Seasonal variation in the abundance of *Noctiluca scintillans* in the Seto Inland Sea, Japan

Kuninao Tada[1], Santiwat Pithakpol[1] & Shigeru Montani[2]

[1]*Department of Life Sciences, Kagawa University, Miki, Kagawa 761 0795, Japan*
[2]*Graduate School of Fisheries Sciences, Hokkaido University, Minato, Hakodate, Hokkaido 048–8611, Japan*

Abstract: The seasonal variation in abundance of *Noctiluca scintillans* was investigated in the temperate coastal sea, Harima Nada, the Seto Inland Sea, Japan for six years. Cell densities >100 cells l^{-1} were often observed during late spring to early summer in the surface layer. Moreover, the growth rates of *N. scintillans* fed *Ditylum brightwellii* were measured in the laboratory under different temperature regimes from 12 to 28°C. *N. scintillans* exhibited a maximum growth rate at 21°C and could not grow in temperatures >27°C. Based on these results, it is considered that the increase in water temperature during the spring period, enhanced the growth rate of *N. scintillans* and led to the high abundances observed in early summer. The subsequent stability of the water column in late spring to early summer also appeared to give rise to an increase in the cell density of *N. scintillans* in the surface layer. In addition, the low abundance of *N. scintillans* in summer, may have been an effect of the low growth rate of *N. scintillans* due to high water temperatures >27°C. The carbon biomass of *N. scintillans* was estimated using the average carbon content of the field samples (0.353 μg C cell^{-1}) and ranged up to about 5 gC m^{-2}. Our estimates suggest that the biomass of *N. scintillans* is not trivial as *N. scintillans* occupied a substatial part of the biomass in heterotrophic populations, even when a red tide due to an *N. scintillans* outbreak is not observed.

Key words: *Noctiluca*, abundance, seasonal variation, Seto Inland Sea, Harima-Nada

Introduction

Noctiluca scintillans is a large heterotrophic dinoflagellate that has a world-wide distribution (e.g. Elbrächter & Qi 1998) and is also well known for its luminescence (e.g. Nicol 1958). *Noctiluca* is also one of the most common red-tide-forming dinoflagellates in temperate and tropical coastal regions and is a frequent cause of water discoloration. *N. scintillans* does not have chloroplasts and hence derives its nutrition by ingesting small particles such as phytoplankton and detritus. The morphology and life cycle of this species has already been reported (e.g. Zingmark 1970; Sweeny 1976). *N. scintillans* has been reported to feed on the eggs of copepods and anchovy (Hattori 1962; Sekiguchi & Kato 1976; Kimor 1979; Nawata & Sibaoka 1983). Okaichi & Nishio (1976) studied its toxicity and reported that *N. scintillans* has very high intracellular ammonium concentrations. Moreover, Montani et al. (1998) reported high concentrations of nutrients inside *N. scintillans*

cells and they examined its fecal pellets. Pithakpol et al. (2000a) also studied ammonium and phosphate intracellular pools in *N. scintillans* and the potential supply of these nutrients to the water column when the cells die. However, little is known about its biomass and its ecological role. To date, there has been little interest in *Noctiluca* , probably because a *Noctiluca* red tide does not kill fish. However, the biomass of *N. scintillans* in temperate coastal regions should not be ignored. Recently, the growth rate of *N. scintillans* was investigated and specific growth rates from 0.07 to 0.46 day^{-1} were reported (Buskey 1995; Nakamura 1998a).

Some field observations of *N. scintillans* have been conducted and long term or short term variations in *N. scintillans* and phytoplankton abundances, as well as the cell diameter of *N. scintillans* have been reported (e.g. Le Fevre & Grall 1970; Schaumann et al. 1988; Uhling & Sahling 1990; Huang & Qi 1997; Murray & Suthers 1999). But few studies have been conducted on its ecology and its role in coastal ecosystems. In the Seto Inland Sea, Japan, *N. scintillans* occurs frequently as a red tide in spring and summer.

Corresponding author: Kuninao Tada; e-mail : tada@ag.kagawa-u.ac.jp

and it is present during most of the year. In phytoplankton ecology, it is important to report the biomass of large cells in terms of carbon as well as the abundance of cells. Recently, Nakamura (1998b) reported the biomass, feeding and production of *N. scintillans* during summer (about one month) in close vicinity to our sampling station and suggested that *N. scintillans* was an important member of the mesozooplankton in terms of biomass and production in the Seto Inland Sea during summer. This paper reports on the seasonal variations of *N. scintillans* biomass in terms of carbon and cell numbers in the Harima-Nada, the Seto Inland Sea, Japan for 6 years. We suggest that the biomass of *N. scintillans* can not be ignored. Namely, *N. scintillans* occupied a substantial part of the biomass of the heterotrophic plankton population, even when a red tide outbreak is not occurring.

Materials and Methods

Field observations and processing of water samples

Field observations were conducted monthly for about 6 years (May, 1992–December, 1997) at Stn. NH (34°28'N, 134°24'W; water depth about 35 m) in Harima-Nada, located in the eastern part of the Seto Inland Sea, using the R/V *Calanus*, Kagawa University (Fig. 1). Vertical profiles of temperature and salinity were measured by a CTD (Alec Model AST-1000) and sigma-t (σ_t) was calculated from these values. Seawater samples were collected from 5 to 6 depths (0, 5, 10, 20 and 30 m depth and 2 m above the bottom), using a 10-l Van Dorn bottle. For *N. scintillans* samples, 1000 ml of seawater from each depth was concentrated by gently sieving through a 72 μm mesh to a final volume of ca. 100 ml and the concentrated samples were fixed with formalin to a final concentration of 3% by volume. *N. scintillans* cells were counted using a magnifying glass. Water samples for chlorophyll a (Chl a) concentration determination were immediately filtered through a Whatman GF/F fil-
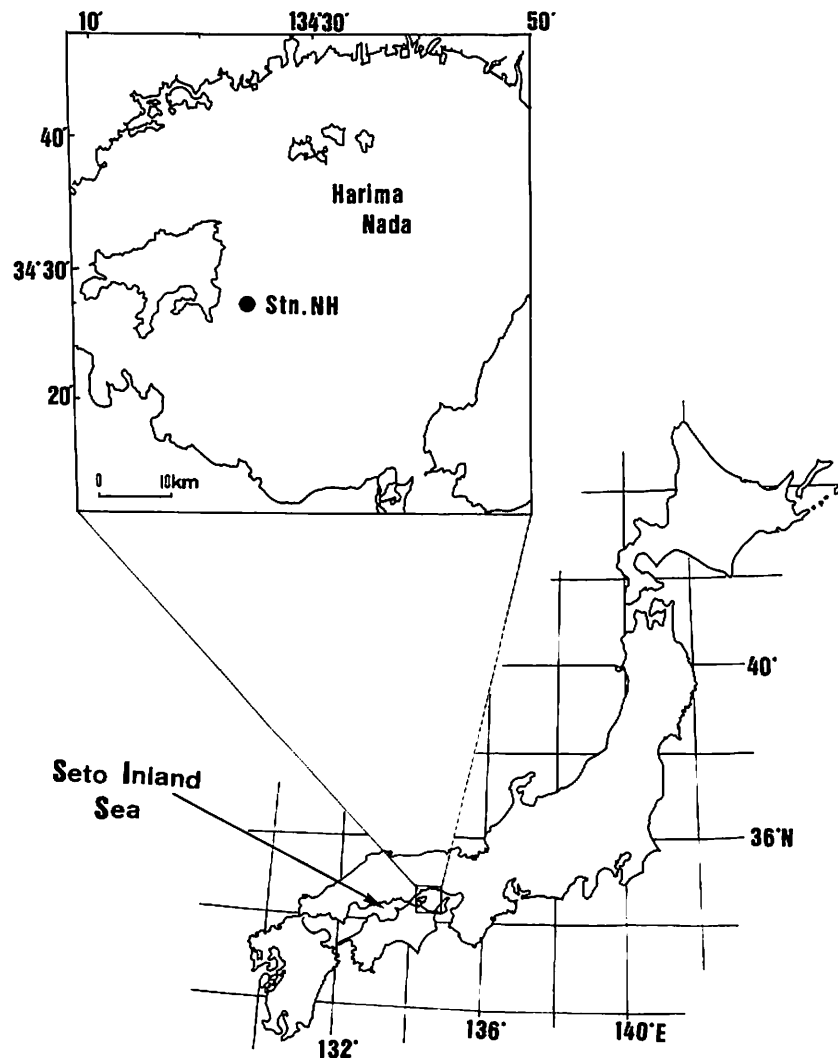

Fig. 1.   The sampling station (NH) in the Harima Nada area, Seto Inland Sea, Japan.

ter and the filters were preserved in N,N-dimethylfor-mamide (DMF) until analysis at −20°C (Suzuki & Ishimaru 1990). Chl *a* concentrations were determined by Lorenzen's (1967) spectrophotometric method (Parsons et al. 1984).

Measurement of the growth rate of *N. scintillans*

We measured the growth rate of *N. scintillans* in the laboratory under different temperatures, using the strain isolated from our field station. At first, *N. scintillans* cells were kept in filtered seawater (0.2 μm Nuclepore filtrate) in a temperature controlled room (21°C) which was illuminated at $100 \mu E m^{-2} s^{-1}$ under a 14L : 10D cycle and the diatom *Ditylum brightwellii* was added as a food source. For this experiment, six temperature regimes were established (12, 16, 21, 25, 27 and 28°C). Prior to the experiment, cells were acclimatized by increasing or decreasing the temperature at a rate of 1°C per day relative to the experimental temperatures. Fifty cells of *N. scintillans* were transferred using a pipette into 50 ml-capacity screw cap test tubes containing about 40 ml of filtered seawater (0.2 μm Nuclepore filtrate) and *Ditylum brightwellii* was added as a food source to the desired cell densities (about 2.7× 10^3 cells ml^{-1}) and, subsequently, the tubes were filled with filtered seawater leaving only a small volume of air in the tube. Initial algal densities were measured by in vivo fluorescence using a Turner Designs fluorometer (Model 10 AU) previously calibrated for these cell densities. Three experimental replicate tubes were attached to a tube rotator (Iuchi Co., Japan TR-118) and rotated at 1 rpm in order to keep the cells in suspension and to create a homogeneous food distribution for *N. scintillans*. These tubes were incubated for 3 days at six different temperature regimes. Controls, which contained either *N. scintillans* cells or algae alone, were also prepared. Time-course measurements of in vivo fluorescence in the vessels were made at 24 hour intervals. *N. scintillans* cells were counted daily and returned to the experimental tubes using a Pasture pipette. The growth rate of *N. scintillans* was estimated from the equation:

$$\mu = \ln(N_t / N_0)/t$$

where μ is the specific growth rate (d^{-1}), N_t represents the final cell number at the end of the incubation at time t (day) and N_0 represents the initial cell number (50 cells). However, due to the limited number of temperature controllable incubators, the experiments were conducted in pairs of temperatures (21°C and another). The growth rate of *N. scintillans* is presented as the relative growth rate at a range of temperatures compared to the growth rate at 21°C. Details of the experimental method are also reported in another paper (Pithakpol 2000; Pithakpol et al. 2000b).

Results

Temporal variations of *N. scintillans* abundance

Although it is well known that seawater looks like tomato juice if there are >10,000 cells l^{-1} of *N. scintillans*, we did not encounter any red tides of *N. scintillans* at Stn NH. However, *N. scintillans* was observed during most of the year. The highest cell density (4050 cells l^{-1}) was found at 0 m on 25 May 1995. *N. scintillans* cell density was >100 cells l^{-1} during late spring to early summer in the surface layer (upper 10 m) in every year. Generally, high cell densities of *N. scintillans* (>100 cells l^{-1}) were observed in warm water periods, although the cell density of *N. scintillans* decreased to <100 cells l^{-1} in August and September when water temperature reached its maximum, exception the case of 1993 (Fig. 2). High cell densities were observed above 20 m depth from March to August in almost every year, although strong stratification of the water column was not found during this period. In 1993, high cell densities were also observed in September and October. On the other hand, low cell abundances were observed during October to the following February in 1993 (Fig. 2). During these observation periods, water temperature varied widely from 8.78 to 28.68°C, although the salinity ranged narrowly from 29.05 to 33.68 in the surface waters. Cell densities greater than 100 cells l^{-1} were usually observed when temperatures ranged from 10 to 25°C, and the salinity from 30 to 33 (Fig. 3).

Growth of *N. scintillans*

During the experiments measuring the growth rate of *N. scintillans*, the abundance of *Ditylum brightwellii* was continuously monitored. It has been reported that growth in *N. scintillans* is correlated with the concentration of prey items (e.g. Buskey 1995; Kiorboe & Titelman 1998; Nakamura 1998a), thus we routinely applied saturated prey concentrations for *N. scintillans* in the experiments.

The growth rate of *N. scintillans* varied from −0.22 to 0.65 d^{-1} when temperatures ranged between 12 and 28°C (Table 1). The growth rate of *N. scintillans* is presented as the relative growth rate at a range of temperatures compared to the growth rate at 21°C (Fig. 4). The relative growth rate increased with the incubation temperature from 12 to 21°C and maximum growth was obtained at 21°C. At temperatures >21°C, the growth rate decreased with increasing temperature. Almost no growth was observed at 27°C and a negative growth rate was obtained at 28°C.

Discussion

Seasonal variation of abundance in *N. scintillans*

The annual average of cell abundances of *N. scintillans* in the surface layer (0–10 m) and of delta sigma-*t* for each month for the 6 year period were examined (Fig.5).: The
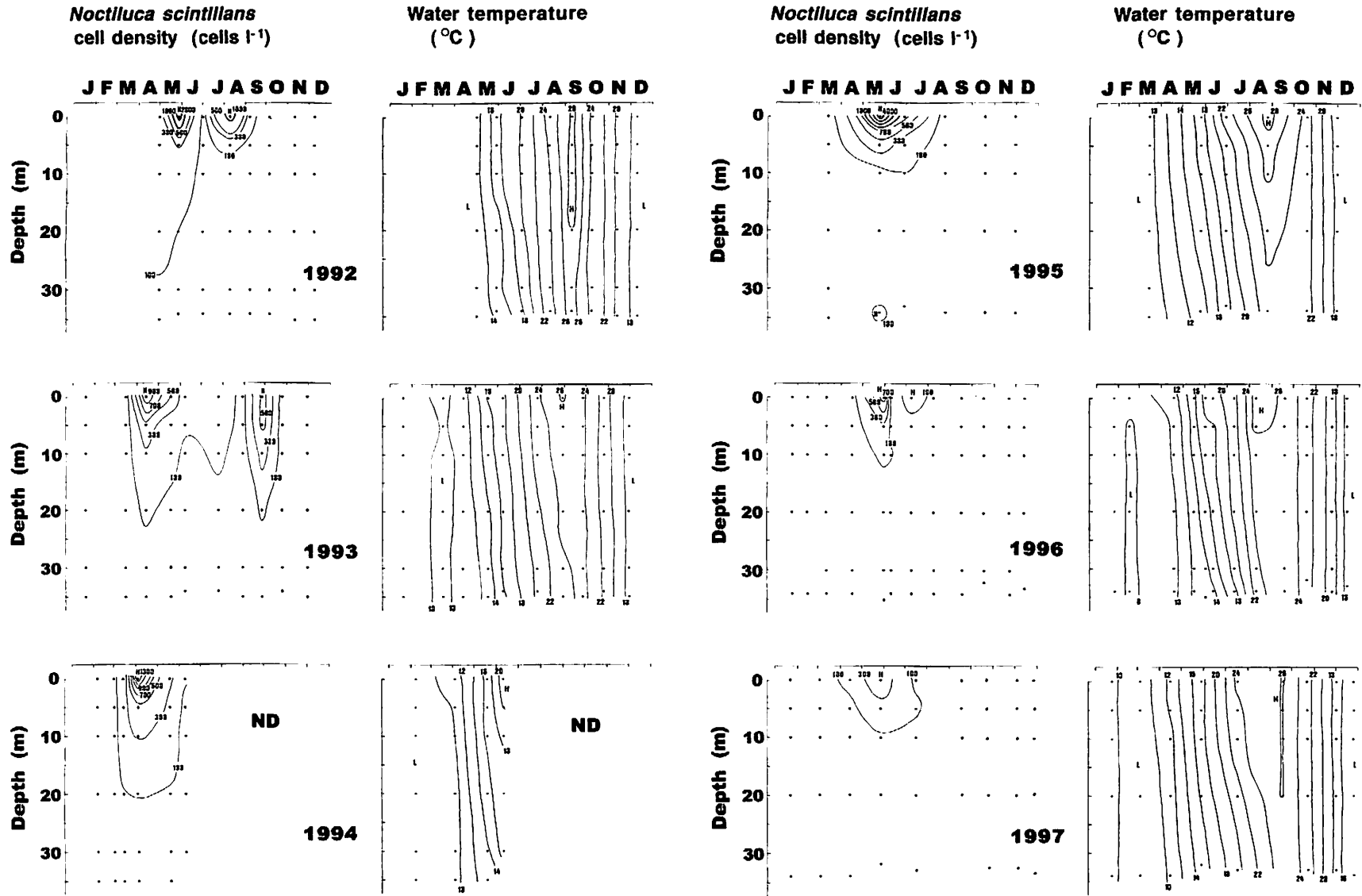
Fig. 2. Temporal variations in cell densities of *Noctiluca scintillans* and water column temperatures at Stn. NH, during 1992–1997 (ND=no data; H=high; L=low).
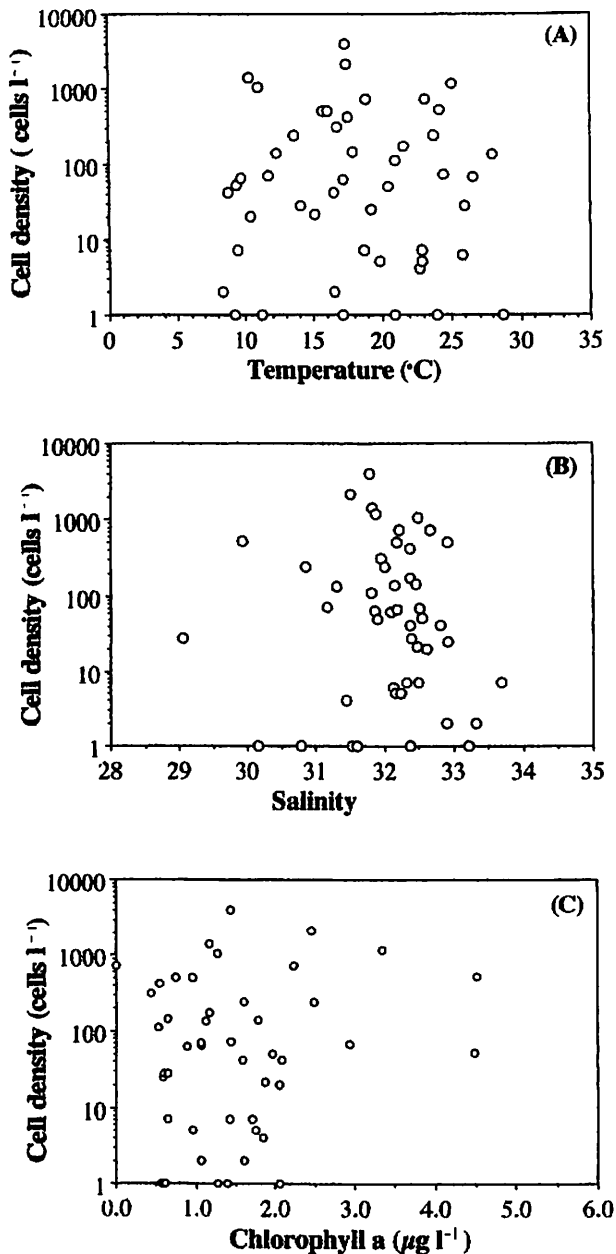
Fig. 3. The relationship between cell density of *Noctiluca scintillans* and surface water temperature (A), cell density of *N.scintillans* and salinity (B), and cell density of *N. scintillans* and chlorophyll *a* concentration (C) over 6-year period (1992–1997).

Table 1 The specific growth rates (d⁻¹) and ±1 standard deviation for *Noctiluca scintillans* fed on the diatom *Ditylum brightwellii* and grown at five different temperatures after 72 hours of incubation (n=3–4).

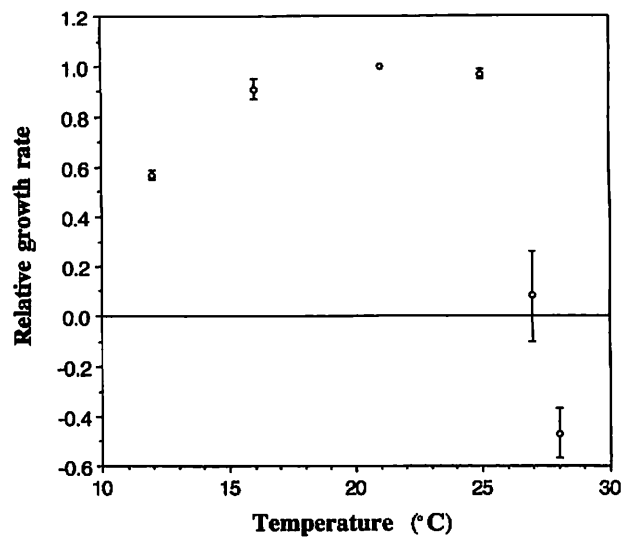| Growth rate at control temperature, 21°C (d⁻¹)	Growth rate at treatment temperature (°C) (d⁻¹)
0.61±0.01	0.35±0.01 (12°C)
0.75±0.04	0.65±0.02 (16°C)
0.67±0.06	0.63±0.03 (25°C)
0.63±0.03	0.15±0.04 (27°C)
0.51±0.01	−0.22±0.05 (28°C)

Fig. 4. Temperature dependent relative growth rate of *Noctiluca scintillans*. The growth rates were normalized from the pairs of experimental temperatures, assuming the growth rate obtained in the control (21°C) was 1 d⁻¹. Error bars show ±1 standard deviation (n=3–4).

delta sigma-*t* is defined as the difference in sigma-*t* between the surface and near the bottom (2 m above the bottom) and is considered to reflect the stability of the water column. The delta sigma-*t* increased with increases in surface water temperature and reached a maximum in July. Cell abundances in the surface layer increased together with the increase in delta sigma-*t* until May. It is generally considered that an increase in the stability of the water column causes *N. scintillans* cells to bloom in the surface layer. The abundance of *N. scintillans* reached a maximum in May and was low during the summer period despite the high delta sigma-

t. On the other hand, the average phytoplankton abundance in the surface layer (0–10 m) was high in March, subsequently decreased, and increased again from June to August. After August the phytoplankton abundance decreased with the decrease in surface temperature. In spite of the high water temperatures, high stability of the water column and high phytoplankton abundance in the summer period, the abundance of *N. scintillans* was high in only April and May and was low during the summer period. We believe that salinity values of the surface water did not influence the biomass of *N. scintillans*, because they varied within a narrow range (29.5 to 33.68). We suggest that the low abundances of *N. scintillans* observed during the summer period was probably due to high water temperatures since the growth rate of *N. scintillans* drastically decreased over 25°C (Fig. 4). It has also been reported that the temperature range of the phagotrophic *N. scintillans* is restricted to water temperatures <25°C (Elbrächter & Qi 1998). Based
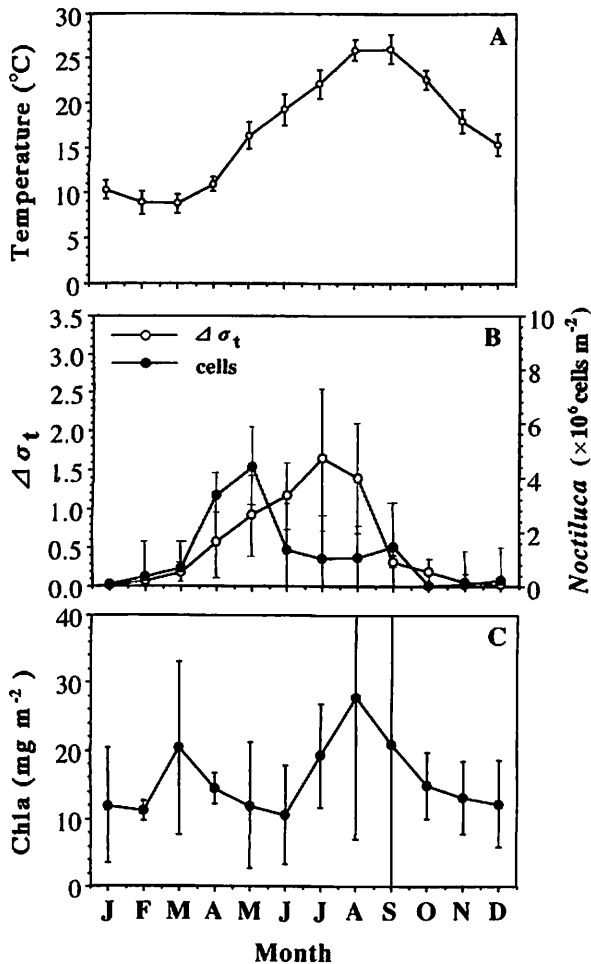
Fig. 5. Seasonal variations of average temperature (a), cell abundace of *Noctiluca scintillans* and delta Sigma-t (σ_t) at Stn. NH (b), standing stock of Chl a in the surface layer (0–10 m) (c). The delta σ_t is the disparity between the value of σ at 0 and 2 m above the bottom. These values are the monthly averages for 6 years (1992–1997). The error bars show ± 1 standard deviation ($n=6$).

on our measurment of the growth rate of *N. scintillans*, maximum growth occurs at 21°C and it is unable to grow at water temperatures >27°C. Our results for the temperature dependence of the growth rate of *N. scintillans* are in good agreement with those in previous studies (e.g. Lee & Hirayama 1992; Uhling & Sahling 1995). Our absolute growth rates for *N. scintillans* (-0.22–0.65 d^{-1}) were similar to those found in previous studies (-0.06–0.35 d^{-1}, Nakamura 1998a; 0–0.52 d^{-1}, Buskey 1995; 0–0.81 d^{-1}, Lee & Hirayama 1992).

Of course, the abundance of *N. scintillans* is not dependent only on water temperature. In our study area, the zooplankton community is dominated by copepods (Uye & Shimazu 1997). Nutritional competition between *N. scintillans* and copepods may be another explanation to explain the changes in the abundance of *N. scintillans*. Although we have no quantitative data on the biomass of copepods in our

study area, we have the impression that copepods influenced the abundance of *N. scintillans*. We usually observed few copepods in the samples from the vertical plankton net tows at Stn. NH, but we observed large numbers of copepods when *N. scintillans* abundance was low. Le Fevre & Grall (1970) also reported that the minimum abundance of copepods corresponds to the maximum abundance of *N. scintillans* and, inversely, the maximum abundance of copepods was found in an area where *N. scintillans* was least abundant.

Estimations of the biomass of *N. scintillans*

We estimated the biomass of *N. scintillans* and phytoplankton in the water column at Stn. NH (Fig. 6). The biomass of *N. scintillans* was estimated, using the average value for the cellular carbon content of field samples (0.353 μg C cell^{-1}) that was obtained from the samples collected by vertical plankton tows at Stn. NH (Tada et al. 2000). On the other hand, phytoplankton carbon was estimated from the standing stocks of Chl a, using the C/Chl a ratio of 56.5 reported for this region (Tada & Morishita 1997). The water column standing stocks ranged from 0 to 4.91 g C m^{-2} for *N. scintillans* and from 0.73 to 8.73 g C m^{-2} for phytoplankton each month. The standing stock of *N. scintillans* sometimes exceeded that of phytoplankton when the *N. scintillans* biomass was high. Moreover, *N. scintillans* abundance tended to become high when phytoplankton abundances decreased (Fig. 6). Such trends have also been observed in Dapeng Bay, South China Sea (Huang & Qi 1997). This suggests that *N. scintillans* may exert predation pressure on the phytoplankton community during periods when there is a large population of *N. scintillans* even when the biomass is not high enough to form a red tide. *N. scintillans* abundance is probably not to affected by grazing pressure from its predators, because it is known that few organisms feed on *N. scintillans* actively.

It is well known that physical forcing such as that by currents is important in order to accumulate large numbers of *N. scintillans* cells, although visible blooms are a result of the coupling of physical and biological processes (Elbrächter & Qi 1998). As described above, we did not encounter a red tide of *N. scintillans* during our observation period and high cell densities of *N. scintillans* (>100 cells l^{-1}) were observed regularly during late spring to early summer in the surface waters in every year. We believe that the variation in *N. scintillans* abundance, as seen in Fig. 2, mainly occurred due to biological processes, except for the extremely high cell densities in the microsurface layer (i.e. 0 m on 25 May 1995). Based on our carbon biomass estimate, the biomass of *N. scintillans* can not be ignored, even when the outbreak of a red tide is not occurring. Considering the estimated biomasses of phytoplankton and *N. scintillans*, and the growth rate of *N. scintillans* obtained in this study, our estimates also suggest that the biomass of *N. scintillans* can not be supported only by phyto-
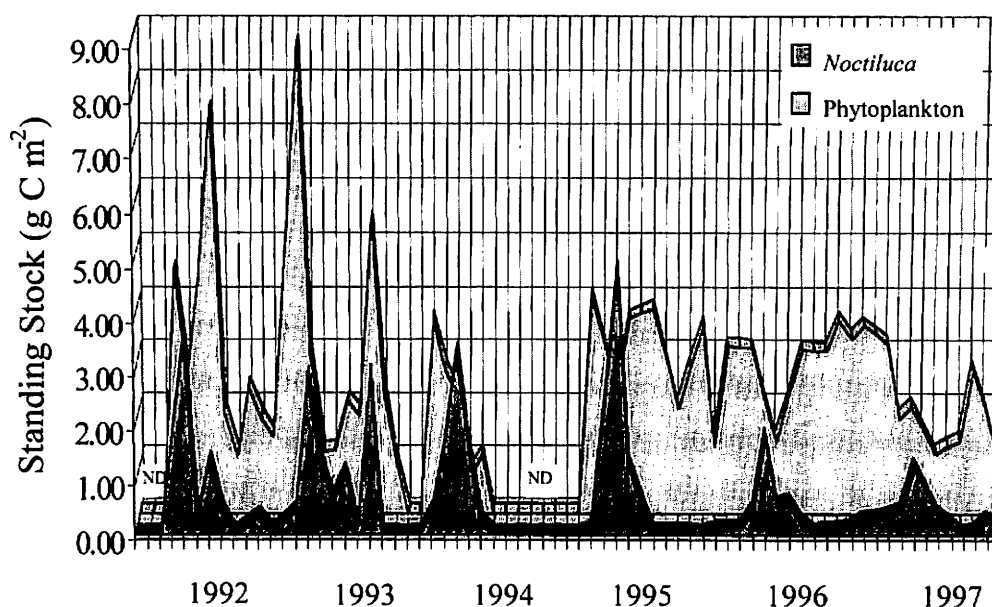
Fig. 6. Seasonal variations in the biomass of *Noctiluca scintillans* and phytoplankton at Stn. NH. The biomass of *N. scintillans* was calculated from the cell number, and assuming a carbon content of $0.338\,\mu g\,C\,cell^{-1}$ (Tada et al. 2000). The carbon biomass of phytoplankton was calculated from the standing stock of Chl a, and assuming a C/Chl a ratio of 56.5 (Tada and Morishita 1997).

plankton. Therefore we propose a hypothesis that *N. scintillans* also feeds on detritus and/or microzooplankton. It is known that *N. scintillans* is a voracious predator; it is not a selective feeder and instead it ingests all particles that it can engulf (e.g. Kimor 1979). However, the growth rate of *N. scintillans* varies widely depending on the species composition of its phytoplankton prey (Tada et al. unpublished data). Although few data are available on how *N. scintillans* fits into the food web (Elbrächter & Qi 1998), we believe that in a temperate coastal environment such as the Seto Inland Sea, *N. scintillans* plays an important role as a grazer. Further research is needed to assess the feeding rate of *N. scintillans* and to evaluate its role as a grazer in coastal ecosystems.

Acknowledgements

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