

The Biology and Prediction of Representative Red Tides Associated with Fish Kills in Japan

Tsuneo Honjo

Red Tide Research Division, Nansei National Fisheries Research Institute, Ohno, Saeki, Hiroshima 739-04 JAPAN

ABSTRACT: The damage to marine resources caused by red tides represents a serious problem for fisheries and allied industries. In Japan, most fish farms have suffered heavy losses from red tides of the dinophyte *Gymnodinium nagasakiense* and of the raphidophytes *Chattonella antiqua*, *C. marina*, and *Heterosigma akashiwo*. Therefore, an extensive series of studies has been conducted in Japan. Elucidation of the mechanisms of outbreak of harmful red tides associated with fish kills and the development of countermeasures for these red tides are urgently needed. Recently, *Heterosigma* red tides have caused severe losses for salmon culture operations in several places around the world. The development of fish farming and the progress of eutrophication on a global scale will accelerate the incidence of harmful red tides. This article is a review of the physiological ecology of these organisms and of the techniques proposed in Japan to predict the outbreaks of harmful red tides in areas that have experienced damage from them.

KEY WORDS: red tide, *Gymnodinium nagasakiense*, *Chattonella antiqua*, *Heterosigma akashiwo*.

1. INTRODUCTION

Many kinds of organisms usually coexist in natural seawater. Red tide is a marine phenomenon in which one or several species among these organisms grow sporadically or gradually to high cell densities and consequently discolor the water. Most harmful red tides that kill fisheries' resources in Japan are formed by certain phytoplankters belonging to the Dinophyceae and Raphidophyceae. Such dramatic phenomena have focused the attention of many Japanese researchers.

Historical records of the outbreaks of red tide date roughly to the eighth and ninth centuries. According to Takano (1987), some discolored water events in the sea were referenced in ancient documents (early Heian era, 797 and 875). The first scientific study in Japan was published around the early 1900s (e.g., Nishikawa, 1900; Nishikawa, 1903; Okamura, 1911). Nishikawa (1900) described the impact of *Peridinium* and *Gymnodinium* red tides on fish and pearl oysters.

Thereafter, studies were published on occasion, but most of them were limited to descriptions of the red tide organisms and of the damage caused by the red tides. Systematic study was initiated from 1966; the red tides of *Gymnodinium nagasakiense* in Tokuyama Bay of the Seto Inland Sea in 1957 and in Omura Bay of western Kyushu in 1965 served as turning points (Hanaoka, 1972). In 1972 in the Harima-Nada of the Seto Inland Sea, 14 million cultivated yellowtail were killed by a *Chattonella* red tide. The amount of the damage reached ¥7.1 billion (\$1 was worth about ¥360 in 1972). In response, the Japanese Fisheries Agency consolidated a monitoring system for red tides centering around the Prefectural Fisheries Experimental Stations.

Before the 1950s there were less than 10 cases of red tide outbreak per year, and the range and duration of those outbreaks were small (Figure 1). It was after the 1960s that red tides occurred frequently on a much larger scale and inflicted obvious heavy damage on fisheries. In those days, Japan was on the threshold of high economic growth, and people began pouring into cities and factories that were built along the coasts. The aquaculture of fish (yellowtail) using net cages in coastal waters had begun to make rapid progress and experienced significant setbacks as a result of the frequent outbreaks of red tides.

In the Seto Inland Sea, nitrate, total nitrogen (ammonia + nitrite + nitrate), and phosphorus concentrations increased rapidly from the mid-1960s (Figure 1) but nutrient concentrations reached a maximum in the mid-1970s. Thereafter, the concentrations decreased gradually. After 1968, the number of red tides increased each year, reaching a peak of 299 in 1976. However, the number of red tides decreased to about 160 per year in proportion to the decrease in nutrient concentrations. Thus, the frequency of red tide occurrences is closely related to eutrophication. People became aware of the necessity of preventing the environmental pollution. In 1972, fishery environmental criteria were established. In 1973, the temporary Regulation for the Protection of the Environment of the Inland Sea of Japan was enacted by the Parliament. Consequently, the Act of Protection of Water Pollution was also put into force. These actions also influenced the decrease of nutrient concentrations in the Seto Inland Sea. Lam and Ho (1989) reported that the increasing red tides were a consequence of accelerated eutrophication at Tolo Harbor in Hong Kong following intensive urban development in the catchment as well as the Seto Inland Sea.

In other countries, harmful or nuisance red tides of *Gymnodinium breve*, *Gymnodinium* cf. *nagasakiense*, *Chrysochromulina polylepis*, *Heterosigma akashiwo*, *Aureococcus anophagefferens*, etc. have been reported (e.g., Okaichi et al., 1989). Furthermore, there is some evidence of a global increase in novel phytoplankton blooms (Smayda, 1990). In Japan, most of the fish damage has been caused by red tides of the dinophyte *Gymnodinium nagasakiense* and raphidophytes *Chattonella antiqua*, *C. marina*, and *H. akashiwo*. Occasional damage has been caused by the dinophytes *Cochlodinium* sp. and *Noctiluca scintillans*. Therefore, an extensive series of studies in Japan has emphasized red tides of *G. nagasakiense*, *Chattonella*, and *H. akashiwo*.

Recently, the frequency of severe damage by *Chattonella* and *Heterosigma* red tides has tended to decrease. Nevertheless, the total damage amounted to about ¥15.5 billion between 1980 and 1990. The constant production of cultured

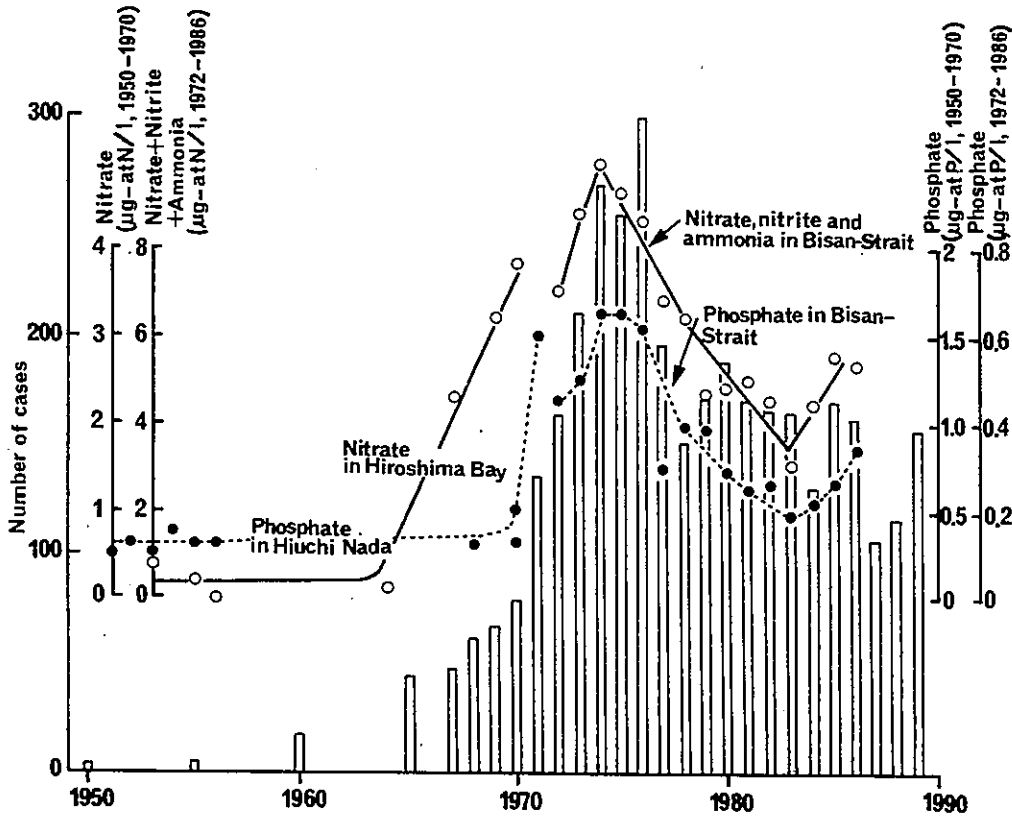


FIGURE 1. Total number of red tides (open bars) and concentrations of nitrogen (open circles and solid lines) and phosphorus (solid circles and dashed lines) in the Seto Inland Sea. Data for nutrients between 1972 and 1989 were furnished by the Okayama Prefectural Fisheries Experimental Station.

fish each year is the goal of greatest concern to fishermen. Red tides have had serious impact on the lives of fishermen. Eradication of red tides is far beyond our power even if we united forces in applying current techniques. Therefore, the development of techniques for predicting these outbreaks is an urgent priority for avoiding the damage. Shirota (1989a,b) reviewed in detail the predictive techniques and countermeasures developed as government efforts by the Japanese Fisheries Agency. This article contains descriptions of a few predictive techniques for *G. nagasakiense* and *Chattonella* red tides as published in Japan.

II. GYMNODINIUM NAGASAKIENSE TAKAYAMA ET ADACHI

A. MORPHOLOGY AND DISTRIBUTION

Gymnodinium nagasakiense Takayama et Adachi is an unarmored marine dinoflagellate (Figure 2A, about 30 μm). This organism has been called by an informal name, *Gymnodinium* sp. Type '65, since its first recording in Omura Bay in 1965 (Iizuka and Irie, 1966). Since then, red tides of this species have appeared in many areas of western Japan. In 1984, along the Kii Peninsula, the damage from a red tide of this organism in the Kumano-Nada amounted to about ¥4.4 billion. Takayama and Adachi (1984) identified this organism as *Gymnodinium nagasakiense*. However, Matsuoka et al. (1989) examined the geographic distribution of *G. nagasakiense* around western Japan and discussed the need for its taxonomic reevaluation in comparison with similar species, in particular *G. nagasakiense*, *G. mikimotoi*, and

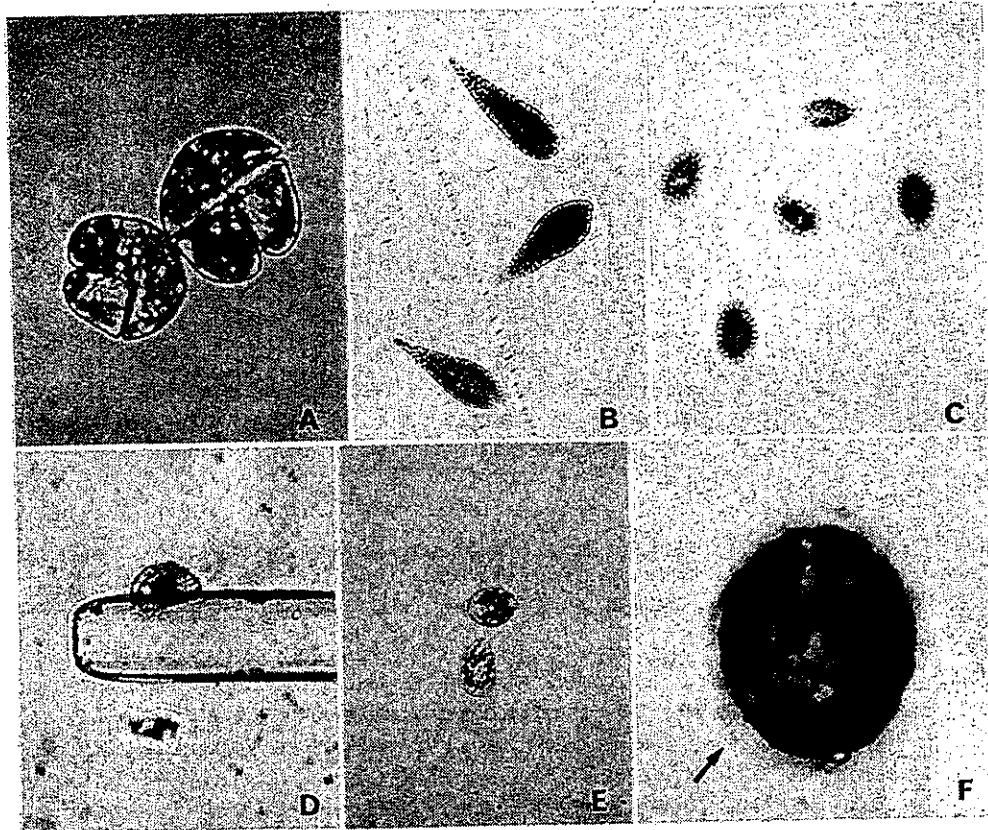


FIGURE 2. Representative red tide organisms in Japan. *Gymnodinium nagasakiense* (A), *Chattonella antiqua* (B), *C. marina* (C), cyst of the genus *Chattonella* (D), preencystment cells (E), and *Heterosigma akashiwo* (F; arrow shows glycocalyx on the cell surface).

Gyrodinium aureolum. Recently, Takayama and Matsuoka (1991) reassessed the morphological and physiological features and proposed that *G. mikimotoi* is conspecific with *G. nagasakiense* and that the former is a senior synonym for the latter. Nevertheless, I will use *G. nagasakiense* here.

Nagasaki et al. (1991a) explored the biochemical taxonomy using a monoclonal antibody which recognizes the cell surface of *G. nagasakiense* (Suo-Nada strain). This monoclonal antibody reacted against all strains of *G. nagasakiense* isolated in Japan but not against *G. breve* (Nagoya Bay strain), *G. catenatum* (Senzaki Bay strain), and *Gymnodinium* cf. *nagasakiense* (Lisbon strain). The isozyme patterns of several strains of *G. nagasakiense* were investigated by Sako et al. (1989), and they pointed out that the analyses were very useful for determining differences in locality and population.

Red tides of *G. nagasakiense* occur widely around western Japan. Occasionally, this organism tends to form red tides over wide areas. Tokyo Bay and Maizuru Bay seem to be the northernmost habitats of this organism along the Pacific Ocean and Japan Sea coasts, respectively. Red tides of *G. nagasakiense* have been recorded in Korea and Hong Kong since the 1980s (Cho, 1981; Lee and Kwak, 1986; Kim, 1989; Wong, 1989; Park, 1991). A similar species has also been observed in Australia (Hallegraeff, 1991).

B. PHYSIOLOGY

Yamaguchi and Honjo (1989) examined the effects of several physical factors on the growth of *G. nagasakiense* (Suo-Nada strain) using axenic cultures. Growth occurred at irradiances greater than 10 $\mu\text{E}/\text{m}^2/\text{s}$ and saturated at 110 $\mu\text{E}/\text{m}^2/\text{s}$. Further, they investigated the growth of this organism at 25 different combinations of temperature and salinity under conditions of light saturation. In that experiment they acclimated the cells to each of the test conditions for about 1 month. Growth was observed at temperatures from 10 to 30°C and at salinities from 15 to 30‰. The highest growth rate was observed at the combination of 25°C and 25‰. The range of salinity that sustained growth at more than 0.5 divisions per day was relatively wide (15 to 30‰) at the optimum temperature (25°C) but was reduced to a much narrower range at suboptimal temperatures (e.g., 20 to 30‰ at 20°C and 25 to 30‰ at 30°C). A statistical test indicated that temperature and salinity interact in their influence on growth. The results revealed that *G. nagasakiense* is a eurythermal and euryhaline organism. The following formula was developed for predicting the theoretical growth rate (μ = divisions per day) from temperature and salinity under conditions of saturated light and ample nutrients:

$$\mu = 1.05753 - 0.3022T + 0.01777T^2 \\ - 0.00035T^3 + 0.00515TS - 0.0001TS^2,$$

where T is the temperature and S is the salinity.

Iwasaki et al. (1990) examined the growth response of *G. nagasakiense* (Gokasho Bay strain) to ecological factors such as light, temperature, salinity, pH, and major nutrients using clonal cultures under axenic conditions. The highest growth rate was obtained at temperatures of 20 to 25°C, salinities of 25 to 26‰, light intensities above

130 $\mu\text{E}/\text{m}^2/\text{s}$, and pH 8.0. Both inorganic and organic nitrogen and phosphorus served as good nitrogen and phosphorus sources. Although several organic substances were utilized and stimulated growth, they did not support growth in the dark. Iron and manganese promoted growth remarkably at a concentration of 200 $\mu\text{g}/\text{l}$. The critical concentration of vitamin B₁₂ was 10 ng/l. Ishimaru et al. (1989) reported that selenium stimulated the growth of *G. nagasakiense* when cultured in a medium based on offshore seawater containing nitrate, phosphate, vitamins (B₁₂, biotin, thiamine), and metals (Fe, Mo, Co, Cu). Growth-promoting substances are present in seawater in and around fish cages (Nishimura, 1982).

C. ECOLOGY

Studies of the ecology of *G. nagasakiense* were initiated by Iizuka and Irie (1966) in Omura Bay. Iizuka and Irie (1969) and Iizuka (1972) investigated the factors related to the occurrence of *G. nagasakiense* red tides in the summer and proposed two factors as being important for those red tides: rainfall and either anoxic bottom water or bottom water having low oxygen concentrations. The importance of the latter was subsequently confirmed through *in vitro* experiments; the organism is resistant to sodium sulfide (Iizuka and Nakajima, 1975), and enhanced growth results from anoxic water (Hirayama and Numaguchi, 1972). Thereafter, Nakata and Iizuka (1987) reported that this organism was present as motile cells at concentrations of 20 to 60 cells per liter in Omura Bay in January and February 1983, and Terada et al. (1987) observed about 10³ cells per milliliter in a harbor of the western Seto Inland Sea when water temperatures were below 10°C. Further, Itakura et al. (1990) and Hosaka (1990) observed *G. nagasakiense* motile cells in Hiroshima Bay and Tokyo Bay during the winter. However, the annual life cycle of cell density of this organism was not yet determined.

Honjo et al. (1990) investigated the annual abundance of *G. nagasakiense* motile cells in Gokasho Bay from 1984 to 1987. When plotted against the mean temperature of the water column, the abundance of motile cells showed bimodal patterns as well as clear decreases during the periods of minimum and maximum water temperature. Figure 3 shows an example of the annual cycle of cell density between April 1989 and April 1990.

Exponential growth occurred during both spring and autumn in the bay (Honjo et al., 1990). Spring growth originated from the motile cell stocks that overwintered; autumn growth arose from cells that survived the high-temperature period. Spring growth was initiated when cell densities were low; during April and early May, the rise in temperature above about 16°C resulted in the initiation of growth culminating in summer red tides from mid May through early July. Autumn growth occurred when the water temperature declined from about 22 to 16°C and light intensity reduced gradually; a localized red tide was observed from December 1984 to January 1985. Although overwintering stock sizes appear to be dependent on temperature, growth of *G. nagasakiense* seems to be independent of rising or falling temperatures and seems to occur over a wide temperature range. It is clear that the motile form of *G. nagasakiense* overwinters in Gokasho Bay. Physiological features of this organism when grown *in vitro* at 10°C support the idea that this organism overwinters as motile cells (Yamaguchi and Honjo, 1989). However, the life cycle of

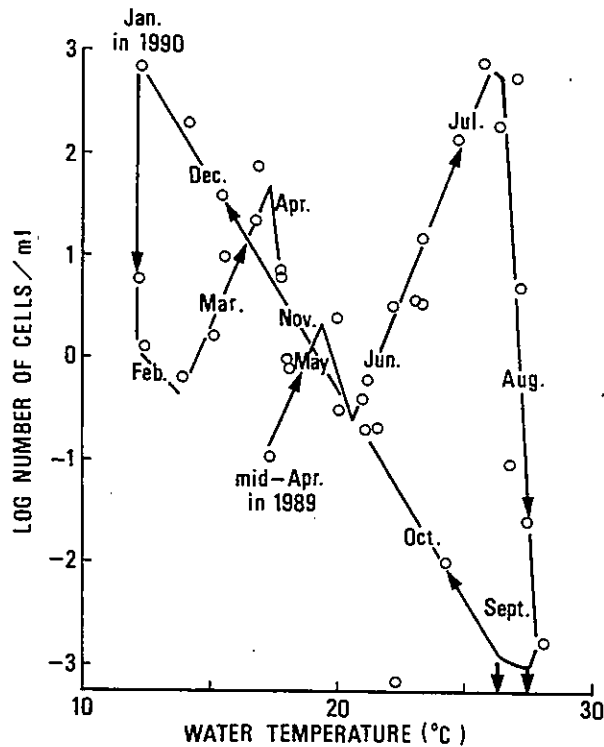


FIGURE 3. Annual cycle of motile cells of *G. nagasakiense* versus the mean temperature of the water column from mid-April 1989 to late April 1990. Motile cells were not observed in 2.5-l water samples during September 1989 (arrows pointing to x axis).

G. nagasakiense and the stock size after the summer red tide have not been elucidated and require future studies.

The maximum growth rate for *G. nagasakiense* has been estimated at 1.0 division per day from both *in situ* and *in vitro* studies (Iizuka, 1979; Iizuka and Mine, 1983; Iizuka et al., 1989). However, spring growth rates in Gokasho Bay were considerably lower than that, ranging from 0.32 to 0.47 divisions per day. The low growth rates may be related to the loss of cells to other areas by displacement of seawater. The low rates result in a long interval between the low cell densities observed at the time of growth initiation and those required for red tides. Developing populations must also encounter unfavorable conditions, such as mixing by wind and reduced salinity from rainfall. Despite these suboptimal conditions, red tides frequently develop in Gokasho Bay. An upward shift in vertical distribution during the daytime was noted as the density of the organisms increased; most cells occurred at depths of 5 to 10 m and densities of 10^2 to 10^3 cells per milliliter but at 0 to 2 m, densities were 10^3 to 10^4 cells per milliliter. The ability to maintain themselves in the middle layer at low cell densities may be important in avoiding the frequently unfavorable conditions encountered at or near the surface.

Downward diurnal vertical migrations were clearly observed at population levels of 10^2 to 10^3 cells per milliliter (Honjo et al., 1990). From the data, the speed of downward migration was estimated to be about 1.3 m/h. A greater speed of 2.2 m/h, has been observed (Y. Koizumi, Ehime Prefectural Fisheries Experimental Station, personal communication). These figures suggest that the population can, at a minimum, migrate more than 8 m downward in 6 h and that most cells at 10 m in the daytime could reach a layer deeper than 18 m by midnight. The mean depth in Gokasho Bay is 12.7 m. Therefore, during the night most cells pass through bottom waters, which contain fairly high concentrations of nutrients and can reach the bottom mud that is extremely rich in nutrients. The interstitial water of the mud and the adjacent seawater contain certain growth-promoting substances (Hirayama and Numaguchi, 1972). Thus, the capability for diurnal vertical migration appears to be another important factor in the development of these red tides, that is, if this organism can take up nutrients during the night.

Dividing cells occurred during the evening and early morning, with the highest proportion occurring around midnight (Honjo et al., 1990). Yamaguchi (1992) investigated DNA synthesis and the cell cycle in *G. nagasakiense* by determining relative DNA contents of individual cells using an epifluorescence microscopy-based microfluorometry system. The nuclei were stained with the DNA-specific fluorochrome 4'-6-diamino-2-phenylindole (DAPI). Because photosynthetic pigment interferes with the fluorescence from the DAPI-DNA complex, the pigment was eliminated by methanol treatment as a first step in quantitative microfluorometry. He determined nuclear DNA contents, cell size distribution, cell density, and frequency of paired cells every 2 h for 24 h using cells grown on a 12-h light:dark cycle. DNA synthesis (S phase) occurred from 1000 to 2200 h and was followed by cell division. The presence of such a distinct S phase strongly suggests that *G. nagasakiense* has a typical eukaryotic cell cycle.

In other waters, such as the Suo-Nada and Omura Bay, the supply of nutritive substances from bottom waters containing low oxygen concentrations may be necessary for growth to high cell density. During a summer season on a weekly basis, Tamori et al. (1991) monitored cell numbers of *G. nagasakiense*, water temperature, salinity, and dissolved oxygen in the Suo-Nada and in the Iyo-Nada, western Seto Inland Sea in order to investigate the mechanism for the occurrence of the spreading of red tides over wide areas. They found that the motile cells appeared initially in the western and southern coastal waters of the Suo-Nada that were characterized by high temperature, low salinity, and the occurrence of an oxygen-deficient bottom layer. Further, they implied that population growth was related to the formation of a pycnocline and that the spread of the red tide resulted from the overturn of stratification induced by strong winds (such as typhoons) resulting in the injection of nutrients from the oxygen-poor bottom water.

Fukami et al. (1992) recently reported that a bacterium, tentatively identified as *Flavobacterium*, caused a remarkably inhibitory effect on the growth of *G. nagasakiense* isolated from Uranouchi Inlet in Shikoku Island. The algal effect of the bacterium was observed on *G. nagasakiense*, but not on *Chattonella antiqua*, *Heterosigma akashiwo*, or a diatom, *Skeletonema costatum*. This finding may be related to the rapid decline of *G. nagasakiense* red tides.

D. PREDICTION

Honjo et al. (1991) monitored water temperature and the number of *G. nagasakiense* motile cells at a station in Gokasho Bay for 6 years. The water temperature was measured at the same depths immediately after plankton sampling. Daily or weekly temperatures for the water column were averaged and mean water temperatures for each month were calculated, along with mean water temperatures for the winter (December–March).

Figure 4 shows changes in the abundance of motile cells of *G. nagasakiense* and in mean monthly temperature. Motile cells were continually observed through the winter (December through March) in 1984–1985, 1986–1987, and 1987–1988. However, motile cells were not observed in 1-l samples during the winter in 1985–1986 and 1988–1989. The Julian days on which mean cell densities of 1 cell per milliliter and 10^3 cells per milliliter in the water column were first reached were estimated

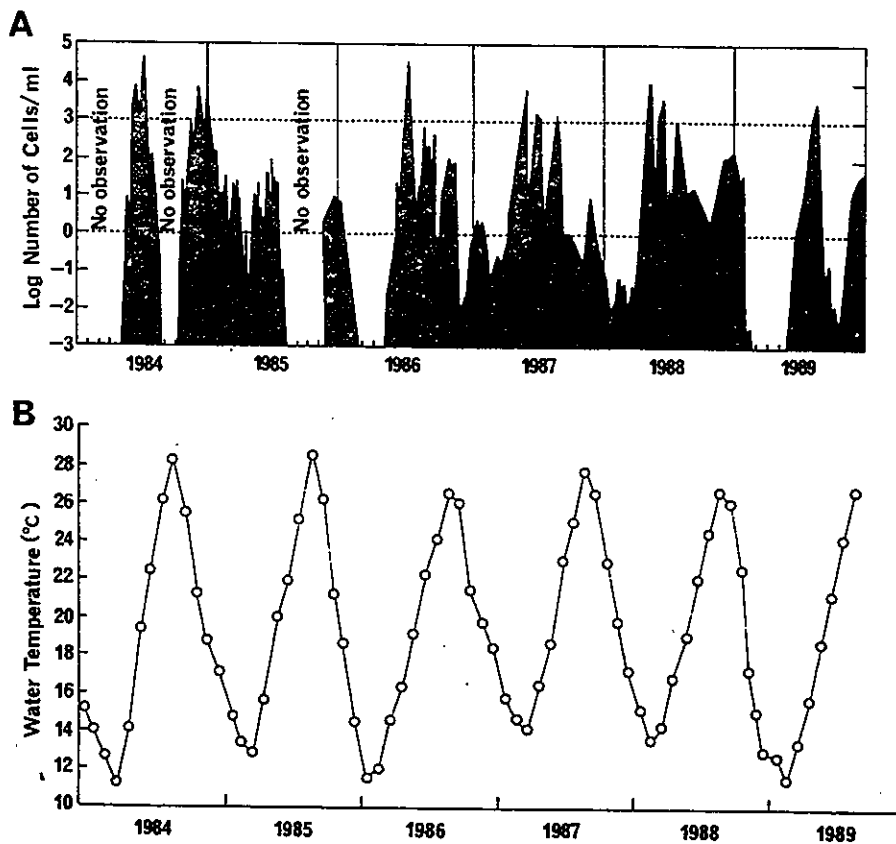


FIGURE 4. (A) Changes in abundance of motile cells of *G. nagasakiense* from May 1984 through 1989, and (B) monthly mean water temperature from December 1983 through September 1989.

from regression lines of the growth curves each year. In 1985, cell densities did not exceed 10^3 cells per milliliter. The mean winter water temperatures were low in 1984, 1986, and 1989, and conversely were high in 1985, 1987, and 1988. Figure 5 presents the two relationships between the Julian days on which cell densities of 1 cell per milliliter and 10^3 cells per milliliter were first recorded and the mean water temperature of the preceding winter. The correlation coefficient between the mean winter temperature and the days on which cell densities reached 1 cell per milliliter was -0.98 and that between the mean winter temperature and the days on which cell densities reached 10^3 cells per milliliter was -0.91 .

Yamaguchi and Honjo (1989) found in *in vitro* experiments that temperature and salinity interacted in their influence on growth of *G. nagasakiense*. However, because winter salinities were nearly constant in the bay during the period of the investigation, the authors suspected that the stock size of the organism in the winter would be primarily dependent on water temperature. Spring growth, culminating in summer red tides, was initiated between April and early May when the temperature rose above ca. 16°C in Gokasho Bay. Once initiated, spring growth was exponential and showed a nearly constant rate from year to year, ranging from 0.32 to 0.47 divisions per day. The time at which cell densities reach 1 cell per milliliter appears to be determined by the size of the overwintering stock which, in turn, is regulated

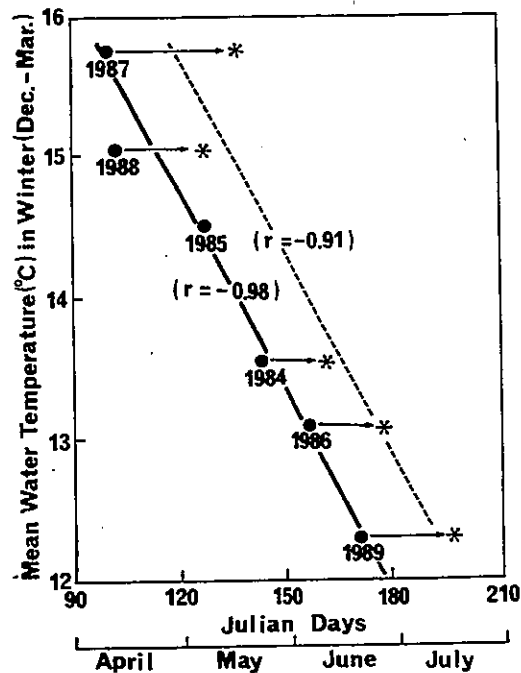


FIGURE 5. Relationships between mean water temperature in the winter and Julian days on which density of *G. nagasakiense* first reached 1 cell per milliliter (solid line) and 10^3 cells per milliliter (dotted line). Figures in parentheses denote correlation coefficients.

by water temperature. The timing of red tides (10^3 cells per milliliter) appears to be linked directly with the duration of the time lag of the early growth phase (1 cell per milliliter) (Figure 6). Because resting cysts of the organism have not been found and because the motile cells can grow at temperatures as low as 10°C (Yamaguchi and Honjo, 1989), they suspect that some *G. nagasakiense* motile cells remain alive even when winter water temperatures are low and that spring growth originates from the overwintering motile cell stocks. These results imply that the timing of red tides is predictable several months beforehand by monitoring water temperatures of Gokasho Bay in the winter. Prediction of the timing of the early growth phase might allow enough time to implement counterplans to avoid damage. In fact, each year the author informs the Nansei town office ruling Gokasho Bay of the estimated time of the outbreak on the basis of the mean winter temperature. The town office communicates this information to fishermen who then know when to stop feeding their cultured fish, which reduces the oxygen demand and considerably decreases the mortality of the fish during red tides.

The demonstrated relationship between mean winter water temperature and the timing of growth may be related to the particular physical and chemical factors of the bay. Recently, Toda et al. (1990) investigated the seasonality of water exchange in Gokasho Bay. The residence time of the water mass is an important factor regulating the stock size of overwintering motile cells. Environmental properties in other bays where *G. nagasakiense* red tides frequently occur are different from those in Gokasho Bay. Therefore, it remains to be determined whether similar signals can be derived from winter temperatures in other localities.

The development of a short-term predictive technique was explored using the diel pattern of cell division of *G. nagasakiense* (Yamaguchi and Honjo, 1990).

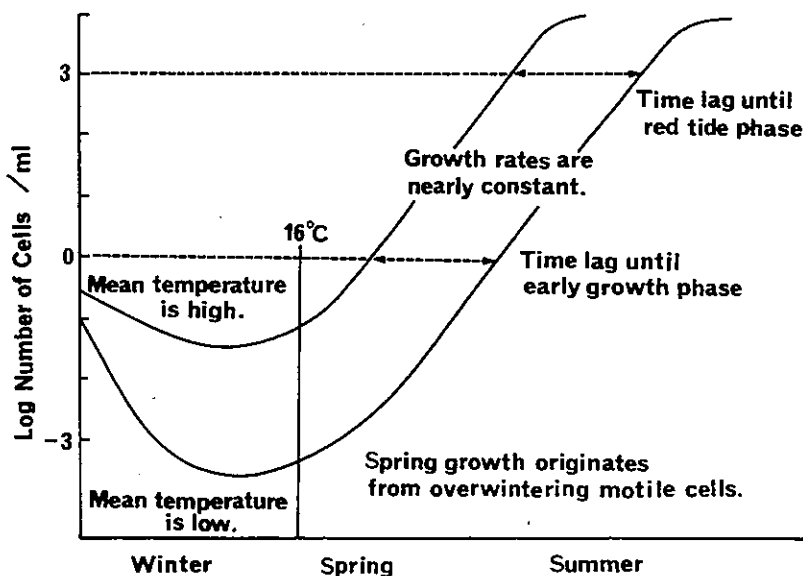


FIGURE 6. Diagram of the timing of *G. nagasakiense* red tides as related to winter water temperature.

G. nagasakiense exhibits a striking phased cell division in light:dark cycles, with the maximum frequency of paired cells occurring between 0400 to 0600 h. Thus prediction of cell increase during the daytime may be possible through observation of the number of paired cells in the early morning.

III. THE GENUS CHATTONELLA

A. MORPHOLOGY AND DISTRIBUTION

Chattonella antiqua (Hada) Ono (Figure 2B) and *C. marina* (Subrahmanyam) Hara et Chihara (Figure 2C) are the species of *Chattonella* that cause harmful red tides in Japan. The size of *C. antiqua* is 50–130 μ 30–50 μ m, and the size of *C. marina* is 30–50 μ 20–30 μ m. A red tide of *Chattonella* was first recorded in Hiroshima Bay in 1969. Hada (1974) initially identified this organism as the genus *Hemieutreptia*. Ono and Takano (1980) classified the genus as *Chattonella* on the basis of light and electron microscopical observations. Morphological classification within the genus *Chattonella* is often difficult because the organisms exhibit various cell sizes, lack rigid cell walls, and change shape in response to environmental conditions.

Nagasaki et al. (1989) and Hiroishi et al. (1988) approached the identification of the genus *Chattonella* by using an immunological technique, preparing specific monoclonal antibodies against the organism. From the reaction spectrum of each strain to the seven kinds of monoclonal antibodies, 18 strains, which had been morphologically identified as the genus *Chattonella*, could be divided into six groups. *C. antiqua* and *C. marina* were separated into different groups. Two strains of *C. marina* isolated from Kagoshima Bay in Kyushu showed very different reaction spectra from those isolated from the Seto Inland Sea region. The antigen molecules, which a monoclonal antibody recognizes, may be glycoproteins and the epitope may be in the carbohydrate side-chain (Nagasaki et al., 1991b).

Chattonella red tides have been reported frequently from western Japan. The distribution of the two species in Japan is similar to that of *G. nagasakiense*. Red tides of the genus *Chattonella* have been recorded in France (Biecheler, 1936; Hollande and Enjumeat, 1956) and India (Subrahmanyam, 1954).

B. PHYSIOLOGY

Many researchers have reported on the growth responses of the genus *Chattonella* to temperature, salinity, and irradiance in the laboratory (Iwasaki, 1971; Nakamura et al, 1982; Nakamura and Watanabe, 1983a; Yamochi, 1984). They described the responses to individual factors in terms of the growth rates or final yields of *C. antiqua* or *C. marina*. Yamaguchi et al. (1991) examined the optimum environmental requirements for growth and survival using 30 combinations of temperatures (10 to 30°C) and salinities (10 to 35‰) under light-saturated conditions. The cells were acclimated previously under each of the test conditions. The maximal growth rates of *C. antiqua* and *C. marina* were obtained at combinations of 25°C and either 20 or 25‰. Neither species survived at 10°C. This result shows that *Chattonella* can not overwinter in many coastal areas of Japan. A statistical test indicated that temperature had the greatest influence on growth rate.

Regarding nutrient requirements, Iwasaki (1971) and Nakamura and Watanabe (1983b) pointed out that *C. antiqua* requires vitamin B₁₂ and, especially, Fe and Mn. Nakamura and Watanabe (1983c) and Nakamura (1985a,c) examined the nitrate and phosphate uptake kinetics of *C. antiqua* grown in light:dark cycles. Further, Nakamura et al. (1988) and Nakamura et al. (1989) monitored concentrations of macronutrients and dissolved Fe and Cu in the surface seawater at two stations in the Harima-Nada, Seto Inland Sea. They detected 3.9 to 10 and 9.3 to 11.2 nM of Fe and Cu, respectively. They confirmed from bioassay experiments that these concentrations of Fe are usually insufficient to support the maximum growth rate of *C. antiqua* except when the organism is dominant. They concluded that Fe, but not Cu, is potentially an important factor in regulating populations of *C. antiqua* in the Seto Inland Sea. Iwasaki (1979) determined that *C. antiqua* can utilize polyphosphate. However, Nakamura (1985b) reported that the alkaline phosphatase activity of *C. antiqua* is very weak and that this organism may not be able to utilize organic phosphomonoesters. Elucidation of this inconsistency on utilization of organic phosphomonoester requires further studies.

C. ECOLOGY

The life cycle of the genus *Chattonella* has been elucidated in detail. Much of this work has been conducted by Imai and his colleagues. Imai et al. (1984a) identified vegetative cells of *Chattonella* in the seawater incubated with sediment samples. After establishing a method of enumerating dormant cells in marine sediments (Imai et al. 1984b), Imai and Itoh (1986) made the initial discovery of *Chattonella* cysts in surface sediments in the Suo-Nada. The cysts were mostly hemispherical, 25 to 35 μm in diameter and 15 to 25 μm in height, and were markedly smaller than the usual vegetative cells (Figure 2D). Living cysts showed autofluorescence of chloroplasts under blue-light excitation regardless of their ability to germinate. Most cysts adhered to solid surfaces such as diatom frustules and sand grains (Imai and Itoh, 1988). The maximum number of cysts observed in samples collected from several areas was around 500 per cubic centimeter of sediment (Imai et al., 1986; Imai, 1990; Itakura et al., 1991).

Imai (1989) investigated cyst formation in *C. marina* using culture experiments. N limitation under low light conditions (1000 lx or less) was effective in inducing cyst formation. He followed the process of cyst formation and published some interesting photographs. The induced cysts were quite similar in terms of morphological characteristics to those observed in sediments from the Inland Sea. The germination of cysts occurred at 22°C and 3500 lx on a 14 h L: 10 h D photocycle after storage at 11°C in the dark for more than 4 months. The majority of cysts were uninucleate. Nakamura and Umemori (1991) also observed cyst formation in *C. antiqua*. Nakamura et al. (1990) speculated that the cysts of *C. antiqua* are products of sexual reproduction. Very recently, however, Yamaguchi and Imai (submitted) of the Nansei National Fisheries Research Institute confirmed that *Chattonella* cysts have a DNA content of 1 c, vegetative cells have a DNA content from 2 to 4 c, small precystment cells (Figure 2E, about 30 μm) induced in the culture have 1 c, and excysted cells from natural cysts within 24 h of germination have 1 and 2 c. From these results, they concluded that *Chattonella* has a diplontic

life cycle and that cyst formation occurs through meiosis of the vegetative cells. Reduction of fluorescence intensity from stained nuclei may have led Nakamura et al. (1990) to an incorrect conclusion.

The germination of *Chattonella* cysts has been investigated using natural cysts (Imai and Itoh, 1987; Imai et al., 1989, 1991b). Temperature was the principal factor influencing germination of the cysts. The incidence of cyst germination was zero at 10°C and very low at 15 and 18°C. It increased at 20°C, reached maxima at 22 and 25°C, and then decreased markedly at 30°C. Maturation and dormancy of cysts were also affected by temperature. At temperatures of 11°C or less, more than 4 months of storage were required for cyst maturation. No maturation was observed at 20°C or greater. Imai and Itoh (1987) summarized the annual life cycle of *Chattonella* as follows: (1) in early summer vegetative cells originate from the germination of cysts in sediments; (2) the vegetative cells form cysts during the summer season; (3) the cysts spend a period of spontaneous dormancy until the next spring; (4) the duration of post dormancy, an enforced dormancy due to low temperature, follows until early summer, although the cysts have already completed spontaneous dormancy in spring.

The population dynamics of *C. marina* were investigated by Honjo (1987). That species showed high growth potential, 1.4 to 2 divisions per day, when cells collected from Gokasho Bay were isolated individually in small tissue chambers and were cultured in the laboratory. Therefore, it was expected that *Chattonella* red tides may form rapidly under natural conditions. However, Ono (1989) reported that the population growth rate during a *Chattonella* red tide did not exceed one division per day.

Chattonella antiqua is capable of diurnal vertical migration and can take up nutrient salts during the dark period (Nakamura and Watanabe, 1984). However, *C. antiqua* migrates to a depth of only about 10 m, where nutrients were consumed primarily by diatoms. Therefore, physical events for supplying nutrients to the upper layer may be necessary for the occurrence of *Chattonella* red tides. Based on oceanographic data, Fukase (1992) considered the mechanism of occurrence of *Chattonella* red tides in the Harima-Nada to be as follows: (1) a large quantity of seawater originating from the Kii-Strait flows suddenly into the Harima-Nada through Naruto-Strait; (2) many *Chattonella* cysts are then released from the sediment; and (3) these cysts germinate and grow rapidly. According to H. Ohtsuka of the Tokushima Prefectural Fisheries Experimental Station (personal communication), *Chattonella* cells must grow rapidly just after certain sporadic environmental events, such as typhoons, which supply nutrients from the bottom sediments to the surface layer.

Imai et al. (1991a) isolated a bacterium with algicidal activity against *C. antiqua*. This bacterium was tentatively identified as *Cytophaga* sp. When a suspension of about 10⁸ bacteria per milliliter was added to a *C. antiqua* culture, the *Chattonella* cells settled to the bottom of flask and were completely killed within 2 to 3 d. Nakamura et al. (1992) examined the grazing effects of a heterotrophic dinoflagellate, *Gyrodinium dominans*, upon *C. antiqua* in laboratory batch culture. He reported that *G. dominans* fed on *C. antiqua* at an ingestion rate of 3.8 prey per cell per d. These findings may relate to the abrupt decrease in cell numbers during the death phase of *C. antiqua* red tides.

Previously, *Chattonella* was believed to be a naked flagellate. Yokote and Honjo (1985) demonstrated the presence of glycocalyx on the surface of *C. antiqua* cells using electron and light microscopy (Figure 7). Histochemical analysis of this structure indicated that the glycocalyx consists of at least two different types of acidic complex carbohydrates, sulfated and nonsulfated, together with a neutral carbohy-

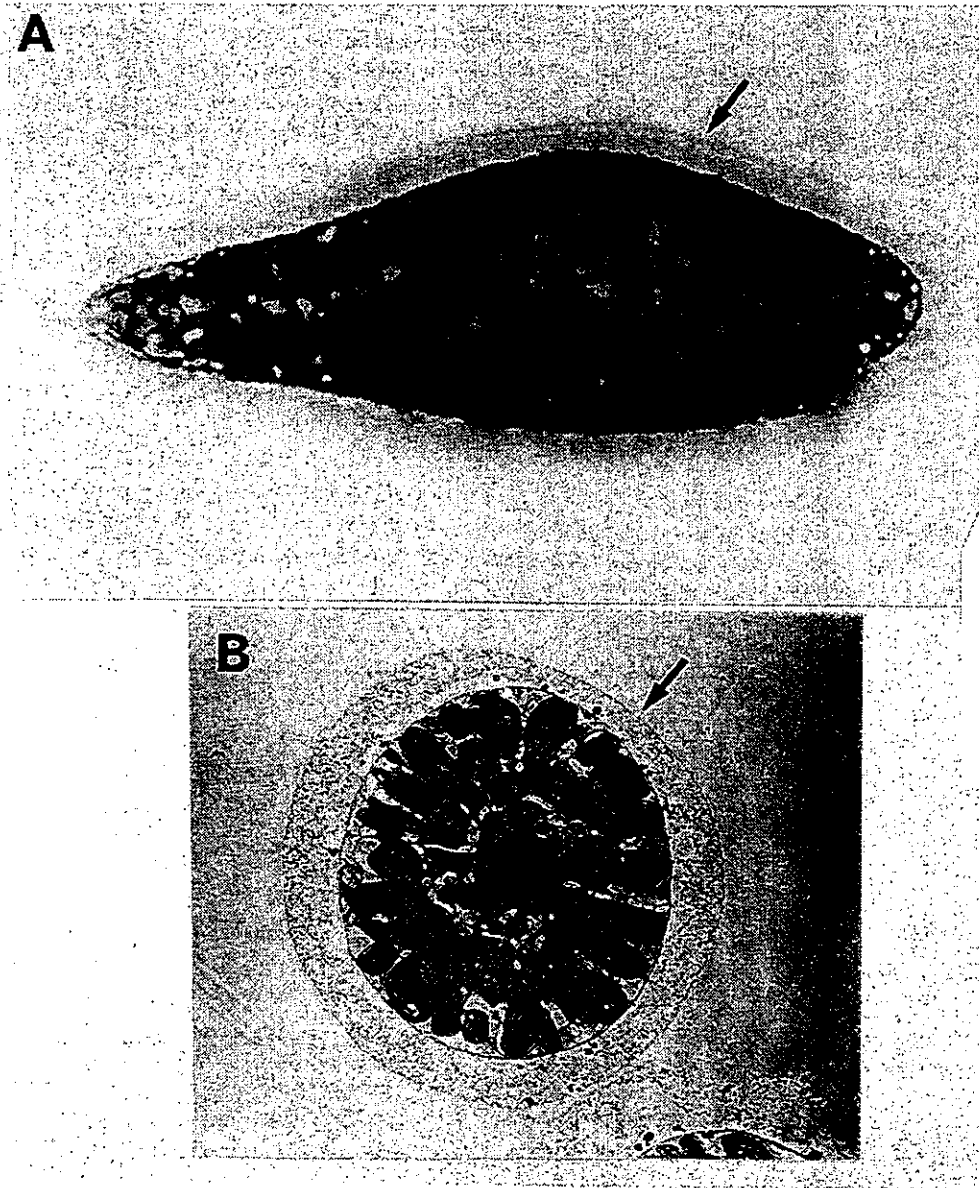


FIGURE 7. Light (A) and electron (B) micrographs of a *Chattonella* cell. Arrows illustrate the glycocalyx on the cell surface.

drate-protein complex with 1,2-glycol groups and with α -D-mannosyl and α -D-glucosyl residues. The functions of the glycocalyx is discussed in the section on the ecology of *H. akashiwo* red tides.

D. PREDICTION

Iwata et al. (1989) analyzed the relationship between vertical stability and the outbreak of red tides in the Harima-Nada using data from a 14-year period. They used the vertical difference in density, which is calculated from water temperature and salinity, as an index for the prediction of red tides of the genus *Chattonella*. They found that the red tides tended to occur in years when the vertical differences in density were small in May and July. They calculated the average deviation from mean bottom water temperature from February to July. The mean bottom water temperature was typically high in the years when *Chattonella* red tides occurred. They suggested that the combination of the vertical difference of density in July and the average temperature deviation from the mean between February and July may allow prediction of *Chattonella* red tides (Figure 8A). Yanagi (1989) found that the average air pressure difference between Fukuoka and Osaka in December, January, and February (winter) was a good parameter for predicting the outbreak of *Chattonella* red tides in the Harima-Nada during the following summer. He also found that the average water temperature difference between the depths of 10 and 40 m in the central part of the Harima-Nada in May and June was a useful parameter. Combining these parameters, he proposed a hypothesis in which *Chattonella* red tides tend to occur in years when the westerly monsoons are not strong and when density stratification is weak (Figure 8B).

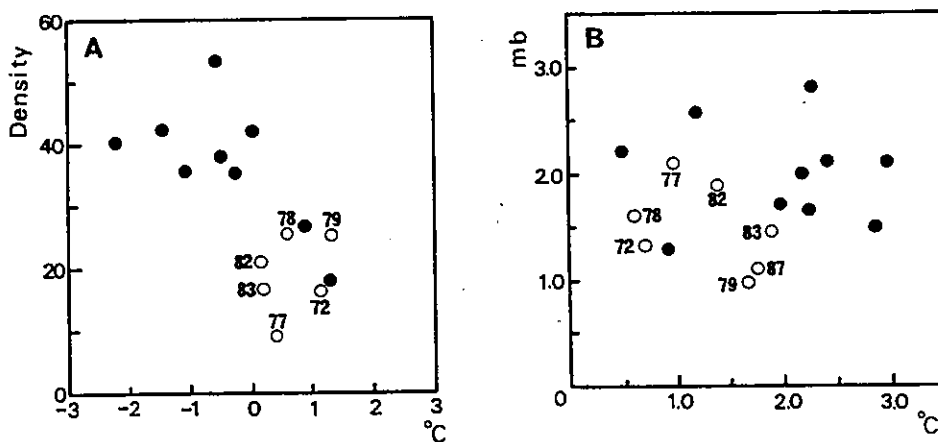


FIGURE 8. (A) The relation between the vertical difference in density ($\times 1000$) in July and the average deviation from the mean bottom water temperature ($^{\circ}\text{C}$) during the period from February to July (modified from Iwata, 1989), and (B) the relation between the average difference in air pressure (mb) in the winter between Fukuoka City and Osaka City and the average difference in water temperature ($^{\circ}\text{C}$) in early summer between the depths of 10 and 40 m in the central part of the Harima-Nada (modified from Yanagi, 1989). Open circles denote the years when *Chattonella* red tides occurred; black circles denote when they did not occur.

Red tides of both *G. nagasakiense* and *C. marina* occur in the Suo-Nada. However, these organisms seldom occur at the same time. Imai (1990) showed that from 1982 to 1987 the years resulting in *Chattonella* and *G. nagasakiense* red tides were distinguishable by the cumulative irradiation time and amount of precipitation during the period between June 1 and July 10; *Chattonella* red tides occurred in years when the irradiation time was long and the amount of precipitation was small. Results of *in vitro* experiments on the effects of light intensity on *Chattonella* and *G. nagasakiense* support his observation (Yamaguchi and Honjo, 1989; Yamaguchi et al., 1991).

IV. HETEROSIGMA AKASHIWO (HADA) HADA

A. MORPHOLOGY AND DISTRIBUTION

The cells of *Heterosigma akashiwo* are yellow-brown and ovoid, being slightly compressed dorsoventrally (Figure 2F). The cell size is $8-25 \times 6-15 \mu\text{m}$. This organism lacks a cell wall and the cell is bound only by a single membrane. The delicate structure of the cell has hindered study of its surface morphology. In Japan, *H. akashiwo* has been referred to by other names, such as *Entomosigma akashiwo* (Hada, 1967) and *H. inlandica* (Hada, 1968), and it has been confused with the chrysophyte *Olisthodiscus luteus* (Carter, 1937) in many countries. Hara and Chihara (1987) reported that *E. akashiwo* and *H. inlandica* are synonymous with *H. akashiwo*, and most red tides ascribed to *O. luteus* actually involve *H. akashiwo*.

Heterosigma akashiwo has been reported in temperate and subtropical embayments in Japan (Iizuka and Irie, 1968; Hara and Chihara, 1987), Korea (Park, 1991), Singapore (Taylor, 1990), New Zealand (Chang, 1990), England (Lackey and Lackey, 1963), Belgium (Conrad and Kufferath, 1954), eastern and western areas of North America (Lackey and Clendenning, 1965; Tomas, 1982; Taylor, 1990), Bermuda (Tomas, 1982), and Chile (Taylor, 1990). In Japan red tides of *Heterosigma* show a tendency to occur in embayments. Most areas where the red tides occur frequently are within the zone where currents are less than 0.5 knots. Tabata and Honjo (1981) investigated the relationship between the displacement rate of seawater (0.1 to 10 times/d) and the appearance of this species, using outdoor continuous-culture vessels of 80 l volume. The experimental vessels were provided with running seawater containing natural plankton. They found that *Heterosigma* cells appeared only at displacement rates less than 2 times per day; the optimum rate for *Heterosigma* appearance was 0.1 times per day.

In the Seto Inland Sea, the total damage from *H. akashiwo* red tides amounted to about ¥2 billion over the 16-year period from 1972 to 1987. Most of this damage has been to fish culture operations. Mortalities of yellowtail and red sea bream have been recorded for the Seto Inland Sea, while mortalities of salmon have been recorded in New Zealand (Chang et al., 1990), Canada (Taylor, 1990), the U.S. (Gaines and Taylor, 1986), and Chile (Taylor, 1990).

B. PHYSIOLOGY

Tomas (1978a) reported from culture experiments that the temperature range for growth of *O. luteus* (Narragansett strain) was from 15 to 30°C. Mori et al. (1982) and Watanabe and Nakamura (1984b) found that the optimum temperature range for a Tanigawa strain was 15 to 25°C and that the strain could grow slowly at 12°C. This helps to explain why *Heterosigma* red tides tend to occur from May through late June. However, the range of suitable salinities differs among culture strains: 30‰ for a Fukuyama strain (Iwasaki et al., 1968), 10‰ for a Gokasho strain (Iwasaki and Sasada, 1969), 27 to 28‰ for a Hakata strain (Honjo and Hanaoka, 1973), 12 to 40‰ for a Narragansett strain (Tomas, 1978a), 12 to 28‰ and 12 to 30‰ for a Tanigawa strain (Mori et al., 1982; Watanabe and Nakamura, 1984a), and 8 to 34‰ for a Tokyo strain (Hosaka, 1992). This suggests that these strains have become physiologically adapted to the ranges of salinity in their habitats.

The basic nutrient requirements for growth of *H. akashiwo* are inorganic nutrients and vitamins (Iwasaki et al., 1968; Iwasaki and Sasada, 1969; Mori et al., 1982; Watanabe and Nakamura, 1984a). The organism also requires trace metals, especially iron and manganese (Honjo, 1974; Iwasaki, 1979). Kohata et al. (1982) and Watanabe et al. (1982a) studied the kinetics of phosphate uptake. The strain from Tokyo Bay was not able to use glycerophosphate and urea (Hosaka, 1992). Environmental events which supply the above-mentioned nutritive substances may be river run-off, formation of oxygen-deficient bottom water, and disturbance of the bottom sediment by wind.

C. ECOLOGY

Growth originates from cell stocks that have overwintered as motile forms or germinated from resting spores. Motile cells have been observed at densities of 100 cells per liter in water samples collected during January to March which is the period of lowest water temperature (Yamochi, 1989). Thus, *Heterosigma* may overwinter as motile cells. On the other hand, Tomas (1978b) and Yamochi (1989) found that motile cells aggregate and metamorphose to nonmotile cells. Yamochi suspended dialysis tubes containing wild *Heterosigma* cells in surface water for 3 d and observed benthic cells on the walls of the tubes. More recently, Imai and Itakura (1991) investigated the distribution of dormant *Heterosigma* cells in sediments in northern Hiroshima Bay, where red tides of *H. akashiwo* occur almost every year. They found densities of dormant cells ranging from 56 to 5950 cells per cubic centimeter in April and from 56 to 29,300 cells per cubic centimeter in March. Also Imai and Itakura of the Nansei National Fisheries Research Institute (personal communication) examined the morphology of *H. akashiwo* cysts from bottom sediments in the same area.

Yamochi (1989) examined the nutrients limiting growth of *H. akashiwo* in Osaka Bay using the algal assay procedure. Growth was enhanced by the addition of chelated iron to filtered seawater, but the addition of nitrogen, phosphorus, or vitamins had no appreciable effect. The concentration of soluble iron in surface water was highest in the inner part of Osaka Bay where salinity was less than 30‰ and where *Heterosigma* red tides occurred frequently. Therefore, he concluded that

the increased supply of chelated iron from river run-off may be a critical factor in red tide outbreaks of *H. akashiwo*.

In Hakata Bay, growth of *H. akashiwo* is usually initiated in the late spring or early summer when a pycnocline is formed in the middle layer (Honjo, 1974). Oxygen concentrations in the bottom water decrease rapidly after the formation of the pycnocline. The pH of the anoxic bottom water decreases to about seven. As a result, nutritive substances (inorganic nutrients, metals, and organic constituents, including growth-promoting substances) are released from the bottom sediments and high levels of these substances accumulate in the anoxic bottom waters. Culture experiments revealed a close relationship between the *in situ* oxygen content of the bottom water and the growth rate of *H. akashiwo*. The species migrates toward the surface early in the morning at a velocity of 1 to 1.3 m/h. Downward migration occurs in the afternoon, and aggregations form in the bottom layer at night. The organism can cross a steep pycnocline gradient during its migration and can take up nitrate and phosphate in the dark (Yamochi et al., 1982; Yamochi, 1989; Watanabe et al., 1982b, 1984). Thus, *Heterosigma* populations have access to these nutritive substances in the bottom water through diurnal vertical migration.

The interstitial water in bottom sediments also contains ample nutritive substances that accelerate the growth of *H. akashiwo*. Honjo and Hanaoka (1974) and Honjo (1974) found that a suspension obtained by swirling a surface sample of bottom sediment in culture medium promoted growth. The bottom sediments promoting the growth contained relatively high levels of organic matter (Honjo and Hanaoka, 1972). Disturbance of the bottom sediment by wind may also lead to red tide outbreaks in coastal waters.

Red tides of this species develop so rapidly and dramatically that many workers have studied the growth of *H. akashiwo* *in vitro* and *in situ* as an approach to the enigma of red tide formation. The results, however, have conflicted. Tabata and Honjo (1983) attempted the culture of marine flagellates using an outdoor continuous-flow system with a 70 m³ tank. The tank was provided with a continuous flow of coastal seawater enriched with NO₃⁻ (15 µg-atN/1) and PO₄³⁻ (1 µg-atP/1). A halocline was made in the middle layer of the tank by passing seawater diluted with tapwater into the upper layer at night and by passing nondiluted seawater into the lower layer in the daytime for several days. Detailed description of the tank was provided by Tabata and Honjo (1983). A supply of natural plankton was provided by the coastal seawater coming into the tank. A massive red tide of *Heterosigma* occurred in the tank. The researchers pointed out that important factors for the successful mass culture of *H. akashiwo* in the tank were an adequate supply of nutrients and protection against loss of the organism from the tank. Most of the *Heterosigma* cells actively migrated to the bottom layer into which seawater did not flow at night.

Honjo et al. (1978a) investigated *Heterosigma* growth dynamics in a fishing port and observed growth rates greater than 1 cell division per day. In the above-described outdoor tank, population growth could be divided into rapid and exponential phases (Honjo and Tabata, 1985). They observed that *H. akashiwo* has the potential for high growth rates, 2 to 5 cell divisions per day, and that there is a strong tendency for large cells to dominate at the beginning of the rapid and exponential growth phases and to be replaced by small cells toward the end of these phases.

They also conducted culture experiments in flasks. A portion of a *Heterosigma* patch in a tank was cultured in a 1000-ml volumetric flask hung in the tank. The growth rate in the flask was 2.3 cell divisions per day. Other *Heterosigma* cells collected from the tank were individually cultured in small tissue chambers. In those chambers, the highest growth rate was 3.3 cell divisions per day; the growth rates of large cells inoculated into the chambers were much higher than those of small cells. The high growth potentials were observed especially at low cell densities in the range of 10 to 1000 cells per liter. Further investigation of environmental and physiological conditions triggering rapid growth is necessary.

The organism prefers dark-associated cell division. In the tank, cell division of *H. akashiwo* first occurred a little before sunset and continued through the night until the next morning. Similarly, in the small chambers cell division began just before the onset of the dark period and continued for 4 to 5 h into the next light period. Seven *Heterosigma* cells divided three times during the night in the small chambers, with a cell division interval of about 6 h (Figure 9).

Puiseux-Dao (1981) and Chisholm (1981) reviewed the chronobiology of cell division in unicellular algae. They discussed the cell division cycle of flagellates in terms of the length of the circadian period but did not consider the rapid division cycles of *H. akashiwo*. The raphidophyte *C. marina* has also shown a high growth potential. Further studies are needed to clarify whether or not the Raphidophyceae are unique among red-tide flagellates in their ability to grow at over 1.0 cell division per day.

The surface structure of *H. akashiwo* cells was examined by light microscopy after the cells were processed by current techniques for carbohydrate and protein histochemistry (Yokote et al., 1985). The results indicated that: (1) *H. akashiwo* has a glycocalyx on its cell surface (Figure 2F); (2) this extracellular structure consists of acidic complex carbohydrates together with a neutral carbohydrate-protein

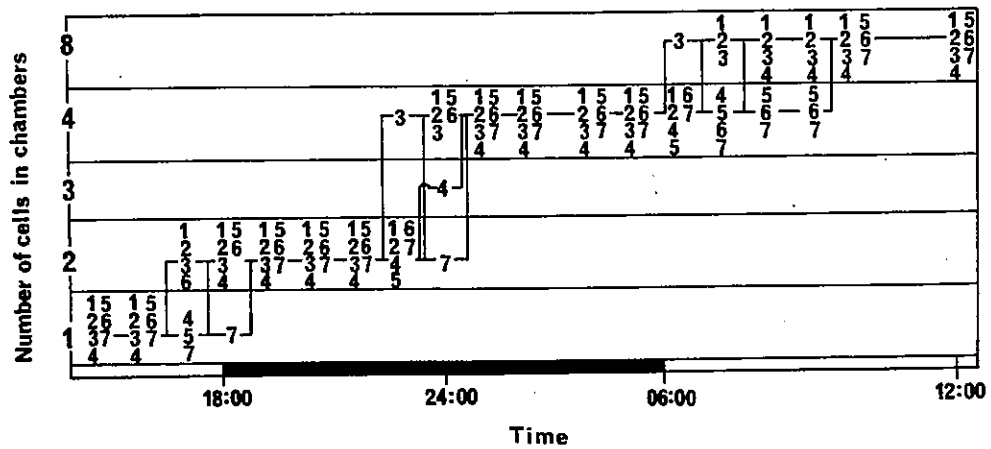


FIGURE 9. Time course of *Heterosigma* cell division in individual cultures growing in small tissue chambers. Almost all began their first division between 1700 and 1900 h. Although their subsequent division times were not uniform, they all stopped dividing at the beginning of the next light period. Numbers 1 through 7 identify the chambers.

complex; (3) a particular moiety of acidic complex carbohydrates involved in the glycocalyx is presumably hyaluronic acid, or a closely related substance; and (4) the histochemical nature of the glycocalyx on the cell surface of *H. akashiwo* is comparable with that of *C. antiqua*, except for a difference in the nature of the neutral carbohydrate-protein complex. The biological importance of surface carbohydrates in animal and plant cells has come to light in recent years. Acidic complex carbohydrates in particular have been considered to perform such physiologically important functions as the maintenance of structural integrity, selective absorption, lubrication, filtration, ion-exchange, and anticoagulating action (Ogston, 1970). Further, glycoproteins are known to be important components for cell recognition and reception of chemical information (Hughes, 1975; Shinohara, 1977). Since the glycocalyx has affinity for colloidal iron, *Heterosigma* and *Chattonella* cells may be able to utilize colloidal iron if they possess certain substances such as siderophores (Murphy et al., 1976; Simpson and Neiland, 1976). The functional roles played by the surface carbohydrates of phytoplankton remain unclear. However, there would seem to be a good possibility that analogous cases in animal and plant cells apply to the surface carbohydrates of phytoplankton as well.

Pratt (1966) reported from weekly observations over a 7-year period that the phytoplankton of Narragansett Bay are alternatively dominated from May through October by brief blooms of *Skeletonema costatum* and *Olisthodiscus luteus*. Through *in vitro* experiments he found that filtrates from more than 10^5 cells per milliliter of *O. luteus* inhibited the growth of *S. costatum*, while those from lower cell densities enhanced the growth of the diatom. Honjo et al. (1978b) noted from daily observations at a fishing port in Hakata Bay that cell numbers of *S. costatum* suddenly decreased when the density of *H. akashiwo* exceeded about 10^4 cells per milliliter. In culture experiments using enriched filtrates of seawater from an *in situ* bloom of *H. akashiwo*, they found that the growth of *S. costatum* was temporarily suppressed. Those studies imply that an allelopathic relationship between the organisms occurs during high cell densities of *H. akashiwo*. Stuart (1972) and Sakshaug (1977) examined the physical features of the allelopathic substances, but experienced difficulty in determining the molecular weight. Since that time, this interesting problem between these phytoplankters has not been investigated further. The study of allelopathy is important for elucidating the mechanisms of the monospecific bloom events which characterize *Heterosigma* red tides.

Tomas and Deason (1981) reported that *O. luteus* was an unsatisfactory food for two *Acartia* species. Uye and Takamatsu (1990) examined feeding interactions between the inshore marine copepods *Pseudodiaptomus marinus* and *Acartia omorii*, and 15 red-tide flagellates. They found that *H. akashiwo*, *Gymnodinium nagasakiense*, and *Chattonella marina* were entirely rejected by *A. omorii*. Such feeding rejection of copepods imply that the major red tide organisms in Japan have reduced grazing pressure and thus have an advantage in terms of the formation of red tides.

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