Plankton Biol. Ecol. 46 (1): 37–47, 1999

plankton
biology & ecology

Population development of the red tide dinoflagellate *Gymnodinium mikimotoi* in inshore waters of Japan

BON KIMURA[1], MASATO KAMIZONO[2], TAKUYA ETOH[2], YOSHITSUGU KOIZUMI[3], MASATADA MURAKAMI[4] & TSUNEO HONJO[5]

[1]*Department of Food Science and Technology, Tokyo University of Fisheries, Konan, Minato, Tokyo 108–8477, Japan*
[2]*Fukuoka Fisheries and Marine Technology Research Center, Buzenkai Laboratory, Buzen 828–0022, Japan*
[3]*Ehime Prefectural Fisheries Experimental Station, Uwajima 798–0104, Japan*
[4]*Department of Food Science and Technology, National Fisheries University, Shimonoseki 759–6595, Japan*
[5]*Department of Agriculture, Kyushu University, Higashi, Fukuoka 812–0053, Japan*

Received 27 April 1998; accepted 6 August 1998

Abstract: Blooms of the dinoflagellate *Gymnodinium mikimotoi* were investigated in the Suo-Nada inshore waters in the western Seto Inland Sea, Japan, during the summers of 1992 and 1993. In 1992, a bloom of *G. mikimotoi* (>1000 cells ml^{-1}) occurred following temperature stratification of the water column associated with the appearance of low dissolved oxygen (<50% of saturated concentration) and a marked increase in NH_4^+ in the bottom water (14–15 July). In 1993, a marked increase in NH_4^+ in both surface and bottom water and an increase of the *G. mikimotoi* population were observed following mixing of the water column induced by strong wind (20 m s^{-1}) of a typhoon (11 August). Blooms of the organism (2500 cells ml^{-1}) occurred in nearshore coastal areas one week after mixing during frequent rainfall, when a pycnocline was formed in the middle layer (18 August). Bioassay experiments in 1993 showed that the marked increase in NH_4^+ in the bottom water was favorable for growth of *G. mikimotoi*. River loading of NO_3^- after rainfall did not directly stimulate the growth of *G. mikimotoi*. The following scenario was suggested: (1) release of NH_4^+ and other micronutrients by biological regeneration at the sea bottom stimulate population development of *G. mikimotoi*; (2) such release occurs following two different events, strong stratification of the water column accompanied by the appearance of low oxygen concentrations in the bottom layer, and a mixing of the water column induced by strong wind; (3) after mixing of the water column, stratification allows the development of *G. mikimotoi* blooms.

Key words: *Gymnodinium mikimotoi*, bloom, Suo-Nada Sea, bioassay, dinoflagellate

Introduction

Gymnodinium mikimotoi is an unarmored marine dinoflagellate and one of the noxious red tide flagellates causing mass mortalities in cultured and wild fish and shellfish populations in western Japan (Honjo 1987, 1994; Matsuoka et al. 1989; Yamaguchi 1994). This species was first reported as *Gymnodinium* sp. type-'65 (Iizuka & Irie 1966). Thereafter it was named *G. nagasakiense* (Takayama & Adachi 1984). Recently this has been proposed to be a synonym of *G. mikimotoi* Miyake et Kominami ex Oda (Takayama & Matsuoka 1991). Also this species is thought to be closely related to the European type *Gyrodinium aureolum* Hulburt (Hallegraeff 1993). A bloom of *G. mikimotoi* was first reported in 1965 at Omura Bay, on the western coast of Kyushu Island, Japan (Iizuka & Irie 1966). Since then the blooms have occurred nearly annually during the summer along the coasts of various regions of western Japan, including the Suo-Nada Sea on the eastern coast of Kyushu Island. During the summers of 1985 (Matsuoka et al. 1989) and 1991, red tides covered a wide area of shoreline in and around the Suo-Nada Sea including the Iyo Nada Sea (western Seto Inland Sea), causing serious damage to aquaculture. Although the frequency of large-scale red tide events has gradually been decreasing since the late 1980s due to effluent controls, local blooms of this alga still occur frequently during the summer, particularly nearshore. Motile cells of this species are also observed in the winter

Corresponding author. Bon Kimura; e-mail, kimubo@tokyo-u-fish.ac.jp

(Nakata & Iizuka 1987; Terada et al. 1987; Itakura et al. 1990) and throughout the year (Honjo et al. 1990).

In earlier investigations, Iizuka & Irie (1969) and Iizuka (1972) proposed two hypotheses to explain the initiation of *G. mikimotoi* blooms in Omura Bay: rainfall for July blooms and either anoxic bottom water or bottom water having low oxygen concentrations for September blooms. Particularly frequent occurrences of September blooms have been limited to regions in the vicinity of the anoxic central part of the bay, and Iizuka (1972) suggested a close relationship between bloom development and the appearance of anoxic bottom water. In a recent study in the Suo-Nada Sea, Tamori et al. (1991) hypothesized that many *G. mikimotoi* blooms that occurred in this area were initiated in the inshore and southwestern region. Also, a following study (Koizumi et al. 1994) revealed that the red tide expanded offshore into other regions of the Suo-Nada Sea through diffusion of the upper mass as a consequence of the breakdown of stratification in the inshore area. Aside from this information on the timing and location of bloom occurrence and the mechanisms of diffusion, little is known about the causative nutritional and environmental conditions for the initial increase in cell density of *G. mikimotoi* in inshore waters.

In a two-year (1992, 1993) study in the Suo-Nada Sea, as part of a monitoring program set up by both national and regional agencies, we investigated environmental conditions favoring the formation of blooms of *G. mikimotoi* in nearshore coastal waters.

Materials and Methods

Investigation area

The Suo-Nada Sea is a large enclosure of water surrounded by the main island of Japan (Honshu) and by Kyushu Island, with an outlet to the Japan Sea consisting of a narrow strait (Fig. 1). In the study area, located nearshore in shallow water (less than 15 m), the effect of offshore currents from the open sea (Pacific Ocean) is relatively weak, resulting in a muddy bottom. Although the water is only about 8 to 14 m deep in this area, the marked increase in water temperature in late July generally involves temperature stratification, with Δt of up to 3°C. These factors often lead to oxygen deficient water at the sea bottom in this area, and this is easily disturbed by mixing of the water column (Tamori et al. 1991; Kamizono 1997). The concentration of dissolved inorganic nitrogen (DIN) is generally low, ranging from 0.2 to 10 μg-at l^{-1}, with an average of 2 μg-at l^{-1}. The average PO_4^{3-} is 0.1 μg-at l^{-1}, being undetectable at times. Overall, the level of eutrophication in the Suo-Nada Sea is generally low compared to other Japanese coastal areas with frequent red tide incidents (Endo et al. 1987).

The site selected for this study, Stn 11, was located 3 km (depth: 8 m) off the coast of Yukuhashi-city on Kyushu Island (Fig. 1). The station is in a region where red tides have been recorded frequently since monitoring began in 1973. Outbreaks of red tides in the Suo-Nada Sea are believed to originate from populations present in nearshore areas, including Stn 11 (Tamori et al. 1991; Koizumi et al. 1994). Other stations were also used for monitoring *Gymnodinium mikimotoi* to give a geographic distribution of the blooms along the coast (Fig. 6).

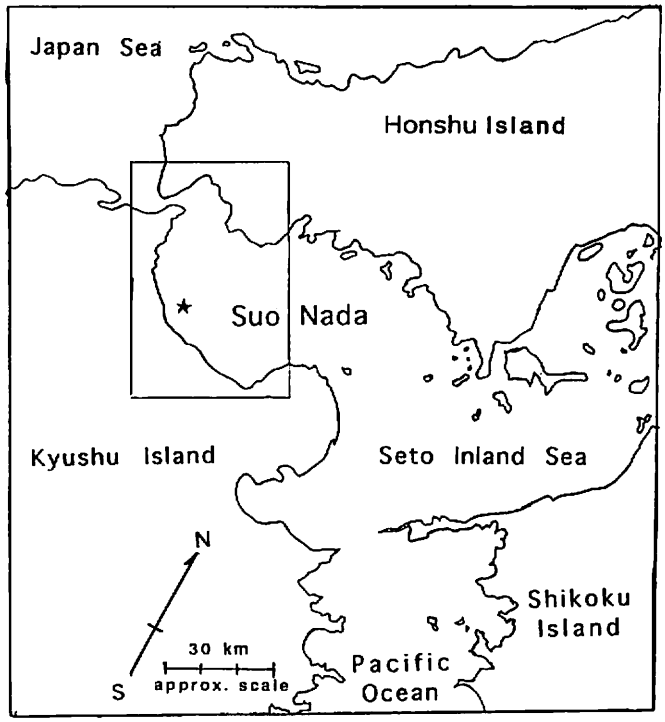

Fig. 1. Study area showing sampling stations in the Suo-Nada Sea. *Star* indicates location of Stn 11.

Sampling

Sampling was performed at weekly intervals, unless otherwise noted, from late May to late August in 1992 and 1993. On each sampling occasion, a vertical profile of salinity and temperature was recorded with an STD (Aleck Electronics Co. Ltd., AST1000M). Further analyses were conducted on samples of surface water (0.5-m depth) and bottom water (1 m above the sea bottom). Samples for determination of dissolved oxygen concentrations were collected in 100-ml Winkler bottles. Seawater samples for nutrient analysis were filtered through Whatman GF/F glass-fiber filters and stored frozen for analysis of ammonium (NH_4^+/NH_3, hereafter referred to as NH_4^+), combined nitrate and nitrite ($NO_2^-+NO_3^-$, hereafter referred to as NO_3^-), and for orthophosphate (PO_4^{3-}) concentrations using a Technicon Auto Analyser TRAACS-800. Particulate organic carbon and nitrogen concentrations were assessed on filtered samples (Whatman GF/B, precombusted at 450°C for 2 h) using an autoanalyzer (Sumitomo Chemical Co. Ltd., NC 80) . Chlorophyll *a* was measured by spectrophotometric analysis of particulate material collected on glass-fiber fil-

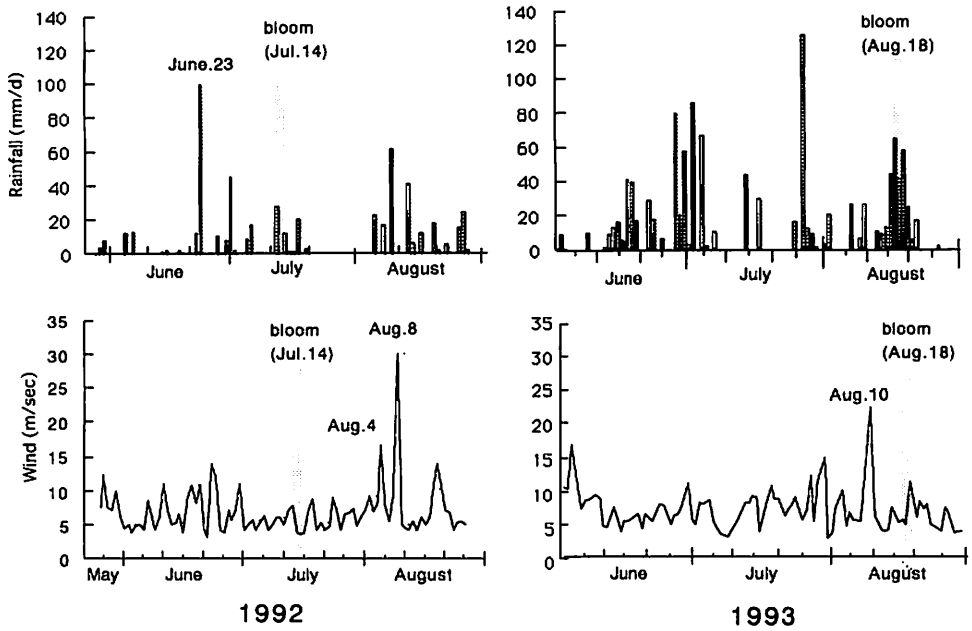

Fig. 2. Variation in rainfall and wind velocity in the Suo Nada coastal area during the summers of 1992 and 1993. Shaded areas indicate the period of *Gymnodinium mikimotoi* blooms.

ters (GF/C) by gentle suction and extracted into 90% acetone. The data for wind and rainfall were obtained from the Fukuoka Fisheries and Marine Technology Research Center, which is situated on the coast of the Suo-Nada Sea about 7 km from Stn 11. The population density of *G. mikimotoi* was evaluated by counting the cells in the samples using a hemocytometer.

Algal bioassay experiments

Bioassay experiments were performed in 1993 to evaluate the growth potential of *G. mikimotoi* in coastal surface and bottom water. Within 3 h after the water samples had been collected and maintained in sterilized 500-ml Pyrex glass bottles on ice, they were filter-sterilized through a 0.22-μm membrane filter (Millipore filter GS). An axenic culture of *G. mikimotoi* was kindly provided by Dr Mineo Yamaguchi (Nansei National Fisheries Research Institute). Prior to the bioassay experiments, the alga was incubated in 1/2.5 diluted SWM-3 medium (Chen et al. 1969; Ito & Imai 1987). The culture medium consisted of aged natural seawater (29 to 31 psu, filtered through Whatman GF/C glass-fiber filters) enriched with 800 μM $NaNO_3$, 40 μM KH_2PO_4, trace elements and vitamins. One hundred-ml Erlenmeyer flasks (precombusted for 1 h at 450°C) were used as bioassay vessels. One tenth-ml aliquots of the precultures in exponential phase were inoculated into 40-ml seawater samples collected on each sampling occasion (average initial cell numbers; 12 ± 6 cells ml^{-1}, nutrient carry-over; N, 2.0 μM; P, 0.1 μM). Samples were taken at regular intervals (2–3 d), and cells were enumerated with a hemocytometer. After confirmation of the growth (7–14 d of incubation), 1-ml aliquots of these preadapted cultures were further inoculated into another set of 40-ml filter-sterilized seawater samples which had been stored at 4°C in the dark. We calculated the amount of nutrient carry-over after the 2nd transfer from precultures to be acceptable to determine accurately the growth potential of the Suo-Nada Sea coastal waters. Considering that the average nutrient levels in the Suo-Nada Sea are ca. 2 μM of N and 0.1 μM of P, the effect of nutrient carry-over on the bioassay method (nutrient carry-over was 0.05 μM of N, and P, 2.5×10^{-3} μM of P) was minimal. All the cultures were incubated at 25°C, in a 12 h light: 12 h dark cycle, with average light intensity of 150 $\mu E m^{-2} s^{-1}$. Samples were taken at regular intervals (2–3 d), and cells were enumerated as described above. Total experimental time was determined by the length of time required for the algal populations to achieve maximum yield. This time was generally about 8–14 d. Triplicate cultures were used for growth measurements and the data represent the mean values of the maximum yield.

Results

Physical parameters

In 1992, the average rainfall per month (174.5 mm) during the period from June to August (Fig. 2) was lower than that calculated from data during the same period over the previous 5 years (1987 to 1991) (199.6 mm), and the average salinity (32.0 psu) (Fig. 3) was higher than the average value for the previous 5 years (31.1 psu). The wind velocity was generally below 10 $m s^{-1}$, though it reached 17 $m s^{-1}$ on 4 August and 30 $m s^{-1}$ on 8 August with the passage of two typhoons (Fig. 2). During July, both the surface and bottom water temperature increased markedly (Fig. 3). A

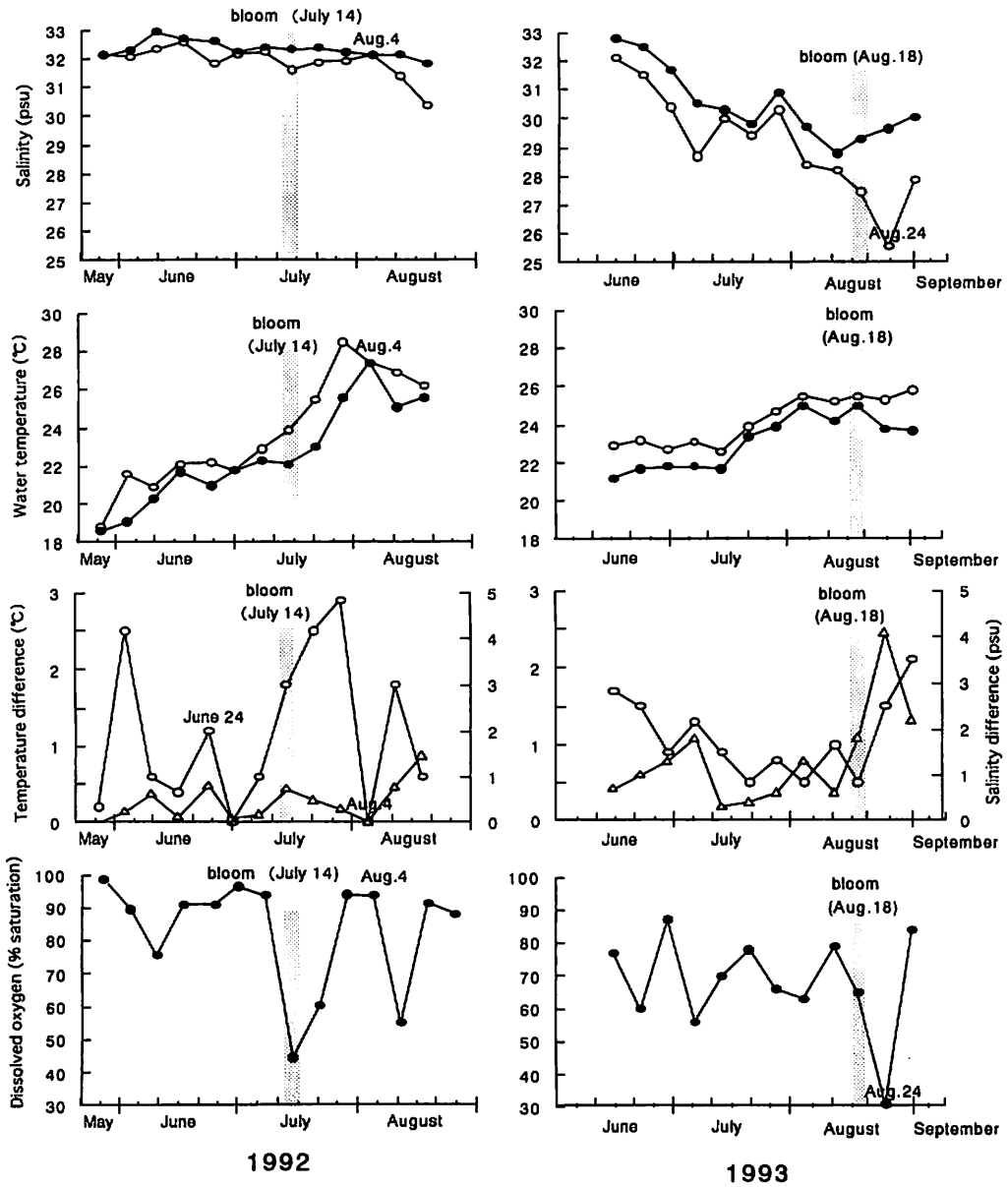

Fig. 3. Variation in water-column characteristics in the surface (○) and bottom (●) water at Stn 11 in the Suo Nada coastal area during the summers of 1992 and 1993. Data for water temperature (○) and salinity (△) differences between surface and bottom water are also presented. Shaded area as in Fig. 2.

strong temperature stratification, indicated by the temperature difference between surface and bottom water, formed throughout July and was broken up by the typhoon on 4 August (Fig. 3). On 14 July, as temperature stratification was beginning, oxygen-deficient water appeared at the sea bottom (Fig. 3).

In 1993, the average rainfall per month (378.5 mm) (Fig. 2) during the period from June to August was almost twice as much as that calculated from the data from 1987 to 1991. The salinity declined throughout this period (Fig. 3), and the average salinity (29.9 psu) during this period was lower than that calculated from 1987 to 1991. The wind velocity was generally below $10\,m\,s^{-1}$, except on 10 August when, with the passing of a typhoon, it reached more than $20\,m\,s^{-1}$ (Fig. 2). The water temperature did not increase markedly throughout the summer (Fig. 3), due to the high frequency of rainfall. A pycnocline formed from 18 to 24 August (Fig. 3) following frequent rainfall (Fig. 2). Although not low enough to be classified as oxygen deficient (<50% of the saturated concentration), the bottom water did not contain high levels of dissolved oxygen (60–80% of the saturated concentration) throughout the summer (Fig. 3). A marked decrease in dissolved oxygen in the bottom water was observed on 24 August, associated with the salinity stratification (Fig. 3).

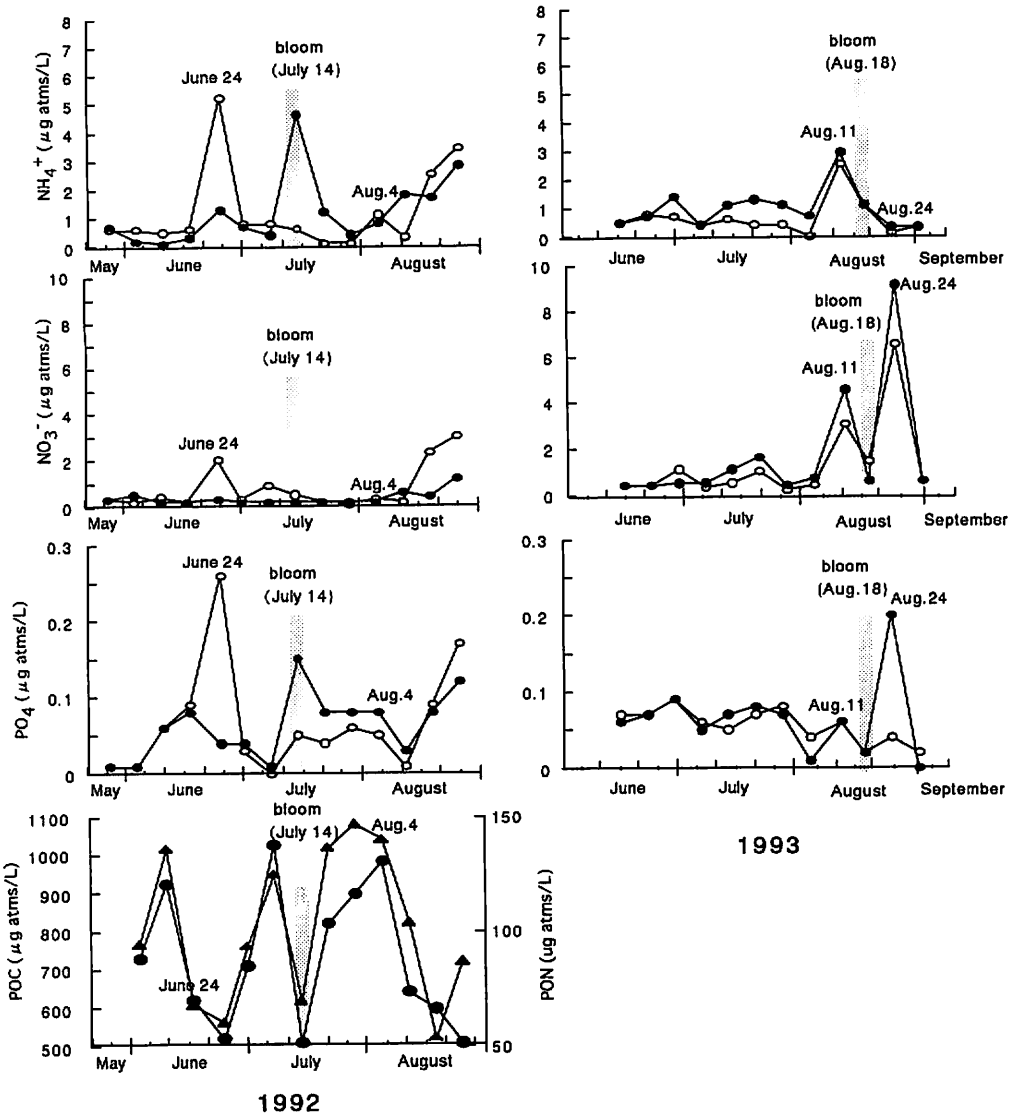

Fig. 4. Variation in nutrients in the surface (○) and bottom (●) water at Stn 11 in the coastal area of the Suo-Nada Sea during the summers of 1992 and 1993. Combined nitrate and nitrite is referred to as NO_3^-. Data for POC (particulate organic carbon, ●) and PON (particulate organic nitrogen, ▲) in the bottom water are presented for 1992 alone. Shaded area as in Fig. 2.

Nutrients

In 1992, peaks in NH_4^+, NO_3^-, and PO_4^{3-} concentrations in the surface water occurred on 24 June (Fig. 4) following heavy rainfall the day before (Fig. 2). These inorganic nutrients were introduced by river inflow as indicated by the slightly increased difference in both temperature and salinity between surface and bottom water on this date (Fig. 3). On 14 July, conspicuous peaks in NH_4^+ and PO_4^{3-} concentrations occurred in the bottom water (Fig. 4) following temperature stratification and the accompanying appearance of oxygen-deficient water (Fig. 3). The stratification at this time is speculated to be due to a marked increase in water temperature (Fig. 3). A significant portion of the DIN was NH_4^+; NO_3^- was almost undetectable in the bottom water (Fig. 4).

In 1993, a single peak in NH_4^+ and two peaks in NO_3^- occurred in both surface and bottom water during the summer period (Fig. 4). The first peak in NO_3^- concentration and the sole peak in NH_4^+ concentration occurred on 11 August, the day after the passage of the biggest typhoon of the season, with a wind velocity of more than $20\,m\,s^{-1}$ (Fig. 2). Although NO_3^- was also detected, a significant portion of the DIN in the bottom water was NH_4^+ at this time. The second peak in NO_3^- concentration in both surface and bottom water and a conspicuous peak in PO_4^{3-} concentration in the bottom water occurred on 24 August (Fig. 4), following frequent rainfall from 14 to 24 August (Fig. 2) and accompanying the appearance of oxygen-deficient water (Fig. 3). There was a change in the composition of the DIN between 11 and 24 August as indicated by the fact that almost no

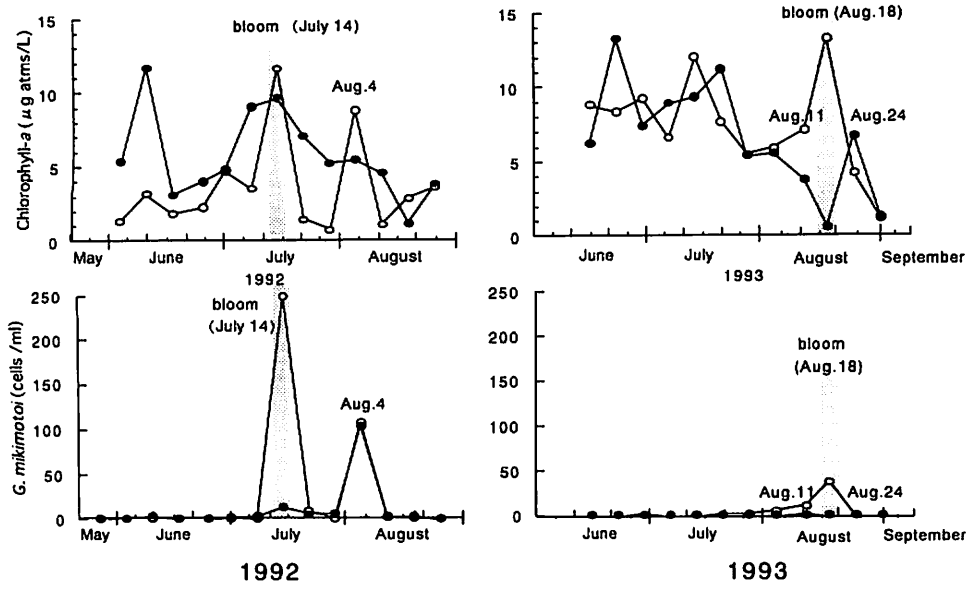

Fig. 5. Variation in chlorophyll *a* and number of *Gymnodinium mikimotoi* cells in the surface (○) and bottom (●) water at Stn 11 in the Suo Nada coastal area during the summers of 1992 and 1993. Shaded areas as in Fig. 2.

NH_4^+ was detected on 24 August. During this period salinity decreased markedly in the surface water, leading to strong salinity-based stratification (Fig. 3). Nitrate (NO_3^-) was apparently introduced by river inflow. The extremely low oxygen concentration (Fig. 3) accounts for the high PO_4^{3-} concentration in the bottom water on this date.

Population increase of *Gymnodinium mikimotoi*

In 1992, a localized bloom of *G. mikimotoi* (14 July) and a small population increase (4 August) were observed during the summer at Stn 11 (Fig. 5). The two peaks in chlorophyll-*a* concentration in the surface water (Fig. 5) were attributed to the *G. mikimotoi* population alone, since virtually no other species of phytoplankton were present during the blooms (data not shown). During other periods, diatoms were the dominant group of phytoplankton, usually accounting for more than 90% of total chlorophyll *a* (data not shown). The bloom on 14–15 July was associated with a marked increase in NH_4^+ (Fig. 4) and a decrease in dissolved oxygen (to below 50% of the saturated concentration, Fig. 3) in the bottom water, following temperature stratification of the water (Fig. 3). The population density of *G. mikimotoi* reached 1000 cells ml^{-1} on the next day (15 July, see Fig. 6A), resulting in a typical red tide. On 15 July, the surface water turned dark brown, although no fish or invertebrate deaths were reported. On 16 July, decay of the bloom proceeded rapidly, with cell density dropping to less than 10 cells ml^{-1} (data not shown).

Another significant increase (ca. 100 cells ml^{-1}) of *G. mikimotoi* occurred at Stn 11 on 4 August 1992 (Fig. 5) in turbulent water stirred up by a typhoon with wind velocity of 15 m s^{-1} (Fig. 2). The sampling was done as the typhoon was approaching the area. At that time, the water column was already turbulent and the research vessel had to return to shore because of increasing wind strength. Stratification was completely eliminated at the time of sampling as indicated by a lack of temperature and salinity differences between the surface and bottom water (Fig. 3) and by the high dissolved oxygen content in the bottom water (Fig. 3). *Gymnodinium mikimotoi* was detectable in high numbers at this time (Fig. 5). During the following period, from 4 to 25 August, no further population development of *G. mikimotoi* was observed (Fig. 5) despite a gradual increase in NH_4^+, NO_3^-, and PO_4^{3-} concentrations in both surface and bottom water (Fig. 4). During this period, another typhoon passed through (8 August), with a wind velocity of more than 30 m s^{-1} (Fig. 2).

In 1993, while Stn 11 was not the central area of the *G. mikimotoi* bloom, population densities at the surrounding stations in the Suo-Nada Sea indicated that the bloom developed between 11 and 18 August (Fig. 6B) during which time frequent rainfall was observed nearshore. The highest count (2500 cells ml^{-1}) was obtained on 18 August at a station located 10 km south of Stn 11 (Fig. 6B). At this time, salinity-based stratification was forming in this region (Fig. 3), and *G. mikimotoi* occurred throughout the southern part of the Suo-Nada Sea (Fig. 6B). The population increase of *G. mikimotoi* was initiated on 4 August and became more rapid on 11 August (Fig.5), the day after the strong turbulence caused by the large typhoon (Fig. 2). The *G. mikimotoi* population was apparently in the process of developing on 11 August (Fig. 5). The maximum count for the in situ *G. mikimotoi* population (37 cells ml^{-1}, Fig. 5) and a marked increase in chlorophyll *a* (Fig. 5) was observed in the surface water on 18 August, when red tides occurred in surrounding areas (Fig. 6B). It was not clear why the cell count for *G. mikimotoi* was low (37 cells ml^{-1}) at Stn 11 relative to the chlorophyll-*a* concentration (Fig. 5). Since we could not find any other dominant phytoplankton species on this date (data not shown), one possibility was

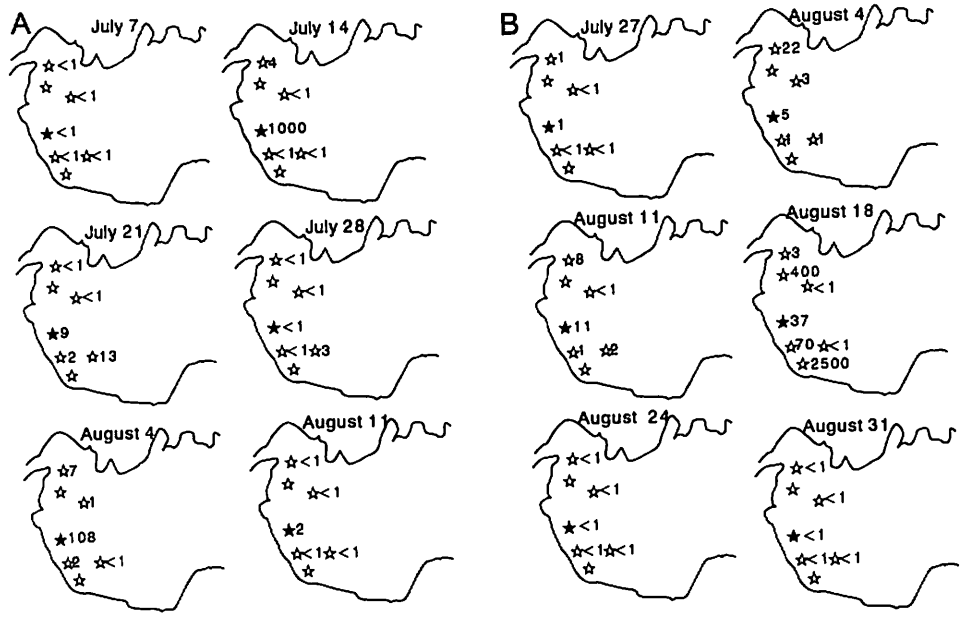

Fig. 6. Spatial distribution of *Gymnodinium mikimotoi* (cells ml^{-1}) in the coastal area of the Suo-Nada Sea during the summer of 1992 (A) and 1993 (B). *Black stars* indicate location of Stn 11. *White stars* indicate locations of other stations. For Stn11 in 1992, the datum of 15 July instead of 14 July is shown (the cell density of *G. mikimotoi* was 249 cells ml^{-1} on 14 July, see Fig. 5).

that cells of *G. mikimotoi* had been lysed by the time of cell counting.

The *G. mikimotoi* population at Stn 11 (Fig. 5) did not respond to the marked increase in NO_3^- either in the surface or bottom water nor PO_4^{3-} in the bottom water on 24 August (Fig. 4), both of which were thought to have been introduced by river inflow.

Bioassay experiments

Bioassay experiments were performed to evaluate the growth potential of algae in coastal waters in 1993. On 11 August the growth potential of *G. mikimotoi* reached a maximum (surface water, 445 cells ml^{-1}; bottom water, 215 cells ml^{-1}), indicating that water conditions were favorable for vigorous growth of the organism (Fig. 7). This increase in growth potential corresponded with the marked increase in NH_4^+ and NO_3^- concentrations in the bottom water (Fig. 4). On 18 August (the date of the red tide nearshore) a decrease in growth potential (Fig. 7) and NH_4^+ concentration in the bottom water (Fig. 4) was observed compared to one week earlier. It was assumed that nutrients had already been consumed by the *G. mikimotoi* population at the time of sampling. Growth potential did not change with the marked increase in NO_3^- and PO_4^{3-} concentrations on 24 August (Fig. 4). The growth potential of *G. mikimotoi* in the surface and bottom water on this date was low (Fig. 7).

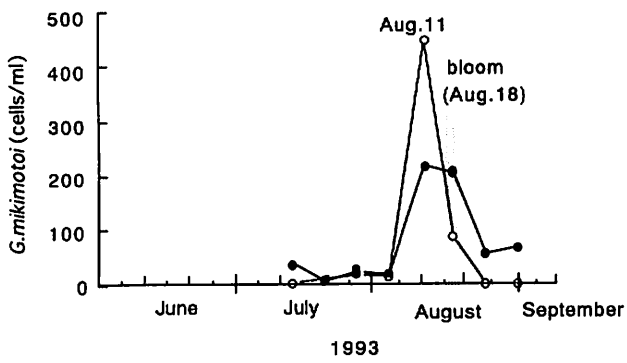

Fig. 7. Variation in growth potential of *Gymnodinium mikimotoi* in the surface (○) and bottom (●) water at Stn 11 in the Suo Nada coastal area during the summer of 1993. Shaded areas as in Fig. 2.

Discussion

The present study suggests that nutrient richness in the benthic zone, as indicated by increases in NH_4^+, should be considered a major indicator of the development of local populations of *Gymnodinium mikimotoi* nearshore in the Suo-Nada Sea. Numerous investigations have presented the hypothesis to explain the initiation of the dinoflagellate blooms in coastal waters. Although some local blooms in coastal areas were apparently due to the advection of blooms initiated in offshore areas (Delmas et al. 1992), most studies have suggested that the availability of terrestrially-derived nutrients account for the local growth of dinoflagellates (Hallegraeff 1993; Rudek et al. 1991). Nitrogenous nutrients have been considered to be one of the major factors controlling the occurrence of many dinoflagellate blooms (Chang & Carpenter 1985; Paerl et al. 1990). In estuarine and coastal waters, nitrogen loading has been suggested as a potent growth-stimulating nutrient

source (Rudek et al. 1991). Nitrogen limitation during summer appeared to be a general phenomenon in Chesapeake Bay (Fisher et al. 1992; Harding 1994). Lagoonal characteristics in a shallow tidal estuary in summer have been documented by Litake et al. (1987), where they suggested that nitrogen was primarily supplied as NH_4^+ by biological regeneration in summer and by river inflow in winter. As for physical characteristics, the literature shows that physical features such as wind-driven currents, tides, upwelling and downwelling, and water stratification are major controlling factors in red-tide formation (Paerl 1988). Particularly, the roles of vertical and horizontal water-column stability have been emphasized (Chang & Carpenter 1985; Delmas et al. 1992). Stratification has been considered an important factor in building up populations of *Gyrodinium aureolum* in the English Channel and the Baltic Sea (Holligan 1979) and in a Long Island estuary (Chang & Carpenter 1985). Stratification has been shown to help maintain patches of dinoflagellates near the pycnocline (Delmas et al. 1992). Our observation on 14 July 1992 indicates that a marked decrease in dissolved oxygen concentration in the bottom water accompanied water temperature stratification (Fig. 3), which was closely associated with the *G. mikimotoi* red tide at Stn 11 (Fig. 5). Furthermore, a marked increase in NH_4^+ concentration in the bottom water (Fig. 4) on the same day implies that NH_4^+ was generated by biological decomposition of organic matter which had accumulated on the sea bottom. The increase in NH_4^+ concentration is speculated to result mainly from benthic biological regeneration, because (1) we did not find any increase in NH_4^+ concentration in the surface water on this date (Fig. 4), and (2) a very high chlorophyll-*a* concentration (Fig. 5), and POC and PON concentrations (Fig. 4) in the bottom water had been observed one week earlier (7 July), suggesting an accumulation of sedimented phytoplankton in the bottom water. Immediately after mass sedimentation of diatoms, the sediment transiently releases NH_4^+ at a high rate (Jensen et al 1990). Also, rapid bacterial turnover of urea into NH_4^+ has been suggested (Therkildsen & Lomstein 1994). Ouchi (Hiroshima Fisheries Experimental Station, pers. comm.) has found a close relationship between the development of *G. mikimotoi* blooms and NH_4^+ increase in the bottom water in Hiroshima Bay. In early studies on *G. mikimotoi* blooms, Iizuka (1972) suggested that the release of micronutrients from a reductive sea floor accompanied by the appearance of anoxic bottom water following strong water stratification events could be one reason for the blooming of *G. mikimotoi* in Omura Bay. Also, Hirayama & Numaguchi (1972) reported that the growth of this species was promoted by an extract from anaerobically decomposed bottom mud from Omura Bay. In the present study, the decrease in dissolved oxygen concentration in the bottom water was unlikely to be the result of the breakdown of the *G. mikimotoi* bloom because the algal population was still in the process of developing (ca. 250 cells ml^{-1}). The population density reached 1000 cells ml^{-1} on the next day (15 July). These observations are in agreement with previous studies, i.e., *G. mikimotoi* blooms after oxygen deficiency (Iizuka 1972) and after an increase in NH_4^+ (Ouchi, pers. comm.) in the bottom water. A connection between algal blooms and preceding anoxic events over a wide area of sea floor was also observed on the Swedish west coast (Graneli et al. 1989).

We did not find an external source of nutrients on 14 July, so during this period it appears that *G. mikimotoi* grew and maintained its population by vertical migration, taking up NH_4^+ and other micronutrients from the sea bottom. Strong stratification creates a distinct advantage for dinoflagellates over less motile phytoplankton taxa, since dinoflagellates can migrate vertically to deeper nutrient-rich water at night. It has been reported that *G. mikimotoi* undergoes a nocturnal downward migration at velocities of between 1.3 (Honjo et al. 1990) and 1.7 m h^{-1} (Iizuka & Irie 1966). A more recent study (Koizumi et al. 1996) revealed that this species can migrate vertically more than 20 m daily at velocities of about 2.2 m h^{-1}. We did not measure the process of vertical migration in the present study. However, since the water was only about 8 to 14 m deep in the study area, *G. mikimotoi* may have exhibited a similar pattern of vertical migration and stayed on or near the sea floor during the night for nutrient uptake as suggested by Honjo (1994).

Another increase in NH_4^+ concentration occurred in the bottom water in 1993 (11 August, Fig. 4), the day after strong mixing of the water column by a passing typhoon (Fig. 2). Results of algal growth potential experiments indicated that the growth potential of *G. mikimotoi* in the bottom water increased markedly on this date (Fig. 7). In a recent study in Gokasho Bay, Iwata et al. (1997) also observed a high growth potential of *G. mikimotoi* in the bottom water 2 to 8 weeks earlier than the development of blooms of this species. In the present study, the bloom in 1993 was formed after a period of rainfall on 18 August (Figs 2, 6B), one week after the marked increase in NH_4^+ concentration and the growth potential in the bottom water. These observations suggest that a wind-induced increase in NH_4^+ concentration and algal growth potential near the sea floor stimulated the population of *G. mikimotoi* to develop. It has been shown that windy conditions will reduce, and sometimes eliminate, the possibility of a dinoflagellate bloom (Hartwell 1975) regardless of nutrient enrichment conditions. Among the microalgae, dinoflagellates appear to be the most sensitive to growth inhibition by small-scale turbulence (Thomas & Gibson 1990). Honjo et al. (1990) found that most *G. mikimotoi* cells occurred at depths of 5 to 10 m at densities of 10^2 to 10^3 cells ml^{-1} in the daytime in Gokasho Bay and indicated that the ability to maintain the population in the middle layer at low cell densities might be important in avoiding frequently unfavorable conditions. In the present study, a population of *G. mikimotoi* developed despite a strong typhoon on 10 August 1993, indicating that the species has tolerance for strong winds during the early stage of population development and adapts well to turbulence where nutrient concentrations are relatively high.

However, the combination of wind strength, depth of water column and population size seems to be important in shallow waters. In the present study, no *G. mikimotoi* population was detected following a powerful typhoon with wind velocity of more than 30 m s^{-1} (8 August 1992, Figs 2, 5). Hence, the *G. mikimotoi* population apparently disappears during extremely strong wind conditions in shallow coastal areas.

The algal bioassay results suggested that NH_4^+ itself was never the sole limiting and thus stimulating physiological factor for *G. mikimotoi*. Although we did not conduct supplementary experiments for the bioassay, the much lower yield obtained by the bioassay of *G. mikimotoi* compared to that calculated from the minimum cell quota of N for this species (Yamaguchi 1994) supported this hypothesis. The dissolved inorganic nitrogen concentration ($NO_3^- + NO_2^- + NH_4^+$) in the surface and bottom water at Stn 11 on 11 August 1993 was 5.5 and 7.4 μg-at N l^{-1} (Fig. 4) which was calculated to support 1400 and 1890 cells ml^{-1} respectively, while the yield actually obtained in the bioassay experiments was 445 cells ml^{-1} and 215 cells ml^{-1}, respectively (Fig. 7). These calculations indicate that it was unlikely that *G. mikimotoi* was nitrogen limited. Similar values for phosphorus indicate that it is also not a limiting factor in the Suo-Nada Sea. We speculate that there exist heretofore unidentified micronutrients present at times near the sea floor which limit or stimulate the growth of *G. mikimotoi*. Hirayama & Numaguchi (1972) have shown that hot-water extracts from anaerobically incubated bottom mud from Omura Bay promoted the growth of *G. nagasakiense* (=*G. mikimotoi*) and in particular, the extracts from summer mud showed much stronger activity than extracts from winter mud. Ishimaru et al. (1989) has emphasized that selenium was an essential factor in the initiation of blooms of this species. We believe that the release from the sea floor of both NH_4^+ and some other micronutrients co-stimulated the development of the bloom. Our findings to date do not allow further speculation on this point.

Both the algal bioassay and the in situ population data presented in this study suggest that an inflow of NO_3^- after rainfall per se was not a direct factor stimulating the increase of the *G. mikimotoi* population. The low growth potential of *G. mikimotoi* in both the surface and bottom water on 24 August (Fig. 7), despite the extremely high NO_3^- and PO_4^{3-} concentrations (Fig. 4), support the above assumption. Other studies (Delmas et al. 1992; Prego 1992) also show that inorganic nutrient input of terrestrial origin does not directly promote the growth of dinoflagellate populations inshore. Paerl et al. (1990) suggested that nitrogen supplied directly by rainfall is capable of enhancing estuarine and coastal phytoplankton production. Chang & Carpenter (1985) suggested that high nitrogenous nutrient availability in a Long Island estuary is a prerequisite for blooms of *Gyrodinium aureolum*. River loading of humic substances and the release of NH_4^+ was suggested to play an important role in algal blooms in an area off the Swedish coast (Carlsson et al. 1993). We have observed that *G. mikimotoi* blooms occur much more frequently inshore (<10 m water depth) than off-shore in the Suo-Nada Sea (data not shown). Since *G. mikimotoi* utilizes both NO_3^- and NH_4^+ in a laboratory culture system (Yamaguchi 1994), it is likely that certain micronutrients related to the growth of *G. mikimotoi* were absent in the river inflow. We believe eutrophication from river inflow to be a prerequisite for *G. mikimotoi* blooms, but it does not trigger the blooms directly.

Our results suggested that blooms of *G. mikimotoi* could occur at relatively low salinities (Fig. 3). The blooms on 18 August 1993 occurred in the middle of a period of frequent rain (Figs 2, 6B). This was in agreement with other studies (Yamaguchi & Honjo 1989; Itoh et al. 1990). Yamaguchi (1994) has shown that the range of salinity tolerable for growth of this species was wide; ranging from 15 to 30 psu, being optimum at 25 psu (25°C) for maximum growth rate and at 30 psu (20°C) for maximum cell yield. The wide salinity range was similar to that which Chang & Carpenter (1985) found for *Gyrodinium aureolum* on field observations in a Long Island estuary. Also, Itoh et al. (1990) suggested that blooms of *G. mikimotoi* are most likely in years of relatively high rainfall and low amounts of accumulated sunlight intensity, based on 15 years of field data from the Suo-Nada Sea (1973–1987). This hypothesis was supported by the observations of the bloom in 1993. However, the bloom on 14 July 1992 was apparently not related to rainfall and salinity decrease, but rather, to oxygen deficient water near the sea floor. Therefore, rainfall and salinity decrease may account for a part of the bloom occurrence in these areas, but are by no means the only environmental factors that determine the bloom development of this species in the Suo-Nada Sea. A study in another area (Gokasho Bay) (Honjo 1987) also suggested that rainfall events and salinity decreases in coastal waters were unlikely to be the sole explanation for the development of blooms of this species.

Stratification and wind mixing theory are not mutually exclusive. It is likely that at different times of the year, different mechanisms control *G. mikimotoi* population development. In the estuary of the Suo-Nada Sea where the water depth is 7 m, temperature stratification of the water column is likely in July when a rise in water temperature is prominent. Since the water is shallow, however, there is likely to be no difference in water temperature between the surface and the bottom in August, thus temperature stratification is less likely during this period. In addition, typhoons are most likely to occur in August. A scenario can be drawn for the initiation of blooms of *G. mikimotoi* in shallow and muddy-bottom coastal areas, based on one or a combination of the following: (1) Temperature stratification favors the development of blooms of *G. mikimotoi*, allowing the organism to form and maintain patches. In particular, strong stratification accompanied by oxygen-deficient bottom water make for favorable growth conditions for organisms releasing NH_4^+ and other micronutrients from the ben-

thic zone. (2) Mixing of the water column induced by strong wind could cause a release of NH_4^+ and other micronutrients of benthic origin, creating conditions favorable for initial *G. mikimotoi* population growth. In this case too, temperature or salinity stratification, if they occur, encourage the species to bloom. The growth of *G. mikimotoi* could be initiated by the increase in NH_4^+ and other growth-stimulating micronutrients near the sea floor that are supplied by benthic biological regeneration in shallow coastal areas.

The scenario stated above applies only to local blooms in relatively nutrient-poor, shallow (less than 10-m depth) and muddy-bottom coastal areas, where oxygen-deficient water near the sea floor could easily form because of the low porosity of the sediment, and disturbance of the mud surface as well as vertical migration of *G. mikimotoi* to the bottom zone is possible. In deeper (more than 10-m depth) areas, different mechanisms may dominate in controlling *G. mikimotoi* population development. We did not observe blooms of *G. mikimotoi* at Stn 15 (14-m depth) or at other deeper, offshore areas in the Suo-Nada Sea throughout the two-year study period, although stronger stratification of waters occurred in deeper sites in this area. Nutrient measurement and bioassay experiments showed that both the nutrient concentration and the growth potential of the bottom water in these deeper areas (Stn 15) were much higher than that in the shallow area (Stn 11) (data not shown). Given the migration velocity of *G. mikimotoi* as reported (Iizuka & Irie 1966; Honjo et al. 1990; Koizumi et al. 1996), a calculation indicates that *G. mikimotoi* can migrate to these deeper areas at midnight to uptake nutrients. However, our observations indicated that stratification at sites deeper than Stn 15 (14-m depth) was not associated with a population increase of *G. mikimotoi* throughout the study period. It is tempting to interpret this as indicating that the vertical migration of this species did not occur or at least could not effectively contribute to the development of the bloom and a more dynamic mixing of the water column involving the bottom waters was necessary for the development of the bloom in these deeper areas. It is, however, beyond the limit of this study to speculate on the exact mechanisms for the development of the bloom in these areas. Further studies are needed to this effect. In any case, populations that develop inshore by the mechanism stated above may spread to offshore areas by diffusion of the upper water mass as a consequence of the breakdown of stratification inshore, as suggested by Koizumi et al. (1994).

In conclusion, we have presented evidence that suggests that an increase of NH_4^+ in bottom water may play an important role in the development of local populations of *G. mikimotoi* in nutrient-poor, shallow coastal areas like the Suo-Nada Sea. In all likelihood, an NH_4^+ increase in bottom water is not the sole cause of the blooms. To determine whether monitoring NH_4^+ concentrations in bottom water assist in predicting the occurrence of *G. mikimotoi* blooms, further studies based on field-based observations are needed.

Acknowledgments

We thank Dr Mineo Yamaguchi of Nansei National Fisheries Research Institute for kindly providing the axenic culture of *Gymnodinium mikimotoi* and for his helpful discussions. We also thank Toshiyuki Sato for technical assistance and Dr Yuzaburo Ishida for encouragement. In addition, we thank Dr Alan W. White for critical review of the manuscript. This project was supported by funds from the Ministry of Agriculture, Japan.

Literature Cited

Carlsson, P., A. Z. Segatto & E. Graneli 1993. Nitrogen bound to humic matter of terrestrial origin—a nitrogen pool for coastal phytoplankton? *Mar. Ecol. Prog. Ser.* **97**: 105–116.

Chang, J. & E. J. Carpenter 1985. Blooms of the dinoflagellate *Gyrodinium aureolum* in a Long Island estuary; Box model analysis of bloom maintenance. *Mar. Biol.* **89**: 83–93.

Chen, L. C. M., T. Edelstein & J. McLachlan 1969. *Bonnemaisonia hamifera* Hariot in nature and in culture. *J. Phycol.* **5**: 211–220.

Delmas, D., A. Herbl & S. Y. Maestrini 1992. Environmental conditions which lead to increase in cell density of the toxic dinoflagellates *Dinophysis* spp. in nutrient-rich and nutrient-poor waters of the French Atlantic coast. *Mar. Ecol. Prog. Ser.* **89**: 53–61.

Endo, T., T. Nishijima, Y. Hata, S. Montani & T. Okaichi 1987. Chemical environment for red tide occurrence, p. 159–210. In *Akashio no Kagaku* (ed. Okaichi, T.). Kouseisha-kouseikaku, Tokyo. (In Japanese.)

Fisher, T. R., E. R. Peele, J. W. Ammerman & L. W. Harding Jr. 1992. Nutrient limitation of phytoplankton in Chesapeake Bay. *Mar. Ecol. Prog. Ser.* **82**: 51–63.

Graneli, E., P. Carlsson, P. Olsson, B. Sundstrom, W. Graneli & O. Lindahl 1989. From anoxia to fish poisoning: the last ten years of phytoplankton blooms in Swedish marine waters, p. 407–427. In *Novel Phytoplankton Blooms—Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms* (eds. Cosper, E.M., V.M. Bricelj & E.J. Carpenter). Springer-Verlag, Berlin.

Hallegraeff, G.M. 1993. A review of harmful algal blooms and their apparent global increase. *Phycologia* **32**: 79–99.

Harding, L. W. Jr. 1994. Long-term trends in the distribution of phytoplankton in Chesapeake Bay:roles of light, nutrients and streamflow. *Mar. Ecol. Prog. Ser.* **104**: 267–291.

Hartwell, A. D. 1975. Hydrographic factors affecting the distribution and movement of toxic dinoflagellates in the Western Gulf of Marine, p. 47–68. In *Toxic dinoflagellate blooms*. Proc. 1st Int. Conf. Mass. Sci. Technol. Found (ed. LoCicero, V. R.). Wakefield, Massachusetts.

Hirayama, K. & K. Numaguchi 1972. Growth of *Gymnodinium* type-'65, causative organism of red tide in Omura Bay, in medium supplied with bottom mud extract. *Bull. Plankton Soc. Jpn* **19**: 13–21.

Holligan, P.M 1979. Dinoflagellate blooms associated with tidal fronts around the British isles, p. 249–256. In *Toxic Dinoflagellate Blooms in North Holland* (eds. Taylor, D. L. & S. S.

Seliger). Elsevier, New York.

Honjo, T. 1987. Environmental factors controlling the red tide of *Gymnodinium nagasakiense*, p. 228–237. In *Akashio no Kagaku* (ed. Okaichi, T.), Kouseisha-kouseikaku, Tokyo. (In Japanese.)

Honjo, T. 1994. The biology and prediction of representative red tide associated with fish kills in Japan. *Rev. Fish. Sci.* **2**: 225–253.

Honjo, T., S. Yamamoto, O. Nakamura & M. Yamaguchi 1990. Annual cycle of motile cells of *Gymnodinium nagasakiense* and ecological features during the period of red tide development, p. 165–170. In *Toxic Marine Phytoplankton* (eds. Graneli, E., B. Sundstrom, L. Edler & D.M. Anderson). Elsevier, New York.

Iizuka, S. 1972. *Gymnodinium* type-'65 red tide in occurring anoxic environment of Omura Bay. *Bull. Plankton Soc. Jpn* **19**: 22–33. (In Japanese with English abstract.).

Iizuka, S. & H. Irie 1966. The hydrographic conditions and the fisheries damages by the red tide water occurred in Omura Bay in summer 1965—II. The biological aspects of a dominant species in the red water. *Bull. Fac. Fish. Nagasaki Univ.* **21**: 67–101. (In Japanese with English abstract.)

Ishimaru, T, T. Takeuchi, Y. Fukuo, & M. Kodama 1989. The selenium requirement of *Gymnodinium nagasakiense*, p. 357–360. In *Red Tides: Biology, Environmental Science, and Toxicology* (eds. Okaichi, T., D. M. Anderson, & T. Nemoto). Elsevier, New York.

Itakura , S., I. Imai & K. Itoh 1990. Seasonal occurence of the noxious red tide dinoflagellate *Gymnodinium nagasakiense* in Hiroshima Bay, Seto Inland Sea. *Bull. Nansei Natl Fish. Res. Inst.* **23**: 27–33 (In Japanese with English abstract.)

Itoh, K. & I. Imai 1987. Rafido so (Raphydophyceae), p. 122–130. In *A Guide for Studies of Red Tide Organisms* (ed. Japan Resource Conservation Association). Shuwa, Tokyo. (In Japanese.)

Itoh, K, I. Imai, S. Itakura, M. Yamaguchi, Y. Matsuo, K. Terada, M. Kamizono & H. Ikeuchi 1990. Environmental characteristics for the red tide occurrence of *Gymnodinium nagasakiense* in Suo Nada, p. 145–158. In *A report on the 5 Year Studies on the Prediction of Red Tide*. Nansei Fisheries Research Institute, Hiroshima.(In Japanese.)

Iwata, Y., I. Sugahara, T. Kimura, K. Silapajarn, M. Sano, T. Mizuguchi, A. Nishimura, M. Inoue & T. Takeuchi 1997. Growih potential of *Gymnodinium mikimotoi* in Gokasho Bay. *Nippon Suisan Gakkaishi* **63**: 578–584. (In Japanese with English abstract.)

Jensen, M. H., E. Lomstein & J. Sorensen 1990. Benthic NH_4^+ and NO_3^- flux following sedimentation of a spring phytoplankton bloom in Aarhus Bight, Denmark. *Mar. Ecol. Prog. Ser* **61**: 87–96.

Kamizono, M. 1997. Studies on the formation mechanisms of oxygen-deficient water mass in the southwestern part of Suo-Nada (Buzen Sea), Japan. *Ann. Rep. Fukuoka Pref. Fish. Exp. St.* **6**: 97–146. (in Japanese.)

Koizumi, Y., K. Takashima, M. Kamizono, T. Eto, T. Baba, S. Hiyama, T. Ikeda, T. Iwao, Y. Hinoshita, M. Uchima, T. Yanuma, T. Uchida & T. Honjo 1994. Features of the annual appearance of *Gymnodinium mikimotoi* and mechanism of expansion of its distribution in the western Seto Island Sea, Japan. *Umi no Kenkyu* **3**: 99–110. (In Japanese with English abstract.)

Koizumi, K., T. Uchida & T. Honjo 1996. Diurnal vertical micgration of *Gymnodinium mikimotoi* during a red tide in Hoketusu Bay, Japan. *J. Plankton Res.* **18**: 289–294.

Litaker, W., C. S. Duke, B. E, Kenney & J. Ramus. 1987. Short-term environmental variability and phytoplankton abundance in a shallow tidal estuary. *Mar. Biol.* **96**: 115–121.

Matsuoka, K., S. Iizuka, H. Takayama, T. Honjo, Y. Fukuya & T. Ishimaru 1989. Geographic distribution of *Gymnodinium nagasakiense* Takayama et Adachi around west Japan, p. 101–104. In *Red Tides: Biology, Environmental Science, And Toxicology* (eds. Okaichi, T., D. M. Anderson & T. Nemoto). Elsevier, New York.

Nakata, K. & S. Iizuka 1987. A note on overwintering of a red tide dinoflagellate *Gyminodinium nagasakiense* (Dinophyceae) in Omura Bay, Kyushu. *Bull. Plankton Soc. Jpn* **34**: 199–201.

Paerl, H. W. 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland wateres. *Limnol. Oceanogr.* **33**: 823–847.

Paerl, H. W., J. Rudek & M. A. Mallin 1990. Stimulation of phytoplankton production in coastal waters by natural rainfall inputs; nutritional and trophic implications. *Mar. Biol.* **107**: 247–254.

Prego, R. 1992. Flows and budgets of nutrient salts and organic carbon in relation to a red tide in the Ria of Vigo (NW Spain). *Mar. Ecol. Prog. Ser.* **79**: 289–302.

Rudek, J., H. W. Paerl, M. A. Mallin & P. W. Bates 1991. Seasonal and hydrological control of phytoplankton nutrient limitation in the lower Neuse River Estuary, North Carolina. *Mar. Ecol. Prog. Ser.* **75**: 133–142.

Takayama, H. & R. Adachi 1984. *Gymnodinium nagasakiense* sp. nov., a red tide forming dinophyte in the adjacent waters of Japan. *Bull. Plankton Soc. Jpn* **31**: 7–14.

Takayama, H. & Y. Matsuoka 1991. A reassessment of the specific characteres of *Gymnodinium mikimotoi* Miyake et Kominami ex Oda and *Gymnodinium nagasakiense* Takayama et Adachi. *Bull. Plankton Soc. Jpn* **38**: 53–68. (In Japanese with English abstract.)

Tamori, H., T. Iwano, M. Kamizono, M. Yoshida, T. Ikeda, T. Baba, Y. Koizumi, M. Uchima, H. Miura & T. Yanuma 1991. Elucidation of area where *Gymnodinium nagasakiense* initially appeared and its environmental features in western Seto Inland Sea. *Nippon Suisan Gakkaishi* **57**: 2179–2186. (In Japanese with English abstract.)

Terada, K., H. Ikeuchi & H. Takayama 1987. Winter distribution of *Gymnodinium nagasakiense* (Dinophyceae) in coastal waters of Suo-Nada, Inland Sea of Japan. *Bull. Plankton Soc. Jpn* **34**: 201–203. (In Japanese with English abstract.)

Therkildsen, M. S. & B. A., Lomstein 1994. Seasonal variation in sediment urea turnover in a shallow estuary. *Mar. Ecol. Prog. Ser.* **109**: 77–82.

Thomas, W. H. & C. H. Gibson 1990. Effects of small-scale turbulence on microalgae. *J. Appl. Phycol.* **2**: 71–77.

Yamaguchi, M. 1994. Pysiological ecology of the red tide flagellate *Gymnodinium nagasakiense* (Dinophyceae)—mechanism of the red tide occurrence and its population. *Bull. Nansei Natl Fish. Res. Inst.* **27**: 251–394. (In Japanese with English abstract.)

Yamaguchi, M. & T. Honjo 1989. Effect of temperature, salinity and irradiance on the growth of the noxious red tide flagellate *Gymnodinium nagasakiense* (Dinophyceae). *Nippon Suisan Gakkaishi* **55**: 2029–2036. (In Japanese with English abstract.)