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Does *Noctiluca scintillans* end the diatom bloom in coastal water?

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ABSTRACT

Weekly field observations were conducted in Harima-Nada in the eastern part of the Seto Inland Sea, Japan from 21 August to 26 October 2015 to investigate the role of heterotrophic dinoflagellate *Noctiluca scintillans* on a coastal ecosystem. We observed a rapid *N. scintillans* growth just after increasing Chl. *a*, of which the dominant species were *Chaetoceros* spp., *Thalassionema* spp., *Thalassiothrix* spp., *Coscinodiscus* spp., *Thalassiosira* spp., and *Stephanopyxis* spp. We also observed under a microscope that *N. scintillans* cells grazed on these species of diatoms. The biomass of *N. scintillans* was estimated to increase from 4.50 to 470 $\mathrm{mgC\,m^{-2}}$ in 7 days. On the other hand, phytoplankton biomass drastically decreased from 2110 to 725 $\mathrm{mgC\,m^{-2}}$. The increase in *N. scintillans* biomass could account for 33.6% of the decrease in phytoplankton biomass. If we assume all the decrease in phytoplankton biomass was due to the *N. scintillans* grazing of diatoms, the 33.6% indicates the apparent gross growth efficiency of *N. scintillans* under a sufficient source of feed. Our results indicated the active feeding of *N. scintillans* on diatoms ends a diatom bloom in coastal water.

1. Introduction

Noctiluca scintillans Macartney (Kofoid and Swezy, 1921) is a heterotrophic dinoflagellate widely distributed in temperate and subtropical coastal regions (Maclean, 1989; Elbrächter and Qi, 1998; Harrison et al., 2011). Red *N. scintillans* often forms red tides in coastal water from spring to summer, and discolors the sea surface water in Japan (Fukuyo et al., 2004; Miyaguchi et al., 2006). Fish are rarely killed due to red *N. scintillans*. Aiyer (1936) reported fish mortality of the Madras coast, and also Okaichi and Nishio (1976) reported that fish were sometimes killed in the Seto Inland Sea, and the toxic substance was identified as ammonia. Also, Pithakpol et al. (2000) estimated that the NH_4-N in *N. scintillans* contributed to the Seto Inland Sea water column NH_4-N ranging from 0 to 119% in the middle of spring when *N. scintillans* red tide was observable. They concluded that the nutrient regeneration by *N. scintillans* should not be ignored, and the temporary enhancement of nutrients at sea surface by *N. scintillans* effectively supports the growth of autotrophs and results in successive phytoplankton blooms. Tada et al. (2004) estimated that the carbon biomass of *N. scintillans* ranged up to about 5 $\mathrm{gC\,m^{-2}}$, and the standing stock of red *N. scintillans* sometimes exceeded that of phytoplankton when the *N. scintillans* biomass was high in the Harima-Nada in the Seto Inland Sea, same study area of this study. Therefore, it is thought that *N. scintillans* plays a significant role in the marine ecosystem because of its high biomass, even if it does not form a red tide (Tada et al., 2004). Further, Nakamura (1998) estimated the production of *N. scintillans* in the Seto Inland Sea, and it was similar to that of copepods in the warm season.

N. scintillans has little prey selection and can feed on bacteria, phytoplankton, protozoans, copepods, copepod eggs, and fish eggs (Hattori, 1962; Takayama, 1977; Nawata and Sibaoka, 1983; Kirchner et al., 1996). It was reported that *N. scintillans* occurs after a diatom bloom in field observations (Kiørboe and Titelman, 1998; Weston et al., 2008), and an abundance of *N. scintillans* correlates with chlorophyll *a* (Chl. *a*) (Fonda-Umani et al., 1983). In the microcosm incubation, the growth rate of *N. scintillans* correlates with Chl. *a*, which is dominated by diatoms (Zhang et al., 2017). Thus, the feeding pressure of *N. scintillans* has possibilities of controlling the dynamics of diatoms. However, there are few approaches to estimate their grazing rate in the field (Nakamura, 1998; Zhang et al., 2017).

In this study, we observed a rapid increase in red *N. scintillans* just after a diatom bloom in the Seto Inland Sea, and we tried to evaluate its

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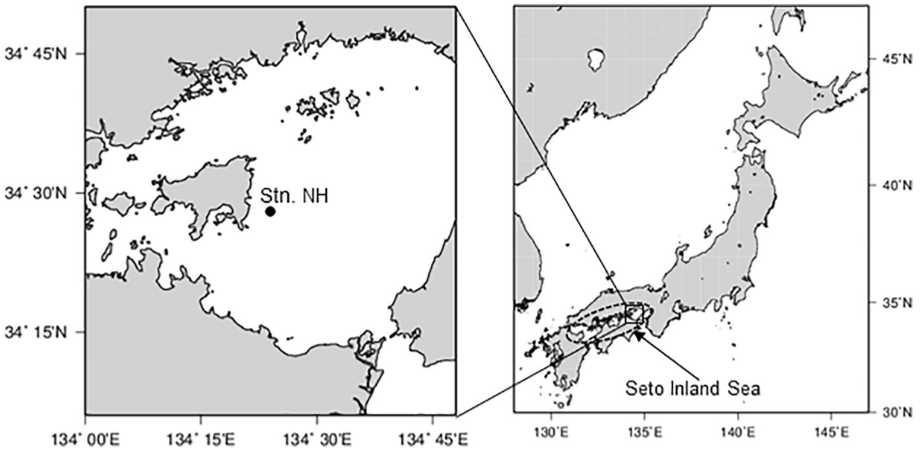

Fig. 1. Sampling station, Stn. NH, Harima-Nada, Seto Inland Sea.

grazing pressure, and also we investigated the role of *N. scintillans* on a coastal ecosystem.

2. Materials and methods

2.1. Field observations

Weekly field observations were conducted from 21 August to 26 October 2015 at Stn. NH (34°28′N, 134°24′E; water depth, about 35 m) in Harima-Nada in the eastern part of the Seto Inland Sea using the R/V *Calanus III* and *Nauplius II*, Kagawa University (Fig. 1). Vertical profiles of temperature and salinity were measured using a CTD (Model AST-1000; Alec Electronics Co. Ltd.), and sigma-*t* was calculated from these values. Seawater samples were collected at depths 0, 5, 10, 20, and 30 m using a 10-L Van Dorn bottle. Water samples for Chl *a* analysis were immediately filtered through a Whatman GF/F filter, and the filters were extracted in 90% acetone and kept in the dark for 12–24 h at 4 °C in a refrigerator until analysis. Chl *a* concentrations were measured following the method of Holm-Hansen et al. (1965), as described in Parsons et al. (1984), using a Turner Designs fluorometer (10 AU, Turner designs Inc.). And the dominant species of diatom bloom was observed and identified under the microscope.

The zooplankton were collected by vertical hauls from a depth of 10 m to the surface using a Kitahara net (0.24 m mouth diameter, 330 μm mesh), because it was reported *N. scintillans* was observed with high density upper 10 m depth (Tada et al., 2004). Unfortunately, we did not use a flow meter. After collection, the zooplankton were immediately preserved in a 10% formalin-seawater solution buffered with borax and brought back to the laboratory. Species composition of zooplankton were identified and counted taking 2 mL of 60–150 mL samples for 3 times under a dissecting microscope (Wild M32, Leica). For the *N. scintillans* collected on 8 and 15 October, food vacuoles were observed and food items were identified under a microscope (×40).

2.2. Estimation of the biomass of *N. scintillans* and phytoplankton

We estimated the biomass of *N. scintillans* and phytoplankton upper 10 m depth at Stn. NH (Fig. 1). The biomass of *N. scintillans* was calculated from cell number and cellular carbon content (0.353 μgC cell^{-1}), which was average value of the monthly data for one year, reported by Tada et al. (2000) at Stn. NH, the same station in this study. The carbon biomass of phytoplankton was estimated from the standing stocks of Chl *a*, assuming a C/Chl *a* ratio of 56.5 reported from this region (Tada and Morishita, 1997).

3. Results and discussion

3.1. Temporal variations of environmental factors, Chl. *a*, *N. scintillans* and zooplankton

During the observation period, the temperature gradually decreased from 27.2 to 22.3 °C, and salinity was relatively stable, ranging from 30.4 to 31.7 (Fig. 2). Judging from the sigma-*t* distribution (Fig. 2), vertical mixing occurred in the latter half of the observation period. Average Chl. *a* concentration was 1.4 μg L^{-1}. From 21 August to 1 October, Chl. *a* concentration was ranging from 0.2 to 2.4 μg L^{-1}. Chl. *a* concentration increased rapidly to higher than 3 μg L^{-1} (max; 5.964 μg L^{-1}) from a depth of 10 m to the surface during the period from 6 to 8 October. During this increase, dominant diatoms were *Chaetoceros* spp., *Thalassionema* spp., *Thalassiothrix* spp., *Coscinodiscus* spp., *Thalassiosira* spp., and *Stephanopyxis* spp. (We will report on the diatom bloom in detail elsewhere). Subsequently, Chl. *a* decreased drastically by 13 October (Fig. 3). Up until 1 October, the density of *N. scintillans* was low at < 295 cells tow^{-1} but suddenly increased to 62,900 cells tow^{-1} on 15 October (Fig. 3).

Total zooplankton individuals ranged from 243 to 2200 inds. tow^{-1} from 21 August to 8 October (Fig. 4). Cladoceans accounted for 59.5% of the total zooplankton on 21 August (Fig. 4). Copepods were dominant from 3 September to 1 October, accounting for 47.8 to 93.2% (Fig. 4). After 8 October, total zooplankton individuals rapidly increased, reaching 64,178 inds. tow^{-1} on 15 October (Fig. 4). This increase was caused by *N. scintillans*, accounting for 98.1% on 15 October (Fig. 4). After that, total zooplankton individuals declined to 2181 inds. tow^{-1} by 26 October. *N. scintillans* also decreased (Fig. 4). It was reported that *N. scintillans* was predated by large Copepoda (*Calanus helgolandicus*) (Petipa, 1960). However, we observed that Copepoda abundance was lower than *N. scintillans*, by two orders of magnitude in 15 October, when *N. scintillans* was high density.

In this study, the *in situ* temperature was under 25 °C after 17 September (Fig. 2), which is suitable for *N. scintillans* growth. Tada et al. (2004) reported that *N. scintillans* can grow well in temperatures from 16 to 25 °C. In this study period, a diatom blooms occurred and subsequently quick *N. scintillans* growth was observed. We believed that *N. scintillans* could grow by feeding on the diatoms in the bloom, and we found at least four species of diatoms which consisted of the bloom in the food vacuoles of the *N. scintillans* (Fig. 5). Actually, we microscopically observed 8–103 cells of *N. scintillans* from each samples on 8 and 15 October, and 75.0–84.3% of *N. scintillans* contained these diatom species in the food vacuoles.