JOURNAL OF PLANKTON RESEARCH | VOLUME 28 | NUMBER 3 | PAGES 313–324 | 2006

Relationship between the bloom of *Noctiluca scintillans* and environmental factors in the coastal waters of Sagami Bay, Japan

HIDEO MIYAGUCHI[1]*, TETSUICHI FUJIKI[2], TOMOHIKO KIKUCHI[3], VICTOR S. KUWAHARA[4] AND TATSUKI TODA[1]

[1]DEPARTMENT OF ECOLOGICAL ENGINEERING FOR SYMBIOSIS, FACULTY OF ENGINEERING, SOKA UNIVERSITY TANGI-CHO, HACHIOUJI, TOKYO 192–8577, JAPAN, [2]MUTSU INSTITUTE FOR OCEANOGRAPHY JAPAN AGENCY FOR MARINE-EARTH SCIENCE AND TECHNOLOGY, 690 KITASEKINE SEKINE, MUTSU, AOMORI 035–0022, JAPAN, [3]DEPARTMENT OF HYDROGRAPHIC SCIENCE, FACULTY OF EDUCATION AND HUMAN SCIENCES, YOKOHAMA NATIONAL UNIVERSITY, TOKIWADAI, HOOGAYA-KU, YOKOHAMA 240–8501, JAPAN AND [4]FACULTY OF EDUCATION, SOKA UNIVERSITY TANGI-CHO, HACHIOUJI, TOKYO 192–8577, JAPAN

*CORRESPONDING AUTHOR: hmiyaguc@soka.ac.jp

Received March 25, 2005; accepted in principle January 15, 2006; accepted for publication January 30, 2006; published online February 2, 2006

Communicating editor: K.J. Flynn

In order to study the mechanism of the bloom formation of Noctiluca scintillans, the relationship between the variation in the abundance of N. scintillans and environmental factors was examined in the coastal waters of Sagami Bay, Japan. Hydrographic (temperature, salinity, water stability), biological (chlorophyll a concentration, zooplankton biomass) and meteorological (rainfall, wind velocity, wind direction) factors were investigated from 1997 to 2004. For all years, the abundance of N. scintillans started to increase from March and reached a maximum in spring between April and May. The abundance in 1997 and 2000 was relatively high compared to the other years while the abundance in 1998 and 2004 was relatively low. A stepwise multiple linear regression analysis showed that the wind direction and rainfall were significantly correlated with the variation in the abundance of N. scintillans. Our results suggest that bloom formation can be separated into a three-step process: (i) initial increase in the abundance of N. scintillans attributed to an increase in optimum hydrographic and biological factors, (ii) N. scintillans is then accumulated by convergence of seawater by the factors of low rainfall and wind and (iii) swarmer-effects suggested enhanced bloom formation. Accumulation is considered to be a key trigger in this process of the formation of large-scale blooms.

INTRODUCTION

The large heterotrophic dinoflagellate *Noctiluca scintillans* is one of the most common “red tide” organisms. *Noctiluca scintillans* is a widespread organism and the blooms occur from spring to summer in temperate and tropical neritic waters all over the world. The seasonality of *N. scintillans* bloom formations has previously been proposed to be regulated by a variety of environmental factors such as hydrographical and biological factors (Elbrächter and Qi, 1998).

Temperature and salinity are variable factors in estuaries and coastal waters and are determinant factors of population dynamics for many phytoplankton species. The growth rate of *N. scintillans* is generally affected by temperature and salinity but *N. scintillans* is known to be a eurythermal and euryhaline organism (Elbrächter and Qi, 1998). Previous studies showed the optimum temperature and salinity ranges for *N. scintillans* were wide and differed between regions (Morton and Twentyman, 1971; Kuroda, 1978; Huang and Qi, 1997; Tada *et al.*, 2004). Thus, it is difficult to clarify the influence of temperature and salinity alone on the bloom formation of *N. scintillans*.

This paper was presented at Plankton Symposium III, held at Figuera da Foz, Portugal between 17 and 20 March 2005, under the auspices of the University of Coimbra and the University of Aveiro, and coordinated by Mário Jorge Pereira and Ulisses M. Azeiteiro.

doi:10.1093/plankt/fbi127, available online at www.plankt.oxfordjournals.org

Availability of phytoplankton as prey is also one of the important factors for the variation in abundance of *N. scintillans* (Elbrächter and Qi, 1998). *Noctiluca scintillans* blooms are also known to occur after diatom blooms (Kiørboe, 1998; Tiselius and Kiorobe, 1998; Dela-Cruz *et al*., 2002). Huang and Qi (Huang and Qi, 1997) reported a negative relationship between the abundance of *N. scintillans* and chlorophyll *a* concentrations during blooms. However, inversely, a positive relationship between *N. scintillans* and phytoplankton biomass has been reported (Schaumann *et al*., 1988; Painting *et al*., 1993). These conflicting reports possibly result from the time lag that happens to occur between predator effects on prey concentrations in field studies. *Noctiluca. scintillans* is also a phagotrophic species feeding on bacteria, detritus, protozoans, copepod and eggs of copepod and fish as well as phytoplankton (Kirchner *et al*., 1996; Nakamura, 1998; Quevedo *et al*., 1999; Strom, 2001). The contributions of qualitative and quantitative prey items to *N. scintillans* bloom remains only partly understood and are not yet clearly established.

Noctiluca scintillans is a large-sized dinoflagellate and can be categorized in the same size range with micro- to meso-zooplankton communities (Elbrächter and Qi, 1998). Further, *N. scintillans* also possibly affect zooplankton communities by competing for food resources (Nakamura, 1998). For example, *N. scintillans* biomass corresponded to more than 90% of the zooplankton during bloom periods (Murray and Suthers, 1999; Miyaguchi *et al*., 2000). Thus, *N. scintillans* blooms subsequently overcome zooplankton biomass by feeding on their eggs and competing for food resources (Nakamura, 1998; Quevedo *et al*., 1999).

Accumulation of buoyant cells caused by convergence of surface seawater is suggested as one of the factors leading to *N. scintillans* blooms or aggregations (Elbrächter and Qi, 1998). At Tai Tam Bay on the southern coast of Hong Kong, the southerly winds following typhoons were responsible for concentrating the red tide on beaches in July (Morton and Twentyman, 1971). In Mirs Bay and Port Shelter around the northern region of Hong Kong, the prevailing northeast monsoon winds promoted the formation of a bloom of *N. scintillans* (Yin, 2003). In the German Bight when the bloom of *N. scintillans* was observed in August 1984, the northwesterly winds from the North Sea toward the German littoral were considered important (Schaumann *et al*., 1988). In the same area, the southeasterly winds from the German littoral toward the North Sea were known to be dominant from April to July, and the accumulation leading to the bloom of *N. scintillans* seemed not to occur during these periods (Uhlig and Sahling, 1990). The accumulation trigger is thought to be influenced by wind direction and topography at each region including the condition of surface seawater change by meteorological factors such as rain, wind velocity, wind direction, tide and ocean current (Smayda, 1997; Dela-Cruz *et al*., 2003). Therefore, not only are the relationship between *N. scintillans* and hydrographic and biological factors significant, but also meteorological factors influencing the accumulation of the cells are crucial factors in bloom formation.

Annual variations in the abundance of *N. scintillans* have also been reported (Uhlig and Sahling, 1990; Huang and Qi, 1997; Pithakpol *et al*., 2000). Observations for twenty years in the German Bight showed annual oscillation with three-year intervals in the abundance of *N. scintillans*. During this study it was found that a year with relatively high abundance is followed by two years of relatively low abundance (Uhlig and Sahling, 1990). Among the three years from 1990 to 1992 in the coastal waters of Hong Kong, the abundance of *N. scintillans* in 1990 was relatively high compared with that in 1991 and 1992 (Huang and Qi, 1997). In the Seto Inland Sea of Japan from 1995 to 1998, the abundance of *N. scintillans* in 1995 and 1998 were relatively high compared with other years (Pithakpol *et al*., 2000). Thus, the abundance of *N. scintillans* shows temporal variability spanning from seasons to years. Blooms of *N. scintillans* have been reported to correlate with environmental factors but the initial trigger of the large-scale bloom formations have not specifically been attributed to the any particular condition. The cause of the large-scale bloom formation in coastal embayments is still controversial.

Sagami Bay, located in central Japan, forms an embayment with its mouth opened toward the Pacific Ocean (Fig. 1). The bay hydrography is primarily related to fluctuations of the Kuroshio Current axis and to water originating from the Sagami and Sakawa rivers as well as water from Tokyo Bay (Hogetsu and Taga, 1977). The coastal waters of Sagami Bay consist of surface water from the center of the bay and these river waters (Nakata, 1985). Shore-parallel foam lines associated with changes in water coloration are often observed in the coastal waters of the bay. The accumulation of shore-parallel foam suggests a possible convergence of marine and river water while the associated change in water coloration suggests that the convergence delineates a front between two water masses (Shimode *et al*., 2005). Because of the variety of water masses, many organisms co-occur in Sagami Bay and these organisms cause the biota of the bay to be diversified. *Noctiluca scintillans* has been known to appear in Sagami Bay for

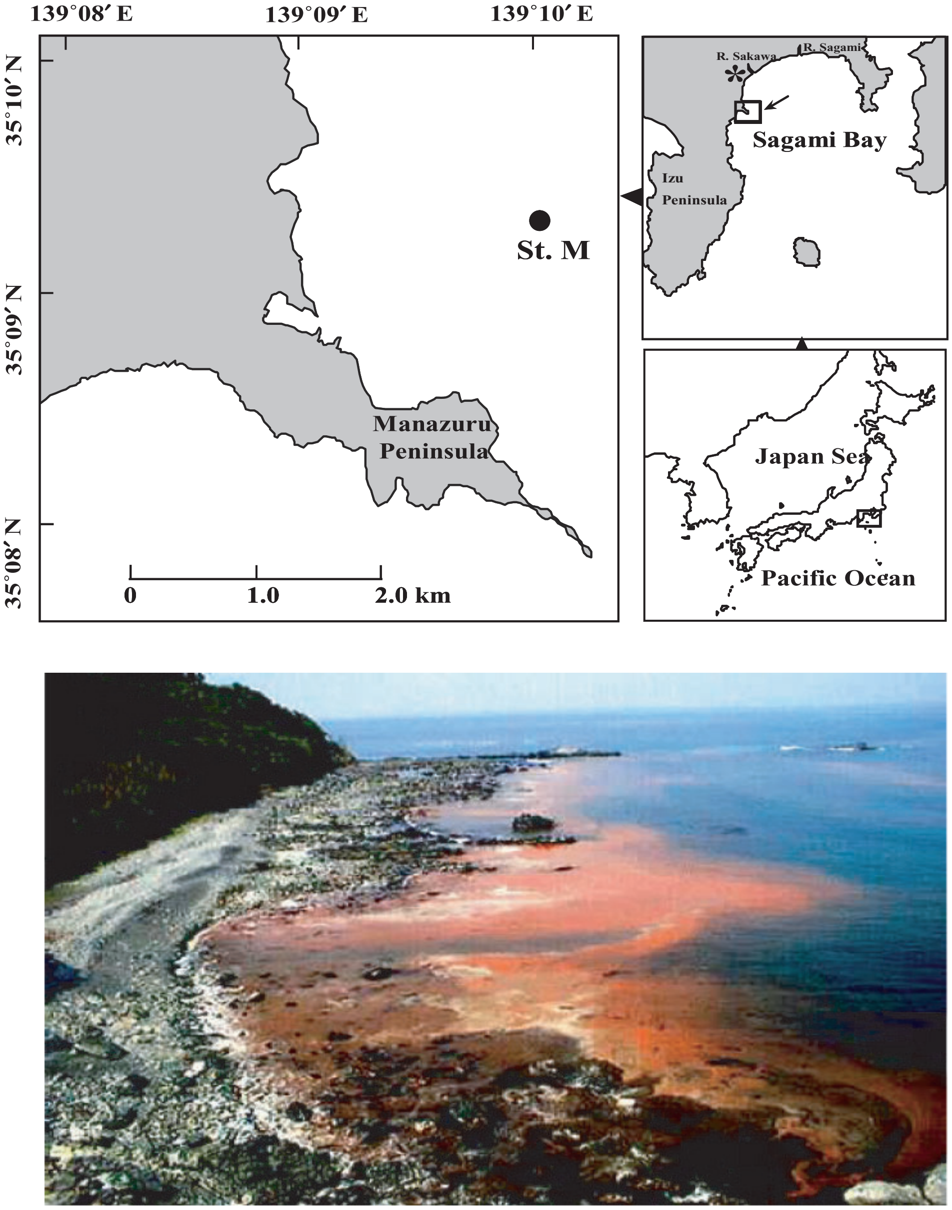

Fig. 1. The location of the sampling site, St. M in Sagami Bay. Asterisk indicating the site of Odawara Office of the Japan Meteorological Business Support Center, which is monitoring the meteorological factors used in this study. Photograph shows a massive *Noctiluca scintillans* bloom which occurred along Manazuru Peninsula in May 2000.

many decades (Nakata, 1985; Miyaguchi *et al.*, 2000). Blooms of the species have been observed almost every year monitored (Fig. 1). However, there is presently little known about the trigger mechanism of the bloom formation in the bay and it is difficult to isolate the controlling factors of the variability in the abundance of *N. scintillans*.

The purpose of this study was to examine the relationship between the blooms of *N. scintillans* in Sagami Bay and environmental factors in order to determine the characteristics of bloom formations. Our goal was to utilize multiple measurements from the bay, including meteorological data, to resolve the complex processes that initiate and sustain bloom formations. We have continually monitored the seasonal and annual variations in the abundance of *N. scintillans* at several stations in the coastal waters of Sagami Bay for the past eight years as part of an ongoing cooperative research between Soka

University and Yokohama National University. The long-term time-series research has made it possible to extract the significant factors influencing the variation and mechanisms which regulate and trigger large-scale blooms of *N. scintillans*.

METHOD

Study area

The present study was conducted at Station M (St. M) near the Manazuru Peninsula, Sagami Bay (Fig. 1). St. M is situated about 2 km north of Manazuru Peninsula (35°09′49″N, 139°10′33″E, depth 120 m). St. M was selected to examine the seasonal and annual variation in net-zooplankton as a long-term station for cooperative research between Soka University and Yokohama National University. St. M is a key temperate sampling station in Japan that has methodically been studied for variability in environmental factors, bio-optics variability, and distribution and abundance of plankton due to its relatively high productivity and biodiversity (Kuwahara *et al.*, 2000; Nagao *et al.*, 2001; Shimode *et al.*, 2005).

Sampling

Samplings were carried out approximately once a month from 1997 to 2004, usually before noon (10:00–12:00 local time) aboard the R/V *Tachibana* of the Manazuru Marine Laboratory, Yokohama National University. Surface seawater was collected by means of a bucket while seawater from 10, 20, 30, 40 and 60 m depths by 5 L Niskin bottles. Net-samples were collected by vertical towing from 50 m depths to the surface using 0.45 m diameter plankton net with a 180 μm mesh size.

Hydrographic, biological and meteorological factors

The environmental factors, temperature, salinity and water stability as hydrographic factors, chlorophyll *a* concentration and other-zooplankton biomass as biological factors and rainfall, wind velocity and wind direction as meteorological factors were investigated from 1997 to 2004.

Temperature was measured with a mercury thermometer and salinity was determined with a light-refraction salinometer (SHIBUYA model S-10). Water density was estimated as sigma-t (σ_t). Water stability was calculated by, $E = 1/\sigma_{t(Avg)} \times d\sigma_t/dD$, where E is water stability, $\sigma_{t(Avg)}$ is the average density of seawater and $d\sigma_t/dD$ is the density vertical gradient (Mengesha *et al.*, 1998).

The seawater was filtered onto Whatman glass fiber filters (Type GF/F) and placed into a 20 mL brown vial containing 10 mL *N*, *N*-Dimethilformamide (DMF) for chlorophyll *a* extraction (Suzuki and Takahashi, 1990). Chlorophyll *a* concentration was measured using the Turner Design fluorometer, Model 10-AU. Net-samples were filtered onto pre-combusted (500°C for 2 h) Whatman glass-fiber filters (Type GF/A) and frozen. Later, frozen samples were dried at 60°C, 24 h in an oven (EYELA NDO-600ND). Measurements of ash free dry weight and organic carbon and nitrogen were performed with the method described by Hirota and Szyper, (1975) and Nagao *et al.* (2001) using the carbon–hydrogen–nitrogen (CHN) analyzer (FISON model NA1500NCS), the balance (SARTORIUS MC5) for weighting of samples, and the oven (ISUZU STR-28K) for combustion. Other-zooplankton (>180 μm) biomass was estimated by subtracting the carbon biomass of *N. scintillans* (see below) during this study period from each monthly carbon biomass of the net-samples.

Rainfall (mm), wind velocity (msec^{-1}) and wind direction were obtained from the Japan Meteorological Business Support Center at the Odawara Office, Kanagawa, Japan (35°15′01″N, 139°09′03″E). The wind direction data were expressed as numerical residuals by the relationship between the directions and the angles.

Cell numbers

Collected net-samples were concentrated through a sieve (100 μm), poured into 50 mL glass vials and preserved with borate-buffered formaldehyde (5% final concentration). The cells numbers of *N. scintillans* in the net-samples were counted and the cell diameters of 100 randomly chosen cells were measured from each sample under a stereomicroscope.

Cell volume, carbon content and biomass

In order to estimate the biomass of *N. scintillans*, the regression equation between the cell volume of *N. scintillans* and carbon content of the cell was calculated. The cell volume was calculated on the assumption that *N. scintillans* cells are spherical (Hillebrand *et al.*, 1999). About 300 cells of *N. scintillans* were sorted and washed by filtered seawater three times to eliminate other phytoplankton and zooplankton species. The cells were gently filtered onto pre-combusted 25 mm Whatman glass-fiber filters (Type GF/F). The carbon contents were measured as described above. The relationship between the cell volume and the carbon content is

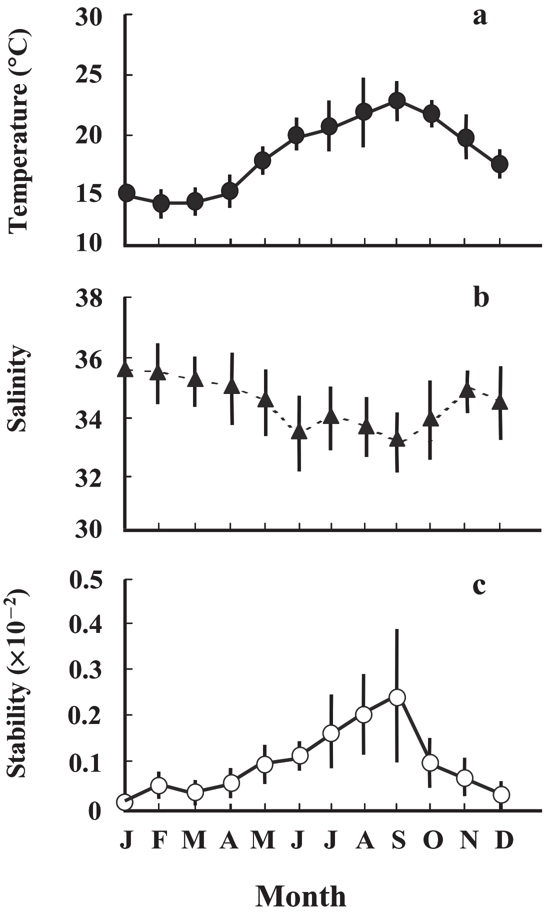

Fig. 2. Monthly average of hydrographic factors from 1997 to 2004; temperature (a), salinity (b) and water stability (c). Vertical bars denote standard deviation.

obtained as the logarithm equation, $C = 0.24 \times \mathrm{Log}_e V - 3.71$ ($n = 9$, $P < 0.01$), where C is the carbon content per single cell of *N. scintillans* (μg C cell^{-1}) and V is the cell volume (μm^3 cell^{-1}).

Statistical analysis

The hydrographic factors; temperature and salinity, and biological factor, chlorophyll *a*, were calculated as the average of 0, 10, 20, 30, 40 and 60 m depth-integrated value for the month throughout the study period. To investigate the hydrographic, biological and meteorological factors characteristics of each year, the residuals were calculated by subtracting the corresponding monthly average of the whole study period from each monthly data. For ranking the significance between the abundance of *N. scintillans* and hydrographic, biological and meteorological factors, a stepwise multiple linear regression analysis was carried out by using the REGRESSION function of analysis tool in EXCEL, Microsoft® (Godhe *et al.*, 2001; Dela-Cruz *et al.*, 2002). In order to avoid the fact that there are different magnitudes of the different factors, all factors were normalized.

RESULTS

Variation in hydrographic, biological and meteorological factors

The average water temperature varied between 14.5°C in February and March, and 22.8°C in September (Fig. 2a). The temperature increased from April to September The average salinity fluctuated between 33.6 in September and 35.9 in January (Fig. 2b). The salinity decreased from April to July during the rainy season. The average water stability ranged from 0.0001 in January to 0.0025 in September (Fig. 2c).

For the monthly average of biological factors, chlorophyll *a* concentration ranged from 0.4 mg m^{-3} in December and January to 2.4 mg m^{-3} in May (Fig. 3a). The average other-zooplankton biomass varied between 2.8 mg C m^{-3} in January and 20.3 mg C m^{-3} in April (Fig. 3b). Two peaks of the other-zooplankton were observed in April and August

For the monthly average of meteorological factors, rainfall ranged between 52.9 mm in December and

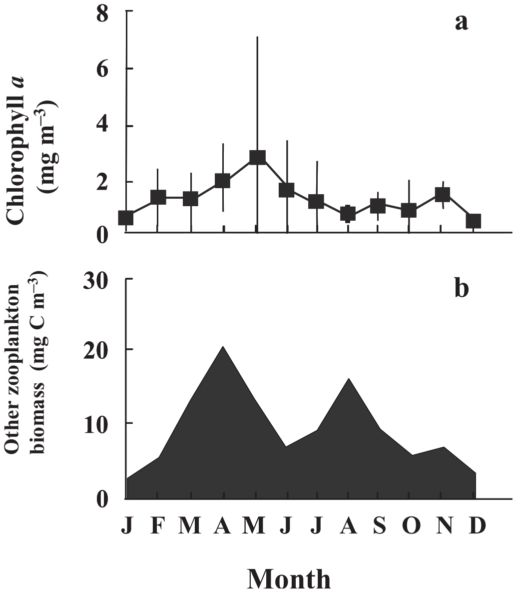

Fig. 3. Monthly average of biological factors from 1997 to 2004; chlorophyll *a* (a) and other-zooplankton biomass (b). Vertical bars denote standard deviation.

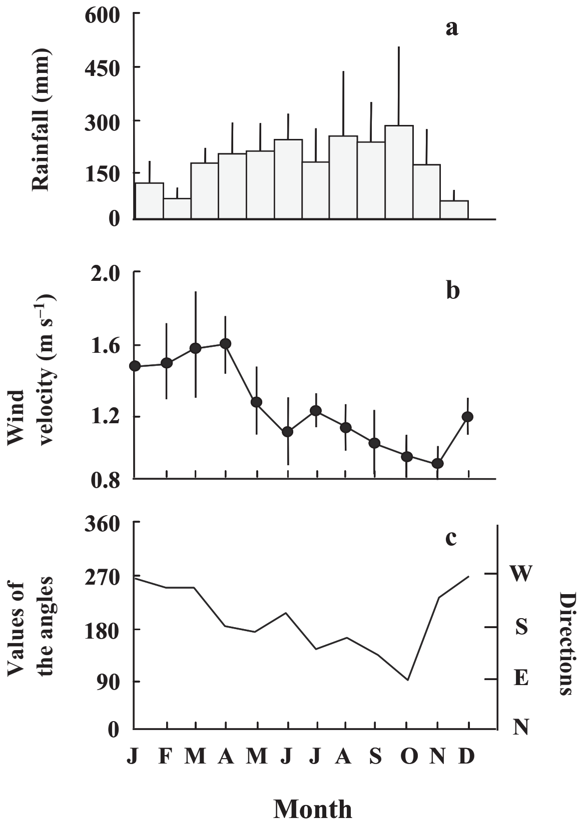

Fig. 4. Monthly average of meteorological factors from 1997 to 2004; rainfall (a), wind velocity (b) and wind direction (c).Vertical bars denote standard deviation.

274.0 mm in October (Fig. 4a). The rainfall tended to be high from August to October during the rainy season when typhoons frequently approach the temperate area around Japan. Wind velocity varied between 0.9 m sec^{-1} in November and 1.6 m sec^{-1} in March and April (Fig. 4b). Wind velocity decreased from May to October The wind directions were from the southwest from January to March and shifted to the southeast after April (Fig. 4c).

Abundance, cell volume and biomass of *N. scintillans*

The abundance of *N. scintillans* started to increase from March and reached a maximum in spring between April and May throughout the study period (Fig. 5a). After reaching a maximum, the abundance abruptly declined and remained low from June to December Abundance of *N. scintillans* showed variation from year to year. The abundance in 1997 and 2000 was higher compared to the other years while the abundance in 1998 and 2004 was relatively low. The maximum abundance was 6.1×10^5cells m^{-3} in May 2000. The minimum cell volume was 2.5×10^7 μm^3 in April 2004 and the maximum was 3.7×10^8 μm^3 in February 2002 (Fig. 5b). The largest cell volume of *N. scintillans* was frequently observed between February and March and then decreased gradually from April to June.

The biomass of *N. scintillans* showed a maximum in the spring between April and May throughout the present study period (Fig. 6). The highest biomass was 294 mg C m^{-3} in May 2000. The biomass in 1997 and 2000 was relatively high compared with other years. During the bloom period in spring, the biomass of *N. scintillans* overwhelmed the other-zooplankton biomass during the entire study period, except in 1998 and 2004.

Deviation of hydrographic, biological and meteorological factors from the monthly averages

The temperature residuals were relatively high in 1998, and were low in 2000 (Fig. 7a). During most of 1998, the salinity residuals were lower than zero (Fig. 7b). The residuals of the water stability were high in the summer of 2000 and the lowest residuals were observed in 1998 (Fig. 7c).

From 1997 to 1999, the residuals of chlorophyll *a* were high and the residuals tended to be lower after 2000 (Fig. 7d). The residuals of the other-zooplankton were high in the spring before *N. scintillans* blooms of 2000 and 2001 while low residuals were recorded in 2003 and 2004 (Fig. 7e).

The rainfall residuals were high in 1998 and 2004, and were relatively low in 1997 and 2000 (Fig. 7f). The wind velocity residuals were high during the spring in 1997 and 2000 and were low during the other years (Fig. 7g). Wind direction was normalized to the south-southeast. The residuals of wind direction were zero in spring 1997 and 2000 and were high in spring 1998 and 2004 (Fig. 7h).

Multiple linear regression analysis

The multiple linear regression equation was estimated as, $D = -0.20\ Wd - 0.14\ R + 0.12\ B + 0.04\ Chl.a - 0.70$ (Table I), where D is the abundance of *N. scintillans*, Wd is wind direction, R is rainfall, B is the biomass of the other-zooplankton and Chl.*a* is the concentration of chlorophyll *a*. The regression coefficients of meteorological factors were higher than that of other factors. Coefficient of correlation of the multiple linear regression equation was 0.73.

DISCUSSION

Seasonal variation in abundance of *N. scintillans*

In the present eight-year study, the seasonal variation in abundance of *N. scintillans* in the coastal waters of Sagami

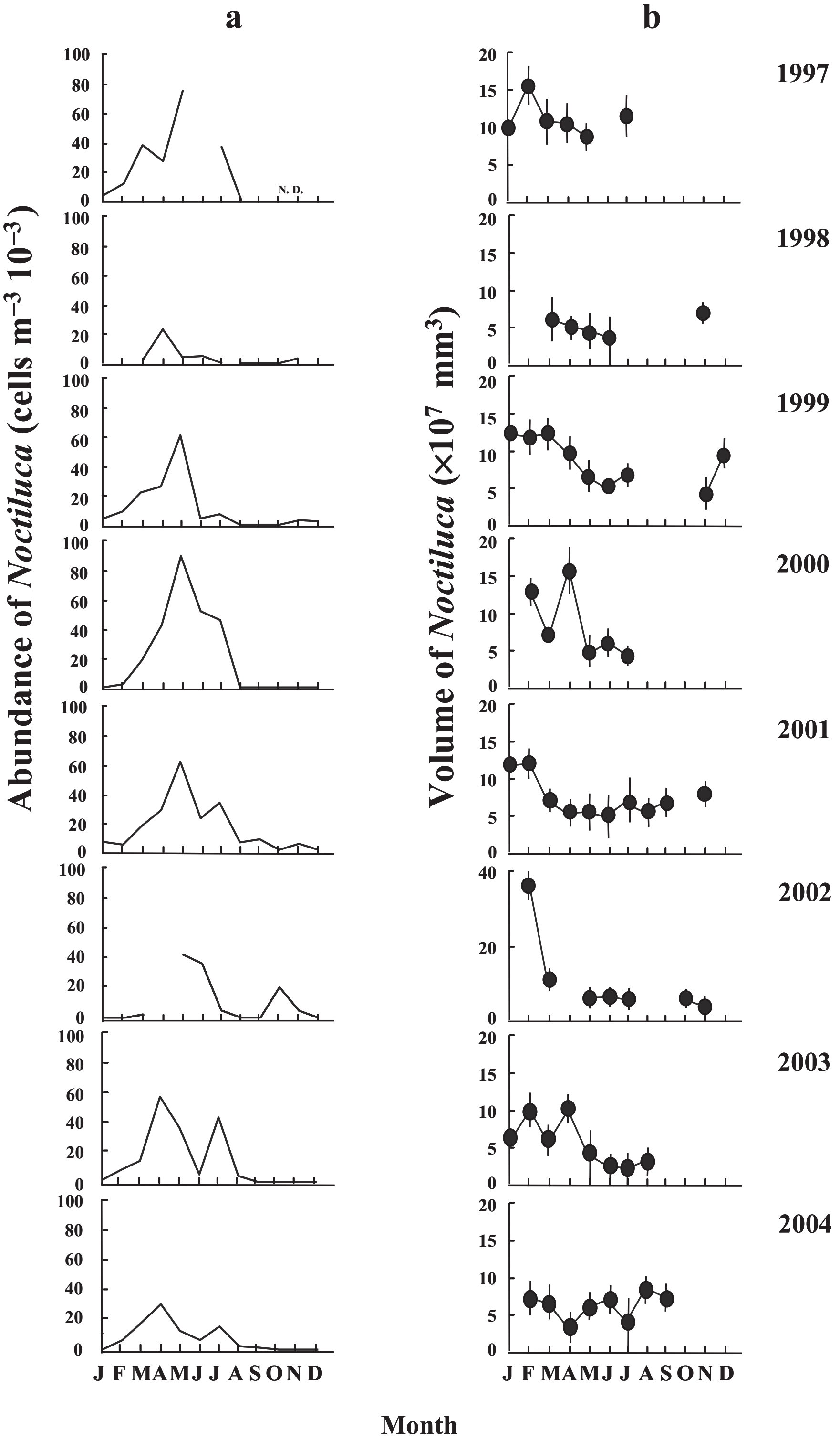

Fig. 5. Abundance (a) and the cell volume (b) of *Noctiluca scintillans* from 1997 to 2004. Vertical bars denote standard deviation.

Bay, Japan, showed a similar pattern every year. The seasonal bloom was observed during the spring, particularly in May (Table II). The seasonal variation patterns were consistent with patterns previously reported in other coastal waters. (Uhlig and Sahling, 1990; Huang and Qi, 1997; Pithakpol *et al.*, 2000). Omori and Hamner (1982) pointed out that high abundance at a single station may not be representative of larger areas. The long-term study in the German Bight suggested the spatial distribution of *N. scintillans* follows a gradient with highest abundances near the coast and decreasing abundances from the German littoral toward the North Sea, but the seasonal variation in *N. scintillans* abundance showed similar patterns at a majority of the sampling stations (Uhlig and Sahling, 1990). In the coastal waters of Sagami Bay, we have also conducted investigations in Manazuru Port (St. A) and at a sampling station of 20 m (St. G.). Results of the present study indicated the seasonal and annual variation in the abundance of *N. scintillans* was also similar to the results of the two other stations (unpublished result). Therefore, the present study of St. M was considered a reliable representation of the overall relationship between *N. scintillans* and environmental factors in the coastal waters of Sagami Bay.

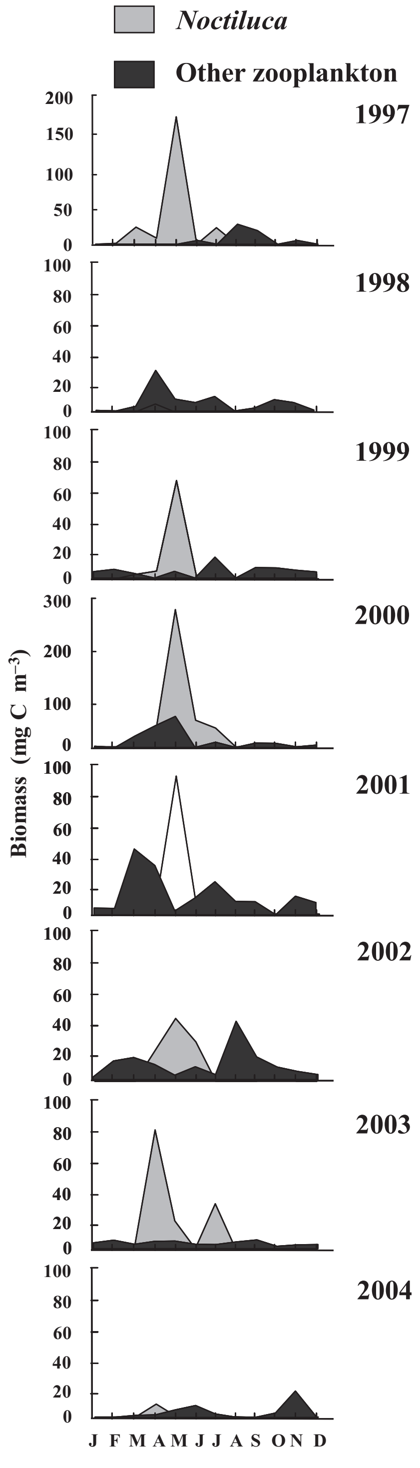

Fig. 6 Biomass of *Noctiluca scintillans* and the other-zooplankton from 1997 to 2004.

In the present study, temperature and salinity during the spring around May was considered the optimum range from 15.2 to 17.8°C and salinity 34.9–35.3, respectively. Previous studies reported the optimum temperature and salinity range for *N. scintillans* to be 10–28°C and 28–36, respectively and differed greatly between study areas (Morton and Twentyman, 1971; Kuroda, 1978; Huang and Qi, 1997; Tada *et al.*, 2004). In Sagami Bay, *N. scintillans* blooms appear to prefer lower temperature and higher salinity relative to those found in previous studies. Huang and Qi (1997) and Tada *et al.* (2004) reported the high abundance of *N. scintillans* correlated with temperature at each sampling site. However, temperature and salinity were not significantly correlated with the variation in the abundance of *N. scintillans* in the present study. Rainfall was high between June and October, and was low during the bloom period of *N. scintillans* in Sagami Bay (Fig. 4a). In Dapeng Bay of Hong Kong, the abrupt decline in salinity due to heavy rain caused a sharp decrease in the abundance of *N. scintillans* (Huang and Qi, 1997). During the terminal period of *N. scintillans* bloom in Sagami Bay, not only the decline in salinity but also the dispersion of the patch by means of heavy rainfall was thought to be one of the contributing factors ending the bloom of *N. scintillans*.

The average water stability at St. M in the present study increased from 0.0001 in January and reached a maximum 0.0025 in September (Fig. 2c). Water stability in the Seto Inland Sea varied from 0.0005 to 0.0035 and

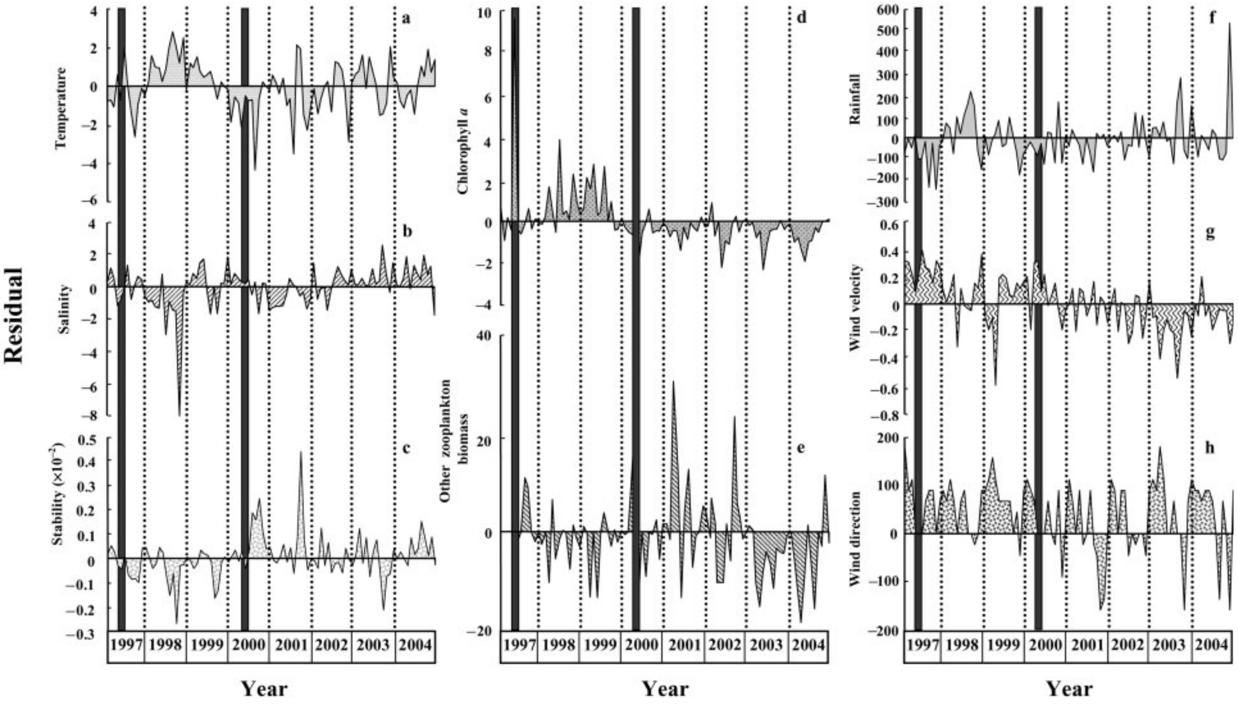

Fig. 7 Deviation of environmental factors from the monthly averages of study period, temperature (a), salinity (b), water stability (c), chlorophyll *a* concentration (d), other-zooplankton biomass (e), rainfall (f), wind velocity (g) and wind direction (h). Vertical dark bars denote the bloom period of *Noctiluca scintillans* in 1997 and 2000.

showed a peak in June and August during the period of *N. scintillans* bloom (Kuroda, 1990). High water-column stability is known to contribute to the variation of phytoplankton composition, abundance and patch formation (Kuroda, 1990; Mengesha *et al.*, 1998; Garibotti *et al.*, 2003). However, water stability was not correlated with the variation in the abundance of *N. scintillans* in the present study and its range was relatively low compared with the Seto Inland Sea. The water stability in Sagami Bay changes rapidly from high to low corresponding to the distribution of the Kuroshio Current and the inflows of the fresh water originating from the Sagami and Sakawa rivers (Hogetsu and Taga, 1977; Nakata, 1985). Although *N. scintillans* abundance did not directly correlate to water stability conditions, it is clear that the transition from low to high stability contributes to bloom formations.

Diatom blooms appeared from March to May in Sagami Bay and were often observed in the body of *N. scintillans*. The contribution of diatoms to total chlorophyll *a* was particularly high when peaks of chlorophyll *a* were observed (Satoh *et al.*, 2000), and microscopic examination showed that the diatoms were mainly composed of *Nitzschia* spp., *Thalassiosira* spp. and *Chaetoceros* spp. (Fujiki *et al.*, 2003). These diatoms were considered the main prey item of *N. scintillans* and may be a contributing factor to the increase in the abundance of *N. scintillans* during the initial phase of its bloom. During the period, the cell density possibly became high to some extent by increase of the growth rate under the favorable food conditions. However, neither a positive nor a negative relationship was observed between the abundance of *N. scintillans* with chlorophyll *a* concentration in the present study. In order to estimate the contributions of prey items to *N. scintillans* bloom, detailed information of the component of its prey items and the time lag in predator effects on prey concentrations are hereafter necessary to be collected in field studies.

In the present study, the biomass of *N. scintillans* dominated other-zooplankton biomass from April to May throughout the study period except in 1998 and 2004. The other-zooplankton biomass was generally high from March to August but a decrease in the biomass was observed in May (Fig. 6). During *N. scintillans* spring blooms, the high abundance seemed to suppress the other-zooplankton biomass. *Noctiluca scintillans* has been known to feed on copepod eggs but copepod population dynamics are not necessarily influenced by the predation of their eggs (Daan, 1987; Quevedo *et al.*, 1999). Competition for food resources between *N. scintillans* and zooplankton has also been hard to identify in previous studies because the grazing rate of *N. scintillans* is

Table I: Multiple linear regression analysis applied to hydrographic and meteorological factors against the abundance of Noctiluca scintillans during the whole study period in Sagami Bay, Japan

Factor	Regression coefficient	*P*	r	Overall *P*
			0.73	<0.0001
Wind direction	-0.20	0.0075		
Rainfall	-0.14	0.021		
Other-zooplankton biomass	0.12	0.025		
Chlorophyll *a*	0.04	0.073		
Constant	-0.70			

apparently low compared to same-sized zooplankton (Nakamura, 1998). Therefore, the ecological conditions causing the decrease in the other-zooplankton biomass by *N. scintillans* blooms are difficult to confirm.

The wind velocity increased in February and then decreased from May to winter in Sagami Bay. The wind directions were southwest from January to March and shifted to south-southeast after April (Fig. 4c). Accumulation is thought to be influenced by wind direction and topography of the region (Morton and Twentyman, 1971; Schaumann *et al.*, 1988; Uhlig and Sahling, 1990; Yin, 2003). The wind from the south-southeast usually blows from the mouth of Sagami Bay toward Manazuru Peninsula (Fig. 1). Thus, the optimal accumulation period of *N. scintillans* cells were considered to occur in the spring season from April to May following the period of increased winds.

The seasonal variation in the abundance of *N. scintillans* was statistically more significant with relation to meteorological factors than hydrographic and biological factors (Table I). The multiple linear regression analysis showed that the most significant meteorological factors relating to the abundance of *N. scintillans* were wind direction and rainfall. These meteorological factors seemed to be directly related to the variation in the abundance of *N. scintillans*.

Annual variation in abundance of *N. scintillans*

In the present study, the abundance of *N. scintillans* showed clear annual variation (Table II). Annual oscillations in the abundance of *N. scintillans* around Helgoland Roads, Germany, occurred at three-year intervals (Uhlig and Sahling, 1990). In this study, the abundance of *N. scintillans* also indicated a similar oscillation; high abundance in 1997 and 2000 and low abundance during other years. Following this oscillation pattern, the year 2003 should have been a high abundance year but was relatively low during April The relatively low wind velocity during the bloom period in 2003 may have influenced a disturbance in the oscillation (Fig. 4).

During the period of high abundance of *N. scintillans* in 1997 and 2000, the rainfall residuals were lower than average and average wind velocity was high (Fig. 7g). Moreover, the wind blowing from the south-southeast was frequently observed in 1997 and 2000. During the bloom period of *N.*

Table II:Summary of the abundance of Noctiluca scintillans and meteorological factors during the bloom period in Sagami Bay, Japan

Year	Abundance of *Noctiluca scintillans*		Meteorological factors during bloom period		
	Bloom period	Maximum ($\times 10^5$ cells m^{-3})	Rainfall (mm)	Wind velocity (m sec^{-1})	Wind direction
1997	May	3.7	137	1.5	SSE
1998	April	0.1	349	1.4	SW
1999	May	1.4	219	1.5	SW
2000	May	6.1	43	1.5	SSE
2001	May	1.9	243	1.2	SSE
2002	May	0.9	189	1.6	ESE
2003	April	1.4	176	1.5	SSE
2004	April	0.1	135	1.6	WSE

scintillans in 1997 and 2000, the winds from the south-southeast and calm surface layers thereafter without any ecological turbulence, such as heavy rain, may have forced the cells of *N. scintillans* to accumulate in the surface layer. The calm surface layer condition may be a key factor in forming and maintaining the high-density conditions of *N. scintillans*.

Noctiluca scintillans has been known to reproduce by binary fission and the formation of flagellate swarmers (Zingmark, 1970). Zingmark (1970) proposed that the swarmers result from meiosis in a gametocyte mother cell and that they represent isogametes which fuse. The resulting zygote was claimed to give rise directly to a vegetative cell, but this was not convincingly proven (Schnepf and Drebes, 1993). More recently, a new reproductive mechanism of *N. scintillans* was proposed suggesting that the vegetative cell of *N. scintillans* was programmed to become the swarmer-mother-cell after a defined number of binary fissions (Sato *et al.*, 1998). However, if the released swarmers from swarmer-mother-cells contact with and stimulate vegetative cells, the program is reset and the vegetative cells continue to conduct binary fissions. This suggests that an intensive multiplication of *N. scintillans* occurred in high-density conditions of the cells, as the encounter rate between vegetative cells and swarmers was high. In years such as 1997 and 2000 when accumulation occurred during the spring, the vegetative cells of *N. scintillans* may have continued to undergo binary fission, resulting in the formation of large-scale blooms of *N. scintillans*. During the spring period, the decrease in the cell volume of *N. scintillans* was observed concurrent with an increase in abundance (Fig. 5b), suggesting that *N. scintillans* may conduct binary fission actively during bloom formation.

Cell volume is also influenced by food availability and the cells swell further upon starvation (Tiselius and Kiørboe, 1998). When the cell volume is small (340–450 μm), it is in good nutritional status and high growth condition, while the large cell volume (400–1200 μm) defines poor condition (Murray and Suthers, 1999; Dela-Cruz *et al.*, 2003). When the cells are well fed, they tend to sink until digestion and defecation have taken place (Omori and Hamner, 1982; Tiselius and Kiørboe, 1998). In the present study, the variation in the cell volume corresponding to depth could not be observed because the net-samples including *N. scintillans* were collected by vertical towing from 50 m depths at St. M. However, net-samples obtained by vertical towing from 15 m depths to the surface at discrete depth intervals of 5 m at St. G showed that the abundance of *N. scintillans* was usually higher in the surface layer than near the bottom layer. The smaller cells of *N. scintillans* were distributed homogeneously near the surface to the 15 m layer during May (data not shown). During the spring at St. M, *N. scintillans* were also considered to be in good nutritional and high growth condition in the coastal waters of Sagami Bay.

Our results suggest that bloom formation can be separated into a three-step process: (i) initial increase in the abundance of *N. scintillans* attributed to an increase in optimum hydrographic and biological factors (temperature, salinity, water stability and chlorophyll *a*), (ii) *N. scintillans* are then accumulated by convergence of seawater by the factors of low rainfall and wind and (iii) swarmer-effects suggested by Sato *et al.* (Sato *et al.*, 1998) enhance bloom formation. Accumulation is considered to be a key trigger in this process of the formation of large-scale blooms. Previous studies concerning the bloom formation of *N. scintillans* have been focused on analyzing the individual factors controlling the variation in the abundance. Based on the findings of the present study, large-scale blooms occur once following the linkage and combination of environmental factors in the three steps of the bloom formation. Thus, the environmental factors regulating the population growth are different between the phases of the bloom formation process. The concept of bloom-phase formation mechanisms was applied to other blooms of *N. scintillans* observed in other areas.

Further studies are required to establish a quantitative method of the number of *N. scintillans* swarmers in the water column in order to estimate accurately the contribution of the swarmers to the bloom formation of *N. scintillans*. In addition, it is necessary to estimate the qualitative and quantitative influence of prey items and competition with zooplankton on the variation in the *N. scintillans* for understanding the ecological significance of the bloom formation in the costal ecosystem.

ACKNOWLEDGEMENTS

We thank Prof. S. Taguchi, Dr A. Shibata, Dr C. Vallet and Prof. H. Yamamoto, Soka University, for investing their time and effort in criticizing this article. We also appreciate Dr E. Nishi and Mr Y. Asakura at Manazuru Marine Laboratory for Science Education, Yokohama National University for their support in collecting samples. We are also indebted to Dr S. C. Y. Leong, Soka University, for valuable comments that improved the article. We also thank Mr N. Nagao, SEAWELL Co., Ltd for many helpful comments on this manuscript.

This research was partially funded by the University–Industry Joint Research Project for Private Universities: matching fund subsidy from MEXT (Ministry of Education, Culture Sports, Science and Technology), 2004–2008.

REFERENCES

Daan, R. (1987) Impact of egg predation by *Noctiluca miliaris* on the summer development of copepod populations in the southern North Sea. *Mar. Ecol. Prog. Ser.*, **37**, 9–17.

Dela-Cruz, J., Ajani, P., Lee, R., *et al.* (2002) Temporal abundance patterns of the red tide dinoflagellate *Noctiluca scintillans* along the southeast coast of Australia. *Mar. Ecol. Prog. Ser.,* **236**, 75–88.

Dela-Cruz, J., Ajani, P., Middleton, H. J. and Suthers, M. I. (2003) Population growth and transport of the red tide dinoflagellate *Noctiluca scintillans*, in the coastal waters off Sydney, Australia, using cell diameter as a tracer. *Limnol. Oceanogr.,* **48**, 656–674.

Elbrächter, M. and Qi, Z. Y. (1998) Aspect of *Noctiluca* (Dinophyceae) population dynamics. In Anderson, D. M., Cembella, A. D. and Hallegraeff, M. G. (eds), *Physiological Ecology of Harmful Algal Blooms*. *NATO ASI Series*, Vol. G 41. Springer-Verlag, Berlin, pp. 315–335.

Fujiki, T., Toda, T., Kikuchi, T. *et al.* (2003) Photoprotective response of xantophyll pigments during phytoplankton blooms in Sagami Bay, Japan. *J. Plankton Res.,* **25**, 317–322.

Garibotti, A. I., Vernet, M., Ferrario, E. M. *et al.* (2003) Phytoplankton spatial distribution patterns along the western Antarctic Peninsula (Southern Ocean). *Mar. Ecol. Prog. Ser.,* **261**, 21–39.

Godhe, A., Noren, F., Kuylenstierna, M. *et al.* (2001) Relationship between planktonic dinoflagellate abundance, cysts recovered in sediment traps and environmental factors in the Gullmar Fjord, Sweden. *J. Plankton Res.,* **23**, 923–938.

Hillebrand, H., Durselen, C. D., Kirschtel, D. *et al.* (1999) BioVolume calculation for pelagic and benthic microalgae. *J. Phycol.,* **35**, 403–424.

Hirota, J. and Szyper, J. P. (1975) Separation of total particulate carbon into inorganic and organic components. *Limnol. Oceanogr.,* **20**, 896–900.

Hogetsu, K. and Taga, N. (1977) Suruga Bay and Sagami Bay. In Hogetsu, K., Hatanaka, M., Hanaoka, T. and Kawamura, T. (eds), *JIBP Synthesis, Productivity of Biocenoses in Coastal Regions of Japan*, Vol. 14. University of Tokyo Press, Tokyo, pp. 31–172.

Huang, C. and Qi, Y. (1997) The abundance cycle and influence factors on red tide phenomena of *Noctiluca scintillans* (Dinophyceae) in Dapeng Bay, the South China Sea. *J. Plankton Res.,* **19**, 303–318.

Kiørboe, T. (1998) Intensive aggregate formation with low flux during an upwelling-induced diatom bloom. *Limnol. Oceanogr.,* **43**, 104–116.

Kirchner, M., Sahling, G., Uhlig, G. *et al.* (1996) Does the red tide-forming dinoflagellate *Noctiluca scintillans* feed on bacteria? *Sarsia,* **81**, 45–55.

Kuroda, K. (1978) The distribution and ecology of *Noctiluca scintillans*. Osaka Bay. *Bull. Jpn. Soc. Fish. Oceanogr.,* **32**, 56–67 (in Japanese).

Kuwahara, S. V., Ogawa, H., Toda, T. *et al.* (2000) Variability of bio-optical factors influencing the seasonal attenuation of ultraviolet radiation in temperate coastal waters of Japan. *Photochem. Photobiol.,* **72** (2), 193–199.

Mengesha, S., Dehairs, F., Fiala, M. *et al.* (1998) Seasonal variation of phytoplankton structure and nitrogen uptake regime in the Indian sector of the Southern Ocean. *Polar Biol.,* **20**, 259–272.

Miyaguchi, H., Fujiki, T., Kikuchi, T. *et al.* (2000) Abundance and size variability of *Noctiluca scintillans*. Manazuru port, Sagami Bay, Japan. *Proceedings of the 11th JSPS Joint Seminar on Marine Science,* pp. 180–189.

Morton, B. and Twentyman, R. P. (1971) The occurrence and toxicity of a red tide caused by *Noctiluca scintillans* (Macartney) Ehrenb., in the coastal waters of Hong Kong. *Environ. Res.,* **4**, 544–557.

Murray, S. and Suthers, M. (1999) Population ecology *Noctiluca scintillans* Macartney, a red-tide-forming dinoflagellate. *Aust. J. Mar. Freshw. Res.,* **50**, 243–252.

Nagao, N., Toda, T., Takahashi, K. *et al.* (2001) High ash content in net-plankton samples from shallow coastal water: possible source of error in dry weight measurement of zooplankton biomass. *J. Oceanogr.,* **57**, 105–107.

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

Nakata, N. (1985) Sagami Bay. In Oceanography Society of Japan, (edn). *Coastal Oceanography of Japanese Islands*. Tokai University Press, Tokyo, pp. 417 (in Japanese).

Omori, M. and Hamner, M. W. (1982) Patchy distribution of zooplankton: behavior, population assessment and sampling problems. *Mar. Biol.,* **72**, 193–200.

Painting, S. J., Lucas, I. M., Peterson, W. T. *et al.* (1993) Dynamics of bacterioplankton, phytoplankton and mesoplankton communities during the development of and upwelling plume in the southern Benguela. *Mar. Ecol. Prog. Ser.,* **100**, 35–53.

Pithakpol, S., Tada, K. and Montani, S. (2000) Nutrient regeneration during *Noctiluca scintillans* red tide in Harima Nada, the Seto Inland Sea, Japan. *Proceedings 15th Ocean Engineering Symposium, 20–21 January, 2000, the Society of Naval Architects of Japan,* pp. 127–134.

Quevedo, M., Gonzales-Quiros, R. and Anadon, R. (1999) Evidence of heavy predation by *Noctiluca scintillans* on *Acartia clausi* (Copepoda) eggs of the central Cantabrian coast. *Oceanol. Acta.,* **22**, 127–131.

Sato, S. M., Suzuki, M. and Hayashi, H. (1998) The density of homogeneous population of cells controls resetting of the program for swarmer formation in the unicellular marine microorganism *Noctiluca scintillans*. *Exp. Cell Res.,* **245**, 290–293.

Satoh, F., Hamasaki, K., Toda, T. *et al.* (2000) Summer phytoplankton bloom in Manazuru Harbor, Sagami Bay, central Japan. *Plank. Biol. Ecol.,* **47**, 73–79.

Schaumann, K., Gerdes, D. and Hesse, K. J. (1988) Hydrographic and biological characteristics of a *Noctiluca scintillans* red tide in the German Bight. *Meeresforschung,* **32**, 77–91.

Schnepf, E. and Drebes, G. (1993) Anisogamy in the dinoflagellate *Noctiluca*? *Helgo. Meeresunter.,* **47**, 265–273.

Shimode, S., Toda, T. and Kikuchi, T. (2005) Spatio-temporal changes in diversity and community structure of planktonic copepods in Sagami Bay, Japan. *Mar. Biol.,* **148**, 581–597.

Smayda, J. T. (1997) What is a bloom? A commentary. *Limnol. Oceanogr.,* **42**, 1132–1136.

Strom, S. L. (2001) Light-aided digestion, grazing and growth in herbivorous protists. *Aquat. Microb. Ecol.,* **23**, 253–261.

Suzuki, R. and Takahashi, I. (1990) An improved method for the determination of phytoplankton chlorophyll using N, N-Dimethylformamide. *J. Oceanogr. Soc. Jpn.,* **46**, 190–194.

Tada, K., Pithakpol, S. and Montani, S. (2004) Seasonal variation in the abundance of *Noctiluca scintillans* in the Seto Inland Sea, Japan. *Plank. Biol. Ecol.,* **51**, 7–14.

Tiselius, P. and Kiørboe, T. (1998) Colonization of diatom aggregates by the dinoflagellate *Noctiluca scintillans*. *Limnol. Oceanogr.,* **43**, 154–159.

Uhlig, G. and Sahling, G. (1990) Long-term studies on *Noctiluca scintillans*. German Bight population dynamics and red tide phenomena 1968–88. *Neth. J. Sea Res.,* **25**, 101–112.

Yin, K. (2003) Influence of monsoons and oceanographic processes on red tides in Hong Kong waters. *Mar. Ecol. Prog. Ser.,* **262**, 27–41.

Zingmark, R. G. (1970) Sexual reproduction in the dinoflagellate *Noctiluca miliaris* Suriray. *J. Phycol.,* **6**, 122–126.