Annual regularity of reduction and restoration of cell size in the harmful diatom *Eucampia zodiacus*, and its application to the occurrence prediction of nori bleaching

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**Abstract:** The diatom *Eucampia zodiacus* is a harmful species that indirectly causes bleaching damage to nori (*Pyropia*) cultivation through the competitive utilization of nutrients during its bloom. In the present study, to develop an understanding of the population ecology of *E. zodiacus* and identify countermeasures to reduce the negative impacts to aquacultured nori, we investigated the cell density, cell size reduction and restoration of *E. zodiacus* at two sampling stations in northern Harima-Nada, eastern Seto Inland Sea, from April 2006 to March 2009. Vegetative cells of *E. zodiacus* were detected almost all-year round in the water column. Cell densities were generally higher at the northwest station (Stn. Aioi), where initial appearances of *E. zodiacus* blooming are often detected in Harima-Nada, than at the northeast site (Stn. Futami). The restoration of cell size occurred once every autumn with great regularity, and the cells with maximum size gradually decreased and reached the minimum size one year later at both sampling stations, suggesting that synchronous changes in cell size occurred throughout the whole area of Harima-Nada. *Eucampia zodiacus* is considered to adapt during the stratified period by reducing its cell size. In the future, predicting the occurrence timing of nori bleaching by *E. zodiacus* will probably be improved by using the data obtained at Stn. Aioi in addition to that at Stn. Futami.

**Key words:** cell size reduction, cell size restoration, diatom, *Eucampia zodiacus*, nori bleaching

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**Introduction**

*Eucampia zodiacus* Ehrenberg is a common marine diatom, often observed in coastal seas such as in Harima-Nada, eastern part of the Seto Inland Sea, Japan. Since the mid-1990s, blooms of *E. zodiacus* have frequently been detected in the water column in the Harima-Nada during the period of intense vertical mixing from winter through early spring (Nishikawa et al. 2011).

Large-scale aquaculture of nori (*Pyropia yezoensis* Ueda) is a big industry in the coastal waters of western Japan, such as the Ariake Sea and the Seto Inland Sea (Watanabe 2009). In the Harima-Nada, nori cultivation is of significant economic importance for fisheries with an average production of around 10–15 billion yen (ca. 80–120 million U.S. dollars) per year, comprising around 50% of the total fisheries production in Hyogo Prefecture. *Eucampia zodiacus* blooms have caused the exhaustion of nutrients in the water column during the nori harvesting season (Imai et al. 2006). The resultant depletion of nutrients has lowered the quality of nori products due to bleaching, causing fisheries damage of several billion yen (Nishikawa 2011). The damage due to *E. zodiacus* has been more protracted and severe than that by other dominant diatoms in the Harima-Nada, because this species is able to grow until the complete exhaustion of the available nutrients in the water column (Nishikawa & Hori 2004, Nishikawa et al. 2009). Therefore, *E. zodiacus* is the predominant harmful organism causing bleaching of aquacultured nori in the...
Currently, there is no feasible countermeasure to combat the bleaching of aquacultured nori in the Harima-Nada. Therefore, predicting the timing of bleaching is one effective strategy to reduce its negative impacts. Nishikawa et al. (2007) investigated the population dynamics of *E. zodiacus* to elucidate its ecology, and Nishikawa & Imai (2011) reported that average cell densities of the *E. zodiacus* populations in autumn exhibited a significant negative correlation with the integrated number of days during the period from the restoration of cell size to the population peak of the bloom. Using this correlation, it was proposed that the best estimation of the timing of nori bleaching could be predicted by monitoring the cell size restoration and mean cell density of *E. zodiacus* in autumn (Nishikawa & Imai 2011). From this viewpoint, increased knowledge on the ecology of *E. zodiacus*, especially concerning the processes of cell size reduction and restoration, is needed to establish the biological background for more precise prediction of the timing of nori bleaching.

In the Harima-Nada, *E. zodiacus* blooming usually starts on the northwest coast (Hori et al. 2006). However, predictions are currently conducted using only data from Stn. Futami, located on the northeast coast (Fig. 1), where cell densities have been relatively low compared to much of the coast of the Harima-Nada (Hori et al. 2006). In the present study, we investigated the cell density, size reduction and restoration of *E. zodiacus*, along with water quality parameters, at two sampling stations, one in the northwest (Stn. Aioi) and one in the northeast (Stn. Futami), in the northern coastal area to improve prediction of the timing of nori bleaching in the Harima-Nada.

### Materials and Methods

Samplings were carried out at two stations in the northern part of the Harima-Nada, in the eastern part of the Seto Inland Sea (Fig. 1). One station is Stn. Futami (34°41′11″N, 134°52′50″E), at a pier about 200 m from the Fisheries Technology Institute of Hyogo Prefecture, located in the northeast part of the Harima-Nada, and the other is Stn. Aioi (34°45′56″N, 134°28′9″E), located at the mouth of Aioi Bay, in the northwest part of the Harima-Nada. Surface water samples were collected at both stations three times a month from October to November and monthly in the other ten months from April 2006 to May 2009 using a clean bucket. The water temperature and salinity were measured using a salinity-temperature sensor (model ACT-RS, JFE Advantech, Japan) at Stn. Futami, and a STD (ACL215-PDK, JFE Advantech, Japan) or SCT meter (model 30, YSI/Nanotech Inc., Japan) at Stn. Aioi. Concentrations of dissolved inorganic nitrogen (DIN, NO$_3$-N+NO$_2$-N+NH$_4$-N), phosphate (PO$_4$-P) and dissolved silicic acid (SiO$_2$-Si) were analyzed with an autoanalyzer (TRAACS-800, BL TEC K.K., Japan), according to the protocols of Manabe & Tanda (1986).

For the enumeration of the cell numbers of *E. zodiacus*, water samples of 5–10 L were concentrated to 10–30 mL.
with nitrocellulose filters (diameter 47 mm, pore size 5 μm, EMD Millipore, USA) using a vacuum/pressure pump (WP6110060, EMD Millipore, USA) (Itakura et al. 1990). Cell counts of E. zodiacus were done with a light microscope using 1-mL concentrated samples. The resulting detection limit was 6 cells L⁻¹. The cell size (length of apical axis) of E. zodiacus was measured using a video-micrometer (model VM-50, Olympus, Japan), with measurements being conducted for 300 cells in each sample. In the period when cell size recovered, the size frequency distribution of E. zodiacus usually exhibited two peaks, below 20 μm and over 40–50 μm, respectively (Nishikawa et al. 2007). Therefore, the mean values of cell size were calculated in each population when size-restored large cells were present with populations of small-sized cells.

Results

The water temperature ranged from 8.6 to 27.0°C at Stn. Futami, and from 8.0 to 29.5°C at Stn. Aioi (Fig. 2A). The temperature values at Stn. Aioi were usually higher than those at Stn. Futami from spring to summer, and the minimum values at Stn. Aioi were lower than Stn. Futami in each year. Salinity varied between 28.2 and 33.0 except in June 2006 (21.3), and showed a similar fluctuation at both sampling stations with no regular pattern (Fig. 2B). DIN, phosphate and silicic acid concentrations ranged from 0.1 to 39.3 μM, 0.06 to 1.02 μM and 0.7 to 104.2 μM, respectively (Fig. 2C–E). Seasonal fluctuation patterns in inorganic nutrient concentrations were similar between the two stations and among the years. Nutrient concentrations generally increased in autumn, and then decreased. Low nutrient concentrations continued throughout the blooming period of E. zodiacus and during the period of stratification from spring to summer. Most of the nutrient concentrations at Stn. Aioi were usually lower than those at Stn. Futami, especially with respect to DIN concentrations, which had values lower than 1 μM during the most part of the spring to summer period at the latter station.

Vegetative cells of E. zodiacus were detected almost year-round at both Stn. Futami and Stn. Aioi (Fig. 3A). The cell densities varied from ND (<6 cells L⁻¹) to over 1.0×10⁵ cells L⁻¹ at both sampling stations, and were low in spring and summer, when most of the values were lower than <100 cells L⁻¹, and generally high from December to early April, with maximum cell densities of 3.0×10⁵ cells L⁻¹ at Stn. Futami and 6.0×10⁵ cells L⁻¹ at Stn. Aioi, respectively. Seasonal trends in the changes of cell density and cell size showed the same pattern between the two sampling stations, but cell densities at Stn. Aioi were generally higher than at Stn. Futami throughout the three-year study period.

The average cell size (length of apical axis) of E. zodiacus ranged from 13.7 to 83.9 μm (Fig. 3B). The cell size decreased to <20 μm every year from October to November. The minimum and maximum cell sizes were observed...
every autumn. Restoration of cell size occurred once every autumn with great regularity just before or after reaching the minimum cell size. Therefore, the length of the apical axis of size-restored large cells started gradually decreasing and reached its minimum size about one year later.

Discussion

The present study revealed that *E. zodiacus* survives as small-sized cells during the stratified period, and restores its cell size in autumn when vertical mixing started to occur. *Eucampia zodiacus* has a higher sinking rate than the other dominant diatoms in the Harima-Nada, such as *Skeletonema* spp. and *Chaeoceros* spp. (Ono et al. 2006). Consequently, vegetative cells of *E. zodiacus* are affected by thermal stratification in the water column more than the other dominant species, and conditions are thought to be unsuitable for their vegetative growth during the stratified period from April to September. It is well known that many planktonic diatoms generally form resting stage cells in conditions unsuitable for vegetative growth. For example, the giant diatom *Coscinodiscus wailesii* Gran, an other causative organism of nori bleaching in the Harima-Nada, forms resting cells from April to August (Nagai 1995) since the sinking rate is high (Ono et al. 2006). On the other hand, *E. zodiacus* either has no resting stage in its life cycle, or the resting period is much shorter than that in other diatoms (Nishikawa et al. 2007). *Eucampia zodiacus* is considered to adapt during the stratified period by reducing its cell size in the Harima-Nada.

In diatoms, cell sizes are usually restored by auxospore formation (Round et al. 1990), and thus size reduction and restoration reflects their life cycle (Ueno 1991). Annual regularities of cell size fluctuations have been reported in natural populations of other diatom species. For example, *Skeletonema costatum* (Greville) Cleve restored its cell size once a year in Hakodate Bay, and twice a year along the southern coasts of Japan such as Tokyo Bay, Dokai Bay and the Ariake Sea (Ueno 1991). Migita (1967) revealed that auxospore formation in *S. costatum* was frequently observed within a specific range of water temperatures (15–25°C) with the maximum at 20°C. The optimal temperature is in close accordance with field data, as to when *S. costatum* restored its cell size (Ueno 1991). It is a general phenomenon for diatoms to form auxospores under suitable conditions for vegetative growth (Ueno 1991). No seasonal periodicity in the cycle of cell-size reduction and restoration was also reported in diatoms such as *C. wailesii*, in which the cycle was 1.5–2.5 years in the Harima-Nada (Nagai 1995). In the present study, water temperatures in October and November ranged from 20 to 25°C in the Harima-Nada, within the optimum temperature range for *E. zodiacus* growth (Nishikawa & Yamaguchi 2006), and nutrient concentrations were relatively high due to vertical mixing in the water column. Moreover, *E. zodiacus* can remain in shallower layers (the photic layer), where it can grow at high growth rates for a longer period. Therefore, it is thought that conditions were optimal for vegetative growth of *E. zodiacus* in this period, and that *E. zodiacus* restored its cell size under such suitable environmental conditions in the Harima-Nada.

The present study revealed that synchronous restoration of cell size occurred every autumn with great regularity at the two sites in the northern part of the Harima-Nada. However, the cell densities at Stn. Futami usually tended to be lower than those at Stn. Aioi. The difference in the abundance of *E. zodiacus* between the two stations is presumably attributable to the difference in physical factors at the sampling stations. In the northern coastal area of the Harima-Nada, bottom depths are mostly shallower than 20 m, and *E. zodiacus* is able to grow throughout most of this depth range (Nishikawa & Yamaguchi 2006). However, Stn. Futami is located relatively closer to the Akashi Strait, and the west-east current velocity at the station is faster than at Stn. Aioi, suggesting that the vegetative cells of *E. zodiacus* at Stn. Futami would tend to be flushed out from the area. In addition, the fluctuation range of water temperatures at Stn. Futami was smaller than that at Stn. Aioi, because the water mass at Stn. Futami was more vertically homogeneous than at Stn. Aioi (Kobayashi et al. 2006). The nutrient concentrations at Stn. Futami were also relatively high, presumably because of the supply of nutrient rich water from the bottom layer of the Harima-Nada or adjacent Osaka Bay.

It is possible that data gathered at Stn. Aioi can be applied to the prediction of nori bleaching events. Nori bleaching caused by *E. zodiacus* has been predicted using two parameters: average cell densities in autumn, and integrated number of days during the period from the restoration of cell size to the population peak of the bloom of *E. zodiacus* (Nishikawa & Imai 2011). Prediction is currently performed using only the autumn data collected at Stn. Futami. The present results indicate that the cell densities at Stn. Aioi are usually higher than at Stn. Futami all-year round, including in autumn during the cell size restoration period. In addition, the coastal areas of northwest Harima-Nada, including Stn. Aioi, are known to be the initial sites where *E. zodiacus* blooming is observed, and the cell densities have been reported to be higher than at other sites in the Harima-Nada during the blooming period (Hori et al. 2006). In the future, the precision of predictions should be able to be improved by using data obtained at Stn. Aioi in addition to that obtained at Stn. Futami.

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