Article

Population Development of the Dinoflagellates *Ceratium furca* and *Ceratium fusus* during Spring and Early Summer in Iwa Harbor, Sagami Bay, Japan

Seung Ho Baek[1,3]*, Shinji Shimode[1,2], Myung-Soo Han[3], and Tomohiko Kikuchi[1]

[1]*Graduate School of Environmental and Information Sciences, Yokohama National University, Yokohama 240-8501, Japan*
[2]*Ocean Research Institute, University of Tokyo, Tokyo 164-8639, Japan*
[3]*Department of Life Science, Division of Natural Sciences, Hanyang University, Seoul 133-791, Korea*

Abstract: To examine the population development of the dinoflagellates, *Ceratium furca* and *Ceratium fusus*, daily field monitoring was conducted between April and July 2003 in the temperate coastal water of Sagami Bay, Japan. During the study period, the concentrations of *C. furca* were always lower than those of *C. fusus*. A sharp increase in the densities of both species was recorded on 5 May showing the maximum cell concentrations (*C. furca* = 14,800 cells L^{-1}, *C. fusus* = 49,600 cells L^{-1}). In the 7 days prior to the May bloom of the *Ceratium* species (29 April to 1 May), the highest density of the heterotrophic dinoflagellate *Noctiluca scintillans* was observed. Additionally, a second bloom of *C. fusus* occurred on 22 July. Here, two causes of the significant increases in the *Ceratium* populations during the two blooming periods (first time; 1 to 8 May, second time; 15 to 22 July) are presented. First, an increase in the nutrients of the surface layer regenerated by the breakdown of blooms by *N.scintillans* could be considered as a major cause of the population increase of the two *Ceratium* species. Second, a decrease in salinity (to 27 psu) was correlated with the later bloom of *C. fusus*. These results suggest that the population development of the two *Ceratium* species requires nutrients regenerated after the reduction of the diatom population by *N. scintillans* and, for *C. fusus*, continuous low salinity conditions, compared to other environmental factors during the rainy season.

Key words: *Ceratium furca*; *Ceratium fusus*; *Noctiluca scintillans*; Bloom process; Environmental factor

1. Introduction

Blooms of toxic and non-toxic dinoflagellates have been widely known to have negative impacts on fisheries, aquaculture, water quality and public health (Anderson 1997; Smayda 1997). Over the last few decades, few studies on the non-toxic dinoflagellates dominating coastal waters have been performed, although many studies on toxic species have been conducted. The dinoflagellates in the coastal waters of the world, *Ceratium furca* and *Ceratium fusus*, do not produce toxins, but have frequently led to damage of aquaculture and natural fisheries (Machida *et al*. 1999; GEOHAB 2001). Numerous investigations have been conducted on *Ceratium* species to explain their blooms, behaviors, and population dynamics in the field (Nordli 1953; Eppley *et al*. 1968; Elbrächter 1973; Nielsen 1991; Bockstahler and Coats 1993; Li *et al*. 1996; Stoecker 1998; Smalley *et al*. 1999; Smalley and Coats 2002) and in the laboratory (Nordli 1957; Qasim *et al*. 1973; Weiler and Chisholm 1976; Weiler and Eppley 1979; Sullivan and Swift 1995; Smalley *et al*. 2003).

Numerous mechanisms have been suggested to explain the formation of harmful algal blooms (HABs). In general, HABs strongly depend on biotic factors such as species competition and differential grazing effects at physiological, cellular, population, community and ecosystem levels for species promotion, and abiotic factors such as light, temperature, salinity, turbulence, and nutrient levels (ECOHAB 1995). Prior experiments (Baek *et al*. 2006, 2007, 2008) were conducted to compare the reproductive ecology of *C. furca* and *C. fusus* with the seasonal changes in Sagami Bay, Japan. Additionally, incubation experiments using isolated cells from the field were conducted. In the bay, concentrations of both species increased significantly

from April to September. This growth increased in the spring and summer seasons with higher temperatures and the lower salinities, and decreased in winter season with the shorter photoperiods, reduced irradiances and lower temperatures. In addition, populations of both species increased after blooms of the heterotrophic dinoflagellate *Noctiluca scintillans,* following spring diatom blooms (Miyaguchi *et al*. 2006; Baek 2007). However, previous evaluations of the *Ceratium* species responses to environmental cues and studies on their population dynamics during blooming periods are inadequate to understand the complex and integrated mechanisms of blooms by the two *Ceratium* species.

Here, the relationships between population dynamics of *C. furca* and *C. fusus* and the environmental factors involved during the blooming season were explored in detail. To determine the optimum environmental conditions for growth of the two *Ceratium* species, daily samplings were carried out in the neritic water of Sagami Bay.

2. Materials and Methods

Study area

Sagami Bay, located in central Japan, forms an embayment with its mouth opened toward the Pacific Ocean on the south (Fig. 1). The water mass of Sagami Bay is strongly influenced by the Kuroshio Current, which runs along the eastern coast of Japan. The warm Kuroshio Current forms the water mass in the upper layer (0-200 m depth) in the central part of the bay. The nutrients in the bay are supplied through the upwelling of deep seawater and the inflows of eutrophic water from the two main rivers (Sagami and Sakawa Rivers) and the Tokyo Bay (Hogetsu and Taga 1977; Iwata 1985). The eutrophic waters are mixed with the Kuroshio Current (oligotrophic waters) flowing into the Sagami Bay. The counterclockwise currents along the coast are dominant in the bay, although the circulation pattern changes rapidly corresponding to the distribution of the Kuroshio Current (Iwata 1985). The nutrient concentrations in the water column are relatively high during the winter and low near the surface in the summer. In particular, during late spring and early summer, maximum freshwater flow provides high nitrogen concentration, pushing the system toward phosphorus limitation (Fujiki *et al*. 2004).
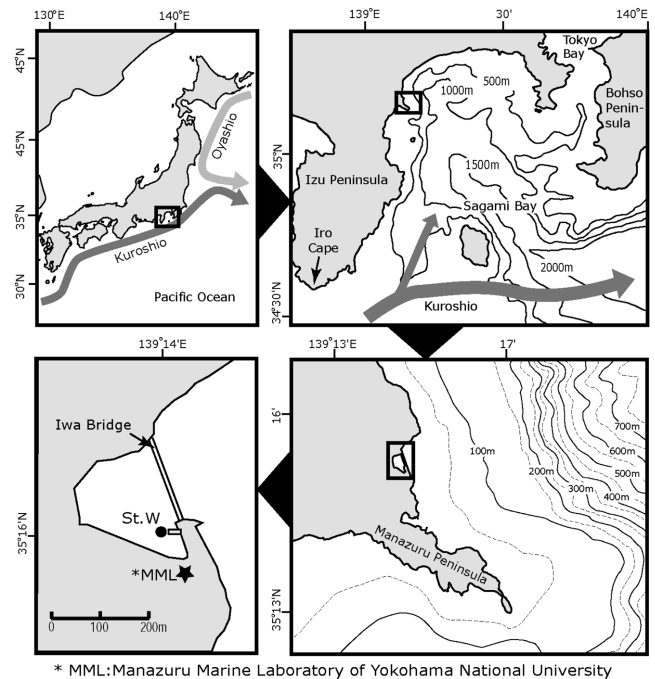
* MML:Manazuru Marine Laboratory of Yokohama National University

Fig. 1. Map of the sampling site (St.W). Manazuru Marine Laboratory (MML) of Yokohama National University is also indicated.

Sampling

Daily sampling was conducted at 9:00 AM for 103 days from 12 April to 23 July 2003 at St. W(Iwa Harbor; 35°09′30″N, 139°09′25″E) in Sagami Bay (Fig. 1). The depth at the sampling station was approximately 4.5 m at high tide and approximately 3 m at low tide. Seawater samples were collected with a bucket at the surface and were taken from a 3 m-depth layer using a 1-L Kitahara-type sampling bottle. Water temperature was measured with a mercury thermometer immediately after sample collection. The collected water was prescreened using a 330-μm mesh to remove larger zooplankton. The salinity of the water samples was obtained using a refrectometer, up to and including the sample of 2 May. Thereafter, CTD (Ocean Seven 31 S/N1202287) was used to measure salinity. Collected water samples were brought back to MML (Manazuru Marine Laboratory of Yokohama National University, Fig. 1).

Daily rainfall, daily irradiance, and wind speed data during the sampling period were obtained from the Nebukawa Office of the Kanagawa Farm-Forestry-Fishery Information Center located approximately 6 km north of the sampling station. In addition to the daily sampling, a detailed investigation on day/night and tidal vertical

migration of *C. furca* and *C. fusus* was carried out from 2 to 6 May 2003, during the period of the first bloom. Water samples were collected 14 times during these 5 days from 0, 1 and 3 m depths by the same method in the daily sampling as previously described. During this period, solar irradiance was measured at 0.5 h intervals using a radiometer (LI-190 SA, LI-COR) placed on the roof of the laboratory (Fig. 1). The irradiance was calculated as μmol m^{-2} s^{-1}.

Nutrient analysis

Samples for nutrient analysis were filtered through a 0.45-μm filter (Millipore Milex HV). The filtered sample water was placed into a plastic tube and frozen. Samples were thawed at room temperature and kept in the dark for measurements following the analytical method of Parsons *et al.* (1984). Nitrite + nitrate (NO_2^- and NO_3^-), and Phosphate (PO_4^{3-}) concentrations were determined using a nutrient auto-analyzer (Bran Luebble, AACA-II).

Chlorophyll *a* concentration

Chlorophyll *a* (Chl.*a*) concentrations were determined by filtering two replicate samples of seawater from both the surface and 3-m layers, using Whatman GF/F glass fiber filters. Chl.*a* concentrations included all size fraction of the chlorophyll from 1 to 330 μm. Each filter was placed into a 10-ml brown vial and was extracted in 10-ml N,N-Dimethylformamide (DMF) at 4°C for 24 h (Suzuki and Ishimaru 1990). The Chl.*a* concentration was determined fluorometrically on a Turner Design fluorometer (model 10AU) according to the method of Holm-Hansen *et al.* (1965).

Measurement of *Ceratium* species and *Noctiluca scintillans* densities

Sub-samples for the estimation of phytoplankton species were immediately fixed with 2.5% glutaraldehyde (final concentration) after filtration through TTTP type 2.0-μm membranes, and stored at 4°C in the dark until cells were counted (using a Sedgwick-Rafter chamber). Live cells of *C. furca* and *C. fusus* were also observed during detailed investigation of vertical migration and morphological changes of the two species. On each sampling day from 2 to 6 May, immediately following filtration of water samples through 20-μm polycarbonate filters, the live cells collected on the filters were observed under 60 x magnification. During this period, the concentrations of the two species

were also determined from the live cell samples. To ascertain the *Noctiluca scintillans* concentration, live cells collected only from the surface water samples during the same study period were counted.

Statistical analyses

During the detailed investigation from 2 to 6 May, the concentrations of *C. furca* and *C. fusus* among day/night or tidal levels were examined using the Mann-Whitney U-test. Concentrations of the two tidal phases were compared using the Kruskal-Wallis test. A weighted mean depth (WMD), adapted from Frost and Bollen (1992), was used to examine the diel and tidal vertical migration of *C. furca* and *C. fusus* collected during the detailed investigation from 2 to 6 May. The WMD was calculated as follows:

$$WMD = (\Sigma n_i d_i) / \Sigma n_i$$

where n_i is cell density at depth d_i, taken to be the midpoint of each stratum at each sampling time.

Results

Environmental factors

Water temperature gradually increased from 13 April to 30 June and thereafter reached a slight decline around 22°C through the end of the sampling period (Fig. 2a). The average of daily irradiance was 13.59 mol m^{-2} d^{-1}, ranging from approximately 1.2 to 28.3 mol m^{-2} d^{-1} (Fig. 2a). Irradiance levels of less than 5 mol m^{-2} d^{-1} were recorded on rainy and cloudy days, and were characterized by high day-to-day variability. Rainfall data showed that almost half of the study period was rainy, and that the maximum rainfall of 109 mm occurred on 31 May (Fig. 2b). Salinity at the surface varied from 23.7 psu on 13 July to 35 psu on 19 April 2003 (Fig. 2b). Although there were not large differences in the water temperature between 0 m (surface) and 3 m depth (bottom) layers, relatively large differences in salinity were recorded between the two layers, especially on 13 July (23.7 psu and 32.7 psu, respectively). In addition, significantly lower salinities were observed after heavy rainfall (Fig. 2b). Daily average wind speeds varied from 0.2 to 2.5 m s^{-1} (Fig. 3a). The total average was 0.81 m s^{-1}, which is much calmer than the annual average of 2003. There was a large temporal variation in dissolved inorganic nutrients. The nitrate + nitrite concentrations varied from
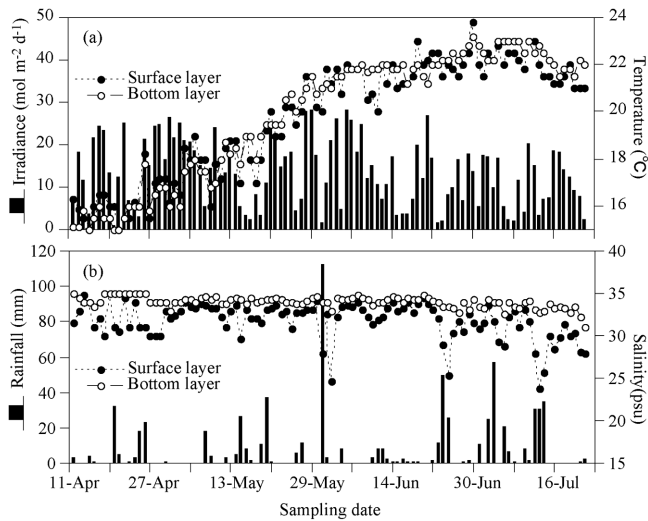
Fig. 2. Temporal variation of abiotic factors at St.W from 12 April to 22 July 2003. (a) Daily irradiance (black bars), surface (closed circles) and bottom (open circles) layer temperatures. (b) Daily rainfall (black bars), surface (closed circles) and bottom (open circles) layer salinities.
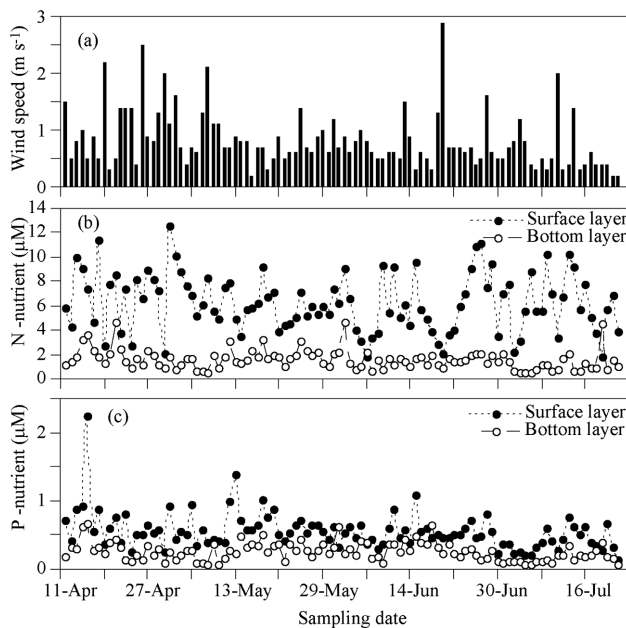

Fig. 3. Temporal variation of wind speed and nutrient concentrations at St.W from 12 April to 22 July 2003. (a) Wind speed. (b) nitrate + nitrite and (c) phosphate concentration in the surface (closed circles) and bottom (open circles) layer.

0.02 to 12.01 µM, with averages of 5.79 µM at the surface and 1.12 µM at the bottom layer (Fig. 3b). The phosphate concentration was generally low, ranging from the lower limit of detection, 0.02 to 2.24 µM. The average phosphate concentrations were 0.57 µM at the surface and 0.26 µM

at the bottom layer (Fig. 3c). The highest phosphate concentrations (2.24 µM) were observed on 16 April under calm weather without wind or rain. These higher nutrients were frequently introduced by river inflow, as indicated by the slightly increased measurement of salinity between surface and bottom water shown by these data.

Biological factors

Average Chl.*a* concentrations at the surface and bottom layers were 1.27 and 1.77 mg m^{-3}, respectively. The Chl.*a* concentration was highest (10.42 mg m^{-3}) on 2 June following the heavy rainfall of 31 May (Figs. 2b, 4a). During the study period, increases of Chl.*a* concentrations were frequently observed 4-6 days later after heavy rainfalls.

When concentrations of the heterotrophic dinoflagellate *Noctiluca scintillans* reached > 500 cells mL^{-1}, red tides formed a figure resembling dense tomato juice. Here, *N. scintillans* concentrations were estimated from the start of the sampling and were found to reach the first red tides between April 29 and May 1. Red tides were observed around the sampling site in 29 April when the highest density (2.3×10^6 cells L^{-1}) of *N. scintillans* was recorded at St.W. However, the cell size of the species was greatly reduced (living cells only; size data was not shown). After reaching the maximum cell density, *N. scintillans* concentrations gradually decreased until 9 May. A second bloom was observed on 12 May. The cells maintained middle-higher densities before a heavy rainfall on 31 May.
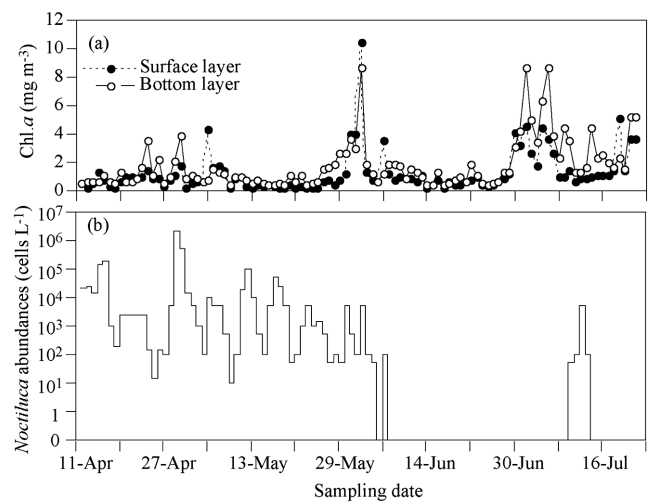

Fig. 4. (a) Chl.*a* concentrations in the surface (closed circles) and bottom layers (open circles) and (b) temporal variations in concentrations of *Noctiluca scintillans* at the surface layer at St. W from 12 April to 22 July 2003.
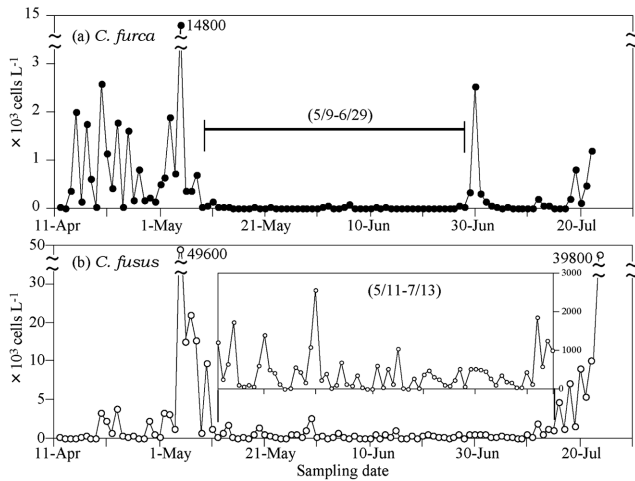
Fig. 5. Temporal variations in concentrations of (a) *Ceratium furca* and (b) *Ceratium fusus* at the surface layer at St. W from 12 April to 23 July.

Following the rainfall, the concentrations were relatively high in the water column during the 5 days after 31 May, and did not rebound until the end of sampling, except from 10 to 13 July (Fig. 4b).

The concentrations of the two *Ceratium* species were characterized by a high day-to-day variability. During the first half of the sampling period, the two *Ceratium* species maintained relatively high densities. The maximum concentrations of the two *Ceratium* species were measured on 5 May (*C. furca* = 14,800 cells L^{-1}, *C. fusus* = 49,600 cells L^{-1}) (Fig. 5a, b). Although *C. furca* and *C. fusus* concentrations rapidly decreased after their blooms, *C. fusus* was almost constantly observed, albeit at a relatively low density, from 11 May to 13 July (ave. 396 ± 484 cells L^{-1}) (Fig. 5b inner frame). The second bloom of only *C. fusus* was recorded on 23 July in the final period of the study. Compared to *C. fusus*, the concentration of *C. furca* was always lower, and additional blooms were not observed at the end of the study.

The relationship between environmental factors and the concentrations of the two *Ceratium* species surrounding the first bloom period (1 to 8 May) is shown in Fig. 6. At this time, the concentrations of the two *Ceratium* species were not significantly correlated with environmental factors such as irradiance, salinity and N-nutrients. In contrast, they were significantly correlated with phosphate levels ($p < 0.05$). Since other species of phytoplankton were greatly lower during the bloom and since peaks of *Ceratium* concentrations were significantly correlated with Chl.*a*
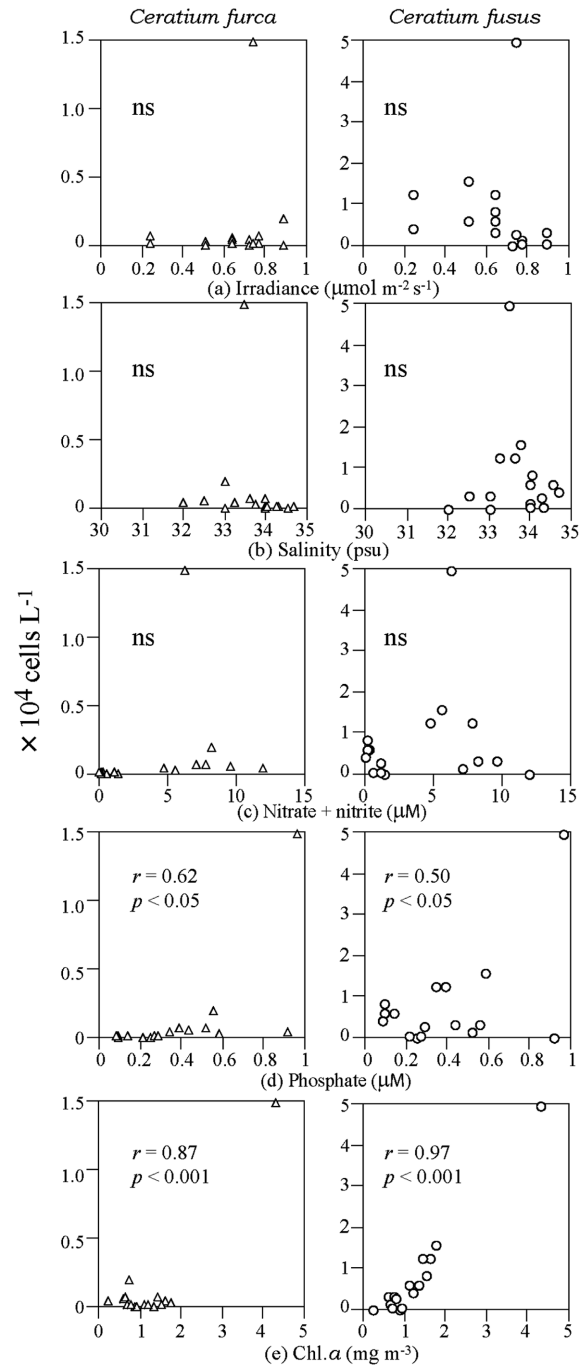

Fig. 6. Relationships between environmental factors and *Ceratium furca* (left) and *Ceratium fusus* (right) concentrations during the bloom period from 1 to 8 May 2003. (a) Daily irradiance. (b) Salinity. (c) Nitrate + nitrite. (d) Phosphate. (e) Chl.*a*. "ns" indicates statistically not significant.

concentrations, Chl.*a* concentrations (> 4 mg m^{-3}) were attributed to the two *Ceratium* species alone (Fig. 6e). The relationship between environmental factors and *C. fusus* population densities during the second bloom by only *C.*
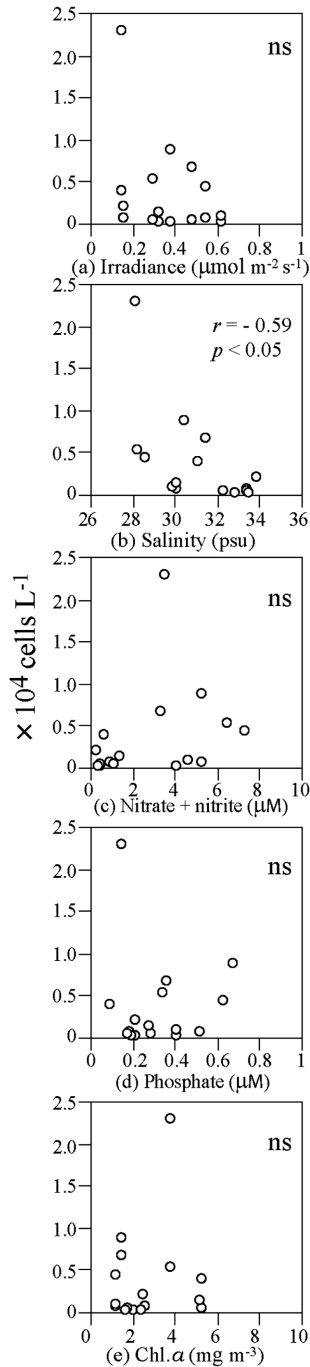
Fig. 7. Relationships between environmental factors and *Ceratium fusus* concentration during the bloom period from 15 to 22 July 2003. (a) Daily irradiance. (b) Salinity. (c) Nitrate + nitrite. (d) Phosphate. (e) Chl.*a*. "ns" indicates statistically not significant.

fusus (15 to 22 July) were was also examined (Fig. 7). A negative correlation between *C. fusus* concentrations and salinity was observed ($p < 0.05$), while the concentrations were not directly correlated with any other environmental factors.
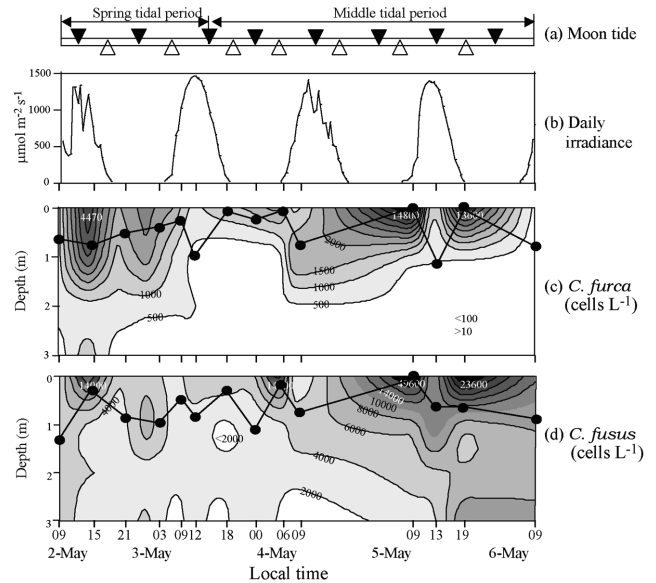

Fig. 8. Effects of (a) tidal conditions and (b) light intensities on the vertical distribution of (c) *C. furca* and (d) *C. fusus* at St. W from 2 to 6 May 2003. White and black triangles indicate high and low tides, respectively. Black circles indicate the WMDs of the two species.

Diel and tidal vertical migration of *C. furca* and *C. fusus*

The densities of *C. furca* and *C. fusus* seemed to decrease in higher daylight irradiance periods, for example at 09:00 on 3 May and 13:00 on 5 May (Fig. 8), although this relationship was not statistically significant (*C. furca*: y = -2.82x + 6101, $r^2 = 0.08$, $p > 0.05$ and *C. fusus*: y = -4.07x + 22134, $r^2 = 0.05$, $p > 0.05$). While higher population densities were observed during high tide, for example at 09:00 and 19:00 on 5 May (Fig. 8), the differences between high and low tide concentrations also were not statistically significant (*t*-test, $p > 0.05$).

The relationship between the WMD of *C. furca* and increasing irradiance was found to be statistically significant (Fig. 9a: $r^2 = 0.74$, $p < 0.001$). In contrast, the relationship between the WMD of *C. fusus* and irradiance was not statistically significant (Fig. 9b: $r^2 = 0.14$, $p > 0.05$). The WMD for *C. fusus* at low tide was significantly higher (deeper) than those at high tide (*t*-test; t-value: -3.093, $p = 0.01$). The averages for the WMD were 0.6 ± 0.30 at high tide and 1.19 ± 0.28 at low tide.

4. Discussion

In Sagami Bay, *C. furca* and *C. fusus* are dominant species; their populations remain at low densities from
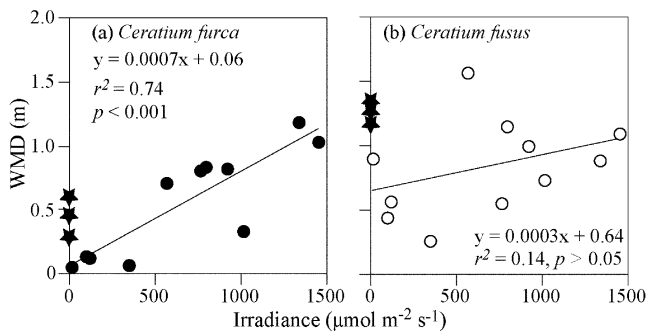
Fig. 9. Relationships between irradiance and WMDs of (a) *C. furca* and (b) *C. fusus* at St. W from 2 to 6 May 2003. Star marks indicate the WMDs of the two species during the dark period. Dark period samples were not included in the regression analysis to understand effect of light on the species distribution patterns.

October to January and tend to increase from April to September (Baek *et al*. 2006, 2007). Similar seasonal variations have been observed in some phytoplankton such as dinoflagellates (Anderson and Rengefors 2006) and raphidophytes (Honjo 1993; Shikata *et al*. 2007). Previous field and laboratory studies have shown that shorter photoperiods, changes in irradiance, and decreases in temperature are the most important environmental factors involved in the decreased concentrations of the two *Ceratium* species (Baek *et al*. 2008). However, the mechanisms behind the summer season population increases are not fully understood. In the present study, a more detailed investigation during the spring and summer seasons provided a better understanding of the population development of the two *Ceratium* species in Sagami Bay.

In coastal regions, phytoplankton communities are subject to control by numerous processes with different time and spatial scales. In 1997, the extensive red tides of *C. furca* were reported from April to July along the coast of Sagami Bay (Machida *et al*. 1999; Kanda *et al*. 2003). As shown by Kanda *et al*. 2003, satellite images of ocean color are available to explain the bloom in 1997. The images suggest that the observed *C. furca* bloom events were changed by shorter time-scales in the bay even when bloom occurred extensively. As mentioned, Sagami Bay is strongly influenced by the Kuroshio Current (oligotrophic waters) and coastal water mass, including those in Tokyo Bay (eutrophic waters). The complicated interactions of the water masses have strong impacts on the structure of the plankton community and biomass (Iwata 1985; Kanda *et al*. 2003). According to the studies by Fujiki *et al*. (2004),

Satoh *et al*. (2000) in the western part and Toriumi (1976) in the eastern part of the bay, *C. furca* and *C.fusus* were one of the most important organisms among the dinoflagellate communities from spring to early summer in Sagami Bay.

Population densities of the two *Ceratium* species increased after the bloom of the heterotrophic dinoflagellate *Noctiluca scintillans* during the first half of sampling period (Figs. 4b, 5). In Sagami Bay, the yearly pattern of seasonal variations in *N. scintillans* concentration is consistent; populations begin to increase in March and reached their maximum in May (Miyaguchi *et al*. 2006). Here, similar results were observed. High densities of *N. scintillans* were observed from the end of April to the middle of May. *N. scintillans* has been known to feed mainly on immobile prey such as diatoms (Nakamura 1998). The availability of phytoplankton as prey is one of the important factors for the variation in the density of its population (Elbrächter and Qi 1998). Additionally, Tada *et al*. (2000) reported that ammonium and phosphate in the intracellular pools of *N. scintillans* was supplied to the water column when the cells died. Thus, because of its high ammonium nitrogen content, *N. scintillans* is thought to act as one of the most important organisms contributing to nitrogen cycles in temperate coastal waters (Okaichi and Nishio 1976; Montani *et al*. 1998; Tada *et al*. 2000). Holligan and Harbour (1977) reported that the peak of *Ceratium* species concentrations invariably follows a diatom bloom, which almost certainly releases organic matter into the water. If there is nutrient competition between diatoms and large dinoflagellates such as *Ceratium* species, motile forms of the *Ceratium* species may escape predation by *N. scintillans*. Therefore, the populations of the two species could develop under relatively lower competitive conditions with high nutrient availability, in which the nutrient might be regenerated by both *N. scintillans* and dead cell of diatoms.

The second bloom by only *C. fusus* on 22 July occurred in the final study period when rainfall was frequent (Figs. 2b, 5b). One explanation is that the population development of *C. fusus* was stimulated by relatively low salinity conditions. Most *Ceratium* species dominating coastal waters have shown a wide range of tolerance to salinity during growth (14 to 34 psu) (Nordli 1953, 1957; Qasim *et al*. 1972; Elbrächter 1973). In previous laboratory experiments, high growth rates of *C. fusus* were observed in salinities from 24 to 30 psu, with the highest growth rate of 0.59 d^{-1} at a salinity of 27 psu (Baek *et al*. 2007). In contrast, no

significant relationship was observed between salinity (from 17 to 34 psu) and *C. furca* (Baek *et al*. 2006). Previous and current results suggest that the growth of *C. fusus* is more robust in lower salinity levels than that of *C. furca*. In this study, the population development of *C. fusus* in the final period coincided with salinity levels around 27 psu.

The effect of heavy rains during the rainy season on the dynamics of phytoplankton biomass in the study site has been reported (Satoh *et al*. 2000). It took approximately 2-3 days after a day of heavy rain for the freshwater causing the low salinity to reach the sampling site (Fig. 2b). Additionally, the low salinity conditions after rainfalls resulted in a large loading of nutrient such as DIN to the coastal region as compared to P (DIN: r^2=0.42, <0.001; P: r^2=0.12, <0.001) (Baek 2007). A significant relationship between salinity and N: P ratios was found, with N: P ratios being higher at low salinity (r^2=0.37, <0.001) (Baek 2007). Nitrogenous nutrients have long been considered to be a major factor controlling the occurrence of dinoflagellate blooms (Ryther and Dunstan 1971; Chang and Carpenter 1985; Howarth 1988; Paerl *et al*. 1990). Previously, it was found that the densities and specific growth rates of the two *Ceratium* species also increased under the high N: P ratio conditions in both the field and laboratory (Baek et al. in press). In the present study, although the concentrations of the two *Ceratium* species were not directly correlated with N-nutrients, they gradually increased after the highest nitrate + nitrite levels were recorded on 1 May (Figs. 3b, 5), implying that the high N-nutrient induced the first bloom by *C. furca* and *C. fusus* on 5 May. Thus, the nitrogenous nutrients in the coastal system could be expected to be an important factor for regulating population dynamics of the two *Ceratium* species.

Because *Ceratium* species are motile, their vertical distribution might reflect an ability to position themselves at an optimal depth in terms of light or nutrients; this would be an advantage over nonmotile forms. Hasle (1954) suggested that the rhythmic vertical migration of dinoflagellates is induced by light. Eppley *et al*. (1968) also reported that vertical migration of *C. furca* was observed between the surface and 5-m layers during daytime and nighttime, respectively. Moreover, Eppley *et al*. (1968) and Blasco (1978) suggested that *C. furca* migrate at speeds of approximately 1-2 m h^{-1}. In the present study, the two *Ceratium* densities appeared to decrease under high daylight irradiances (Fig.8), although relationships between

the average concentrations of each species and irradiance were not significant. In particular, the WMD of *C. furca* significantly increased (i.e., they moved toward the bottom) with increasing irradiance ($p < 0.001$). In addition, although *C. fusus* did not show clear diel vertical migration, the WMDs of *C. fusus* seemed to be relatively high (deep) when irradiance was over 1000 µmol m^{-2} s^{-1}. In previous laboratory experiments (Baek 2007), the two *Ceratium* species were not photoinhibited at 796 µmol m^{-2} s^{-1}, but their growth gradually decreased at 900 µmol m^{-2} s^{-1}. Several authors have suggested that some *Ceratium* species migrated downwards from the near-surface waters, avoiding high-light-induced cell damage (Hasle 1954; Eppley *et al*. 1968; Whittington *et al*. 2000). Therefore, our previous and present results indicate that approximately 1000µmol m^{-2} s^{-1} is the threshold of their tolerance to high irradiance and that strong sunlight is one of the main cues for the vertical migration of *Certium* in this filed condition.

Phytoplanktonic organisms in estuaries and coastal areas are subjected to vastly fluctuating physical conditions as a result of tidal currents. Steidinger *et al*. (1973) pointed out that several physical factors, such as tidal conditions, winds, and currents, may act to concentrate species of HAB in specific coastal areas. Pérez and Canteras (1990) reported that while tidal conditions did not affect the specific composition of the phytoplankton community, they did affect the numbers of individuals observed, with the greatest numbers being found at low tide. Here, it was found that the WMDs of *C. fusus* at low tide were significantly higher (deeper) than those at high tide (*t*-test, $p = 0.01$). In contrast, for *C. furca*, the differences in WMDs between the tidal conditions were not significant (*t*-test, $p > 0.05$). These results imply that *C. furca* performs active downward migration in the low tides, whereas the migration behavior of *C. fusus* is not so prominent. This difference might be caused by the swimming ability of the species. In a previous laboratory study (Baek 2007), the swimming speed of *C. furca* was found to be consistently two to three times higher than that of *C. fusus*. However, even with the higher swimming speed of *C. furca*, the speeds of the two species are considered to be not sufficiently higher than tidal currents, which can easily transport the populations in the surface layer to offshore. Therefore, although the *Ceratium* species, at least *C. fusus*, would perform vertical migrations in the study area, horizontal seawater exchanges by physical processes, such as tidal currents and wind, are one

of the main causes to explain the observed population dynamics.

Although genus *Ceratium* such as *C. furca* and *C. fusus* have been previously thought to be photosynthetic dinoflagellates, there are more recently considered as mixotrophs (Li *et al*. 1996; Smalley *et al*. 1999; Smalley and Coats 2002). Mixotrophic species gain their nutrition through a combination of photosynthesis and uptake of dissolved or organic material (Stoecker 1998). Nutrition-uptake species have a competitive advantage over simple photosynthetic organisms, since this manner of feeding would enable the mixotrophic species to take nutrition from organic material when inorganic nutrients are limited (Smalley and Coats 2002; Smalley *et al*. 2003). To determine grazing effect on ciliates by the *C. furca*, Smalley *et al*. (1999) attempted a new method using fluorescent microspheres. Preys thus labeled were phagocytized by *C. furca* and were easily detected within the predator. Also, Smalley and Coats (2002), and Mouritsen and Richardson (2003) reported that population dynamic of the two *Ceratium* species are strongly affected by ciliate prey distribution such as *Strobilidium* spp. in a stratified estuary. Although not investigated for their mixotrophic ability in this study, the advantage of additional nutrient sources in *Ceratium* species might contribute as an important factor in their bloom development.

In conclusion, the data presented suggest that the population development of the dinoflagellates *C. furca* and *C. fusus* probably require 1) low competitive pressure against nutrient uptake induced by predations on the diatom population by *N. scintillans* and 2) regenerated nutrients through the breakdown of blooms by diatoms and *N. scintillans*. Additionally, these result show that the responses to low salinity conditions were different between the two species, suggesting that *C. fusus* had an advantage over *C. furca* under the continuous low salinity conditions during the rainy season.

Acknowledgements

References

Anderson, D.M. 1997. Turning back the harmful red tide. *Nature*, **388**, 513-14

Anderson, D.M. and K. Rengefors. 2006. Community assembly and seasonal succession of marine dinoflagellate in a temperature estuary: The importance of life cycle events. *Limnol. Oceanogr.*, **52**, 860-873.

Baek, S.H., S. Shimode, and T. Kikuchi. 2006. Reproductive ecology of dominant dinoflagellate, *Ceratium furca* in the coastal area of Sagami Bay. *Coast. Mar. Sci.*, **30**, 344-352.

Baek, S.H., S. Shimode, and T. Kikuchi. 2007. Reproductive ecology of the dominant dinoflagellate, *Ceratium fusus* in coastal area of Sagami Bay, Japan. *J. Oceanogr.*, **63**, 35-45.

Baek, S.H. 2007. Physiological and ecological studies on the reproduction biology of the two dominant dinoflagellates *Ceratium furca* and *Ceratium fusus* in the Sagami Bay. Ph.D. Thesis, Yokohama National University, Yokohama (In Japanese)

Baek, S.H., S. Shimode, and T. Kikuchi. 2008. Growth of dinoflagellate, *Ceratium furca* and *Ceratium fusus* in Sagami Bay, Japan: The role of temperature, light intensity and photoperiod. *Harmful algae*, **7**, 163-173.

Baek, S.H., S. Shimode, M.S. Han, and T. Kikuchi. 2008. Growth of dinoflagellate, *Ceratium furca* and *Ceratium fusus* in Sagami Bay, Japan: The role of nutrients. *Harmful algae*. (In press)

Blasco, D. 1978. Observation on the diel migration of marine dinoflagellates off the Baja Californian Coast. *Mar. Biol.*, **46**, 41-47.

Bockstahler, K.R. and D.W. Coats. 1993. Spatial and temporal aspects of mixotrophy in Chesapeake Bay dinoflagellates. *J. Euk. Microbiol.*, **40**, 49-60.

Chang, J. and 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.

ECOHAB. 1995. The ecology and oceanography of harmful algal blooms: A national research agenda. Wood Hole Oceanographic Institute, Wood Hole. 66 p.

Elbrächter, M. 1973. Population dynamics of *Ceratium* in coastal waters of the Kiel Bay. *Oikos*, **15**, 43-48 .

Elbrächter, M. and Z.Y. Qi. 1998. Aspect of *Noctiluca* (Dinophycese) population dynamics. p. 315-335. In: *Physiological Ecology of Harmful Algal Blooms*, ed. by D.M. Anderson, A.D.

Cembella, and M.G. Hallegraeff. NATO ASI, Vol. G 41. Springer-Verlag, Berlin.

Eppley, R.W., O.H. Hansen, and J.D.H. Strickland. 1968. Some observation on the vertical migration of dinoflagelltaes. *J. Phycol.*, **4**, 333-340.

Frost, B.W. and S.M. Bollen. 1992. Variability of diel vertical migration in the marine planktonic copepod *Pseudocalanus newmani* in relation to its predators. *Canadian J. Fish. Aquat. Sci.*, **49**, 1137-1141.

Fujiki, T., T. Toda, T. Kikuchi, H. Aono, and S. Taguchi. 2004. Phosphorus limitation of primary productivity during the spring-summer blooms in Sagami Bay, Japan. *Mar. Ecol. Prog. Ser.*, **283**, 29-38.

GEOHAB. 2001. Global Ecology and Oceanography of Harmful Algal Blooms, Science Plan. SCOR and IOC, Baltimore and Paris. 86 p.

Hasle, G.R. 1954. More on photoactic diurnal migration in marine dinoflagellates. *Nytt. Mag. Bot.*, **2**, 139-142.

Hogetsu, K. and N. Taga. 1977. Suruga Bay and Sagami Bay. p. 31-172. In: *Hydrographic condition*, ed. by K. Hogetsu, M. Hatanaka, T. Hanaoka, and T. Kawamura. University of Tokyo Press, Tokyo.

Holligan, P.M. and D.S. Harbour. 1977. The vertical distribution and succession of phytoplankton in the western English Channel in 1975 and 1976. *J. Mar. Bio. Assoc. U.K.,* **57**, 1075-1093.

Holm-Hansen, O., C.J. Lorenzen, R.W. Holmes, and J.D.H. Strickland. 1965. Fluorometric determination of chlorophyll. *J. Cons. Perm Int. Explor. Mer.*, **30**, 3-15.

Honjo, T. 1993. Overview on bloom dynamics and physiological ecology of *Heterosigma akashiwo*. p. 33-41. In: *Toxic Phytoplankton Bloom in the Sea*. T.J. Smayda and Y. Shimizu. Elsevier, New York.

Howarth, R.W. 1988. Nutrient limitation of net primary production in marine ecosystems. *Ann. Rev. Eocl.*, **19**, 89-110.

Iwata, S. 1985. Chapter 10 Sagami Bay. p. 401-409. In: *Oceanography of Japanese Islands*, ed. by Oceanographical Society of Japan Coastal, Tokai University Press, Tokyo. (In Japanese)

Kanda, J. Fujiwara, S. H. Kitazato, and Y. Okada. 2003. Seasonal and annual variation in the primary production regime in the central part of Sagami Bay. *Prog. Oceanogr.*, **57**, 17-29.

Li. A., D.K. Stoecker, D.W. Coats, and J.E. Adam. 1996. Ingestion of fluorescently-labeled and phycoerythirin-containing prey by mixotrophic dinoflagellates. *Aquat. Microb. Ecol.*, **10**, 139-147.

Machida, M., M. Fujitomi, K. Hasegawa, T. Kudoh, M. Kai, T. Kobayashi, and T. Kamiide. 1999. Red tide of *Ceratium furca* along the Pacific coast of central Japan in 1997. *Nippon Suisan Gakkaishi*, **65**, 755-756. (In Japanese)

Miyaguchi, H., T. Fujiki, T. Kikuchi, V.S. Kuwahara, and T. Toda. 2006. Relationship between the bloom of *Noctiluca*

scintillans and environmental factors in the coastal waters of Sagami Bay, Japan. *J. Plankton Res.*, **28**, 313-324.

Montani, S., S. Pithakpol, and K. Tada. 1998. Nutrient regeneration in coastal sea by *Noctiluca scintillans*, a red tide causing dinoflagellate. *J. Mar. Biotechnol.*, **6**, 224-228.

Mouritsen, N. T. and K. Richardson. 2003. Vertical microscale patchiness in nano- and microplankton distribution in a stratified estuary. *J. Plankton Res.*, **25**, 783-797.

Nakamura, Y. 1998. Biomass, feeding and production of *Noctiluca scintillans* in the Seto Inland Sea, Japan. *J. Plankton Res.*, **20**, 2213-2222.

Nielsen, T.G. 1991. Contribution of zooplankton grazing to the decline of a *Ceratium* bloom. *Limnol. Oceanogr.*, **36**, 1091-1106.

Nordli, E. 1953. Salinity and temperature as controlling factors for distribution and mass occurrence of ceratia. *Blyttia*, **2**, 16-18.

Nordli, E. 1957. Experimental studies on the ecology of *Ceratia*. *Oikos*, **8**, 200-265.

Okaichi, T. and S. Nishio. 1976. Identification of ammonia as the toxic principle of red tide of *Noctiluca miliaris*. *Japanese Bull. Plankton Soc.*, **23**, 75-80. (In Japanese)

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

Parsons, T.R., Y. Maita, and C.M. Lalli. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford. 173 p.

Pérez, L. and J. Canteras. 1990. Influence of tidal conditions and river volume on phytoplankton distribution and composition in the Pas estuary (Northern Spain). *Scient. Mar.*, **54**, 77-88.

Qasim, S.Z., P.M. Bhattathiri, and V.P. Devassy. 1972. The influence of salinity on the rate of photosynthesis and abundance of some tropical phytoplankton. *Mar. Biol.*, **12**, 200-206.

Qasim, S.Z., P.M. Bhattathiri, and V.P. Devassy. 1973. Growth kinetics and nutrient requirements of two tropical marine phytoplankters. *Mar. Biol.*, **21**, 299-304.

Ryther, J.H. and W.M. Dunstan.1971. Nitrogen, phosphorus and, eutrophication in the coastal marine environment. *Science*, **171**, 1008-1013.

Satoh, F., K. Hamasaki, T. Toda, and T. Taguchi. 2000. Summer phytoplankton bloom in Manazuru Harbor, Sagami Bay, central Japan. *Plankton Biol. Ecol.*, **47**, 73-79.

Shikata, T., S. Nagasoe, T. Matsubara, Y. Yamasaki, Y. Shimasaki, Y. Oshima, and T. Honjo. 2007. Effects of temperature and light on cyst germination and germinated cell survival of the noxious raphidophyte *Heterosigma akashiwo*. *Harmful Algae*, **6**, 700-706.

Smalley, G.W., D.W. Coats, and E.J. Adam. 1999. A new method using fluorescents microspheres to determine grazing on

ciliates by the mixotrophic dinoflagellate *Ceratium furca*. *Aquat. Microb. Ecol.*, **17**, 167-179.

Smalley, G.W. and D.W. Coats. 2002. Ecology of the red –tide dinoflagellate *Ceratium furca*: Distribution, mixotrophy, and garzing impact on ciliate populations of Chesapeake Bay. *J. Eukaryot Microbiol.*, **49**, 64-74.

Smalley, G.W., D.W. Coats, and D.K. Stoecker. 2003. Feeding in the mixotrophic dinoflagellate *Ceratium furca* is influenced by intracellular nutrient concentrations. *Mar. Ecol. Prog. Ser.*, **262**, 137-151.

Smayda, T. J. 1997. Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnol. Ocernog.*, **42**, 1137-1153.

Steidinger, K., M. Burklew, and R. Ingle. 1973. The effects of *Gymnodinium breve* toxin on estuarine animals. p. 179-202. In: *Marine pharmacology: Action of marine biotoxins at the cellular level*, ed. by D. Martine and G. Padilla. Academic Press, NY.

Stoecker, D.K. 1998. Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. *Eur. J. Protistol.*, **34**, 281-290.

Sullivan, J.M. and E. Swift. 1995. Photoenhancement of bioluminescence capacity in natural and laboratory populations of the autotrophic dinoflagellate *Ceratium fusus* (Ehrenb.) Dujardin. *J. Geophys. Res.*, **100**, 6565-6574.

Suzuki, R. and T. Ishimaru. 1990. An improved method for the determination of phytoplankton chlorophyll using N,N-dimethylformamide. *J. Oceanogr. Soc. Japan*, **46**, 190-194.

Tada, K., S. Pithakpol, R.Yano, and S. Montani. 2000. Carbon and Nitrogen Content of *Noctiluca scintillans*, in the Seto Inland Sea, Japan. *J. Plankton Res.*, **22**, 1203-1211.

Toriumi, S. 1976. On the seasonal and vertical appearance of *Ceratium* species in Aburatsubo Bay, Kanagawa Prefecture. *Bull. Japanese Soc. Phycol.*, **24**, 55-61.

Weiler, C.S. and S.W. Chisholm. 1976. Phased cell division in natural populations of Marine dinoflagellates from shipboard cultures. *J. Exp. Mar. Biol. Ecol.*, **25**, 239-247.

Weiler, C.S. and R.W. Eppley.1979. Temporal pattern of division in the dinoflagellate genus *Ceratium* and its application to the determination of growth rate. *J. Exp. Mar. Biol. Ecol.*, **31**, 1-24.

Whittington, J., B. Sherman, D. Green, and R.L. Oliver. 2000. Growth of *Ceratium hirundinella* in a subtropical Australian reservoir: The role of vertical migration. *J. Plankton Res.*, **22**, 1025-1045.