significantly influenced subsequent studies in Japan. The main problems faced in these studies were: (1) where the seed beds were in relation to the existence of the cysts, and (2) identification of unfamiliar or unknown organisms.

Since the frequent occurrences of red tide at that time had attracted public attention, studies on red tide were started in each research field, and carried out actively. Biological studies were focused on the following genera because of their economic and social effects, Raphidophyceae: Heterosigma, Chattonella; Dinophyceae: Gymnodinium, Prorocentrum, Protogonyaulax, and Dinophysis.

#### TAXONOMY

As mentioned above, the appearance of unfamiliar or unknown red tide flagellates induced confusion of identification, because there was no workable identification system owing to the lack of detailed information about their ulcrastructure. The espaisas in question, therefore, were reexamined.

Chattonella antiqua was first found in Hiroshima Bay, the Seto Inland Sea, in 1969, and described under Hemieutreptia antiqua by Hada<sup>2</sup>. Takano<sup>3</sup> reported the morphological similarity between Hemieutreptia antiqua and Hornellia marina. Since then, it has been called "Hornellia" in Japan.

Since Hollande and Enjumet<sup>4</sup> had already asserted that Hornellia marina was the same species as Chattonella subsalse, the taxonomic position of this organism has been unclear. One and Takano<sup>5</sup> and Hara and Chihara<sup>6</sup> agreed that Hemieutreptia is synonymous with Chattonella and described it under the new combined name of C. antiqua, because it differs from C. subsalsa in cell size and chloroplast structure. For the smaller form, Hara and Chihara<sup>6</sup> proposed the new, combined name of Chattonella marina (Subrahmanyan) Hara and Chihara.

Heterosigma akashiwo was first isolated in Bingo-Nada, the Seto Inland Sea, and Gokasho Bay in Kii peninsula in 1966. The genus Heterosigma was proposed by Hada to encompass Entomosigma akashiwo Hada and a new species, Heterosigma inlandica, which the author considered to be unarmoured dinoflagellates. Hara and Chihara reexamined these taxa using cultured material, including type cultures of both H. akashiwo and H. inlandica as well as specimens referred to as Olisthodiscus luteus, and maintained in CCAP and UTEX by light and electron microscopy. They concluded that these algae can be accommodated as a single species, H. akashiwo, because of their similarity in appearance and ultrastructure.

In the Seto Inland Sea there was another unknown microalga, tentatively called Botryococcus sp., which was often found to be one of the main causes of red tide harmful to mariculture. Toriumi and Takano<sup>10</sup> proposed this organism as longed to a new genus and species Fibrocapsa japonica Toriumi and Takano.

#### INITIATION OF BLOOMS

In relation to the initiation of blooms, several investigations have been carried out. Cysts of Protogonyaulax tamarensis were found in Tanabe Bay, Kii peninsula, Ise·Mikawa Bays, and Ofunato, Okirai Bays in the northeastern part of Japan. This suggests that this organism is distributed widely in the coastal waters of Japan. Fukuyo<sup>1</sup> evaluated the (1) vertical distribution of Protogonyaulax cells, (2) the numbers of benthic cysts per unit area, and (3) the germination rate of the cysts in Ofunato Bay from January, 1980 to June, 1981. He suggested the high possibility that the cysts play an important role as "seed populations", and that the appearance of swimming cells was controlled by environmental conditions, such as temperature, rather than the resting period. He described the planozygotes, which formed only in the early stage of planozygote formation and could transform to hypnozygotes; planozygotes were formed during exponential growth.

Studies on the cysts of Raphidophyceae have made slow progress because of their fragility. Research on the cysts has been tried in two different ways: laboratory cultures and field surveys. Furuki et al. 2 observed the morphological changes of Chattonella antiqua occurring under various environmental conditions, and described the conditions which induced their transformation to globular form and recovery. Appearance of vegetative cells of Chattonella in seawater incubated with bottom sediment samples suggested that these organisms have a benthic dormant stage, such as a cyst, during the course of their life cycle. From surveys using the extinction dilution method, Imai  $et\ al_{\cdot}^{13}$ ,  $l^{4}$  studied the distribution of the dormant cells in Harima-Nada and Suo-Nada located on the east and west side of the Seto Inland Sea, and confirmed that 1) the dormant cells accumulated in the restricted area, and 2) vegetative cells regularly occurred in the surface when bottom temperature rose to about 20°C. They reported that it is essential to find the seed beds first and then to monitor the rise of bottom water temperature to predict the appearance of cells in the early stage of the Chattonella redtide outbreak. Imai and Itoh<sup>15</sup> also investigated the effect of scorage temperature on the dormancy and maturation of the dormanc ceals of Chattonella. They summarized the annual life cycle as follows: (1) vegetative cells in early summer originate from germination of dormant cells in sediments: (2) they form overwintering dormant cells during the summer season; (2) dermant colle spend a duration of post dormancy, an enforced one due to

low temperature, until early summer. This explanation seems to be well suited to the temperature regime in temperate sea such as the Seto Inland Sea. Finally, they  $^{16}$  found the cysts of *Chattonella* and described their morphological characteristics.

#### NUTRIENTS, GROWTH PROMOTERS, ENVIRONMENT

The physiological characteristics of the primary red tide flagellates appearing in the coastal waters of Japan were already summarized, and shown that each species has specific preferences and tolerances to the environments 17. Recently, interesting studies have been conducted. Takahashi and Fukazawa<sup>18</sup>, in three semi-continuous culture experiments using natural populations, observed two types of responses for macro- and micro-nutrient dependency. The former group includes Skeletonema costatum, Thalassiosira sp. and Eutreptiella sp., whose growth was mainly stimulated by macro-nutrients. H. akashiwo was in the latter group which was stimulated by micronutrients. Gymnodinium sp. showed an intermediate type. Each flagellate showed characteristic growth responses under different nutrient conditions. H. akashiwo maintained a high growth rate even under poor macro-nutrients, and a further growth enhancement was obtained by moderate concentration of ammonia, manganese and vitamin  $B_{12}$ . Gymnodinium sp. growth was stimulated by macro-nutrients, but not as significantly as the diatoms, and also by iron and vitamin B12. The nutrient requirements of Prorocentrum micans were clarified by Iwasaki and Iwasa<sup>19</sup>. Enrichment of the seawater (Ise Bay) with nitrate, phosphate, and vitamin B12 enhanced its growth. Moreover, the addition of chelated iron or acid extract (at pH 3.0) of marine mud greatly increased the growth, though no enhancement was observed following addition of nitrogen and phosphorus. They suggested that vitamin  $B_{12}$ , or anometallic compounds, and organic substances play a significant role in the blooming of this organism in Ise Bay. The same result was obtained in another strain of P. micans by Yamochi $^{20}$  using Osaka Bay water. In the experiment using AGP method, Yamochi $^{20}$ , 21 reported that the addition of chelated iron to filtered seawater (Osaka Bay) enhanced the growth of H. akashiwo, but not the addition of nitrogen, phosphorus or vitamin  $B_{12}$ . Enrichment with a combination of thiamine, vitamin B<sub>12</sub>, and Fe-EDTA increased by 15-fold the yield of Eutreptiella sp. Neither nitrogen nor phosphorus was effective unless sufficient vitamins, Fe-EDTA and Mn-EDTA were added, whereas the growth yield of Chattonella marina was promoted primarily by addition of Fe-EDTA or phosphorus. Nitrogen was effective only after enrichment with Fe-EDTA and phosphorus. The primary importance of Fe-EDTA was detected both in seawater collected in the early phase and at the climax of C. marina red-tide. He suggested that, in the case of eutrophic waters, chelated iron is one of the crucial factors triggering red tide outbreaks. Secondary nutrients, which stimulate the growth of red tide flagellates in coupling with chelated iron, are different from species-to-species.

Regarding iron uptake, Okaichi et  $al^{22}$  using radioactive iron EDTA- $^{59}$ Fe, calculated the half saturation constants for uptake by C. antiqua, Prorocentrum minimum and H. akashiwo. The values were almost the same. However, the time required to reach the maximum rate of uptake was the shortest in C. antiqua.

Although the role of the vitamin B group in red tide formation has been discussed by many workers, knowledge of its distribution and dynamics in Japanese coastal waters is limited. Recently, vitamin concentration of seawater was measured in Harima-Nada<sup>23</sup>, Ise Bay<sup>24</sup>, and elsewhere<sup>25</sup>. Nishizima and Hata<sup>23</sup> measured vitamin B<sub>12</sub>, thiamine, and biotin concentrations in seawater and bottom sediments at 24 stations in Harima-Nada in early summer and autumn. They showed that the distribution of vitamins corresponded with the distribution of heterotrophic bacteria, and was higher in eutrophic areas. From the seasonal variation of vitamins, they suggested that many phyto-

thiamine, and biotin during the growing season. They also examined vitamin dynamics in the water, and found that 1) the vitamin load from polluted inland water was an important source into the sea; 2) release rates of vitamins into the overlying water from the bottom sediments increased with increasing temperature; 3) both bacterial and phytoplankton communities may play the most important role among the agents supplying and removing vitamin B12 in eutrophic waters. Furuki et al. also showed that C. antiqua grew well in coexistence with bacteria in vitamin B12-free medium. Nishizima calculated vitamin B12 half saturation constants for growth, photosynthesis and uptake of several red tide flagellates. C. antiqua required a cell vitamin B12-quota of about 8.5 ng/1 to maximize its growth rate. Each red tide flagellate required about the same vitamin B12 concentration (10-20 ng/1). Consequently, he suggested that the concentration of vitamin B12 in the water was one of the most important factors controlling the outbreak of red tides in the coastal waters.

The relationship between organic or trace metal pollution originating in fish cultivation and the occurrence of red tides has stimulated discussion. Seawater collected near fish farms promoted the growth of Gymnodinium nagasakiense. Growth was also enhanced greatly by the addition of extracts from mackerel meat and yellowtail feces, while seawater enriched with inorganic nitrogen and phosphorus, and nitrogen, phosphorus plus iron did not promote growth. Growth of C. antiqua was enhanced only after addition of chelated iron to seawater, among various nutrients. From these results, Nishimura<sup>27</sup> concluded that dissolved organic matter at the fish culture site contributes greatly to the occurrence of G. nagasakiense red tide.

The macro-nutrient requirement of C. antiqua was reexamined in a nitrogen- or phosphorus-limited semi-continuous culture system by Nakamura<sup>28</sup>. He calculated the half saturation constants of nitrate, ammonium, and phosphate for uptake and growth. The results showed that these values were unexpectedly low in contrast to the cell volume. Comparisons of the half saturation constants with nutrient concentrations in the Seto Inland Sea in summer suggested that phosphate is one of the controlling factors of population growth of C. antiqua.

#### VERTICAL MIGRATION

Flagellates are well known to undertake vertical migrations. Such migrations have the great advantage of permitting the utilization of the nutrients in a water column of 10-20 meters. Hirano29 has pointed out the importance of vertical migration in the development mechanism of red tide from the serial observations of the vertical distribution of Gymnodinium in a tide pool. Recently, more detailed observations of vertical migration have been made almost concurrently by several workers in the laboratory and field. Concerning H. akashiwo and C. antiqua, Watanabe et al 30 showed that the timing of descent and ascent did not coincide exactly with the onset or end of light phase, and nitrate and phosphate uptake were less affected by light conditions in these species than in coastal diatoms. From the combination of diurnal vertical migration in thermally stratified water and the ability to take up nutrients at night, they described that these species have increased ecological advantages over coastal diatoms. Hatano et al 31 observed the speed of migration of H. akashiwo and its adaptation to photoperiodic rhythm using a cylinder (D 4 x L 30 cm). The downward movement commenced at a half an hour before the light at a speed of 3.5 cm/h. Under continuous illumination or darkness, no vertical migration occurred, but a homogeneous distribution was thus obvious. The vertical migration was changed to a new rhythm at a 6L: 6D photoperiod. It took 3 to 5 days to acclimate to a new migration regime.

In field observations at two fishing ports (about 3 and 8 m in depth) in Osaka Bay, Yamochi and Abe<sup>32</sup> obtained interesting experimental results to prove the ecological significance of diel vertical migration of H. akashiwo.

They observed diel changes in the vertical profile of cell numbers together with several environmental factors at intervals of 0.5 to 3.0 hours when H. akashiwo was predominant. They also prepared dialysis bags filled with red water of the organism. One was suspended at the surface, another close to the bottom, and a third one was exposed to the surface in the daytime and the bottom at night. The organism migrated toward the surface early in the morning at a speed of 1.0 to 1.3 m/h. Downward migration was found in the afternoon, and more than 3,000 cells/ml aggregated in the bottom layer. H. akashiwo could cross steep temperature and salinity gradients (6.5°C and 5.7% S) during its diel vertical migration. High values of particulate organic carbon and nitrogen concentration were obtained in dialysis bags suspended in situ at an identical layer with high cell concentration, while the values for surface and bottom bags were comparatively low. These results show that the organism migrated toward the surface to carry out photosynthesis effectively, and to the bottom to utilize nutrients efficiently.

#### POPULATION GROWTH AND INTERACTION OF ORGANISMS

Population density and growth rate of *G. nagasakiense* were measured by Lizuka<sup>33</sup> in Omura Bay in two ways. Cell number was counted in water samples collected from different depth, and in dialysis tubes filled with in situ seawater containing the organism and suspended at 2.5, 7.5, and 12.5 meters for one to two days. The population growth started 11 days before reaching the density of a red tide. The growth rate was highest at the surface layer,  $\mu_2 = 1.05$  on average, and lowest at the bottom. The growth rate at the middle layer was about 60 per cent of the surface; however, high cell density was maintained for a week after the disappearance at the surface. He also discussed the carrying capacity from the information of the maximum cell density of each organism and the maximum chlorophyll-a content in different waters.

Honjo<sup>34</sup> described the competitive relation between S. costatum and H. akashiwo in phytoplankton succession, and suggested that H. akashiwo produced an allelopathic substance inhibiting the growth of S. costatum at a concentration of  $10^7$  cells/l of H. akashiwo. The interrelation among phytoplankton, bacteria, and Chattonella during the process of development of the Chattonella bloom was investigated by Furuki et al<sup>35</sup> in Harima-Nada. The results showed that suitable communities and characteristics of bacteria were required for the growth of Chattonella. In mixed culture of the bacteria and Chattonella in vitamin-deficient medium, they grew well when they could live together symbiotically, and showed no growth when cultured separately. Ishio et al<sup>36</sup>. succeeded in isolating a new species Vibrio algoinfesta, from the bottom sediments of Hakata Bay which produces dinoflagellate growth inhibitor (DGI). Since then, they have been trying to isolate the substance, determine the chemical structure, synthesize the substance chemically, and produce it in high yield.

Concerning grazing pressure, based on a feeding experiment with Acartia clausi, Tsuda and Nemoto suggested that it could detect the biomass peak and alter its feeding behavior rapidly within at most 6 hours. Itoh and Imai measured the grazing rate of five copepods at different concentration of C. marina. The grazing rate of Calanus sinicus increased with increasing cell concentration up to an average maximal rate of 380 cells/animal/h. They suggested that the feeding activity of these copepods on Chattonella presumably influences the initiation or termination of Chattonella red-tide. Uye sinvestigated the grazing of C. antiqua for five species of copepods. Ingestion rate increased linearly with increasing cell concentration untill a maximum level was reached, beyond which the rate was constant. This cell concentration was higher for larger copepods. The weight-specific maximum ingestion rates were higher in the small species. He calculated grazing pressure of the natural copepod community in Harima-Nada by itegration of