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Original article

Distribution and behavior of harmful algae and trace metals in Harima-Nada, Japan

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Introduction

In Harima-Nada, eastern part of the Seto Inland Sea, Nori (*Pyropia*) cultivation is one of the key fisheries, which accounts for about half of the fisheries production volume and 40 percent of the production value [1]. In this sea area, the problem of Nori bleaching by diatom blooms has occurred almost every year since the mid 1980s [2]. It is thought that massive occurrence of large diatoms *Chaetoceros densus* (Cleve) Cleve, *Coscinodiscus wailesii* Gran, *Eucampia zodiacus* Ehrenberg significantly related to the problem in this area [1]. Hence, it is important to elucidate outbreak mechanism of harmful diatom blooms causing bleaching of aquacultured Nori in the region.

Nutrients have been considered as one of the major factors controlling the composition and abundance of microalgal community, and also the occurrence of the blooms. There are many reports about the effect of macronutrients such as nitrogen on the growth of harmful diatoms in Harima-Nada [3,4]. However, the relationship between the trace metal and diatom growth is not clear. Trace metals are known to play important roles as micronutrients in oceanic biogeochemistry and may also limit biological activity in oceanic waters [5]. Therefore, it is desired to simultaneously determine the trace metals in order to understand this relationship.

In the present study, we examined the relationship between harmful algal bloom and the concentration distribution of essential trace metals such as iron in Harima-Nada.

Materials and methods

Samplings were carried out monthly at two stations in Harima-Nada (Fig. 1), eastern part of the Seto Inland Sea from May 2015 to May 2016. At two stations, H30 (northern nearshore site) and H7 (offshore site),

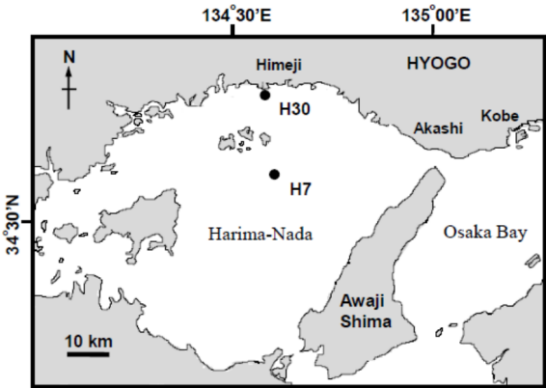

Fig. 1. Location of sampling stations (H30 and H7) in Harima-Nada, eastern part of the Seto Inland Sea.

samples of surface and bottom (1 m above the bottom) water were collected and transferred from sampler to the polycarbonate acid-cleaned bottles. The samples were brought into a clean booth in the laboratory, filtered through 0.2-µm pore-sized Nuclepore filters (Whatman), acidified to pH 2 with HCl (TAMAPURE AA-100, Tama Chemicals) and stored in low-density polyethylene (LDPE) bottles until the analysis. The samples were refrigerated until the analysis.

All reagents used in this study were of the highest purity available. Deionized water purified with an ultrapure water system (Barnstead) was employed throughout the study. Nuclepore filters were cleaned in a mixed acid solution (1 M HCl + 0.5 M HNO_3 + 0.5 M HF) at 100ºC for 2 h and then in hot ultrapure water four times on a hot plate to reduce the potential trace metal contamination. LDPE bottles and tips for micropipettes were thoroughly cleaned with hot 1 M HNO_3, hot 1 M HCl, and then hot ultrapure water twice in a microwave.

Dissolved iron (DFe) concentration was measured using PDTS (3-(2-pyridyl)-5,6-bis(4-sulfophenyl)-1,2,4-triazine disodium salt; ferrozine) method [6] by a 1-m liquid waveguide capillary cell with 400 µm core

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diameter (LPC-100CM, WPI) connected to a tungsten halogen lamp (HL-2000, Ocean Optics) and a miniature multichannel spectrophotometer (USB2000+ VIS-NIR-ES, Ocean Optics) via two fiber optic cables. The concentration of other dissolved trace metals (DNi, DCu, DMn, DZn, DMo) was determined by an inductively coupled plasma optical emission spectrometry (Optima 8300, PerkinElmer) after pre-concentration with a solid-phase extraction system (SPE-100, Hiranuma Sangyo) using a polyamino polycarboxylic acid chelating resin (Nobias Chelate-PA1, Hitachi High-Technologies) according to the procedures of Sakamoto et al. [7].

Chlorophyll-*a* samples were collected on 47-mm glass fiber filters (GF/F, Whatman), extracted with *N,N*-dimethylformamide and determined by fluorometry (10-AU, Turner Designs). Phytoplankton observation was carried out using fresh samples collected from each sampling station on the following day. Species identification and cell count of flagellates were made using 1-ml water samples with an inverted light microscope (TMD300, Nikon). For diatom species, 1% glutaraldehyde fixative and 2- or 7-fold concentrated sample was examined.

Results and discussion

The pH values ranged from 7.5 to 8.2 (Fig. 2A). The pH values tended to be higher in the surface water (0 m at both stations) than in the bottom water (B-1 m) from May to October in 2015, and to be higher in both layers at Stn. 30 (nearshore) than in those at Stn. 7 (offshore) from November 2015 to May 2016.

Chlorophyll-*a* concentration in the epilimnion at Stn. H30 was high from July to October (Fig. 2B). Its maximum concentration of 22.3 μg l^{-1} was observed in October. Chlorophyll-*a* concentration at Stn. H30 was higher compared to that at Stn. H7 throughout the year.

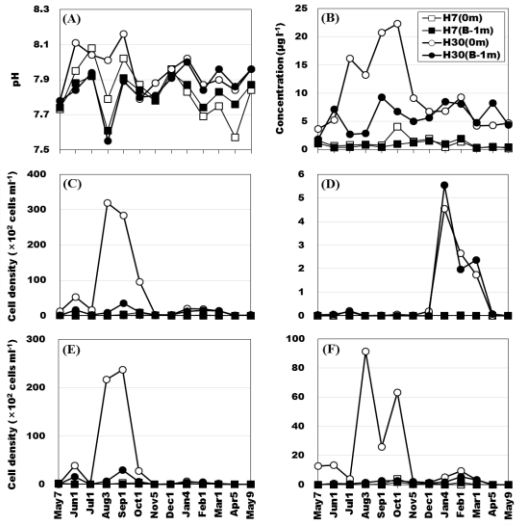

Fig. 2. Seasonal changes of (**A**) pH, (**B**) chlorophyll-*a* concentration and cell densities of (**C**) total phytoplankton, (**D**) *Eucampia zodiacus*, (**E**) *Skeletonema* spp. and (**F**) *Chaetoceros* spp. in surface and bottom water at two stations of Harima-Nada from May 2015 to May 2016.

Table 1. Concentration range of dissolved trace metals in surface and bottom water (nM) at two stations of Harima-Nada from May 2015 to May 2016 (from August 2015 for Mn, Zn and Mo)

Sample		Fe	Ni	Cu	Mn	Zn	Mo
H7 (0 m)	max	81	110	179	35	255	245
	min	DL[a]	21	9.4	5.2	50	12
H7 (B-1 m)	max	336	39	53	47	35	115
	min	DL[a]	7.8	6.5	1.1	9.4	17
H30 (0 m)	max	376	151	45	109	551	101
	min	3.9	31	8.8	15	49	27
H30 (B-1 m)	max	848	91	33	93	47	223
	min	0.8	9.5	5.9	1.8	10	25

[a] DL: Detection Limit.

The seasonal changes in the cell densities of total phytoplankton, *Eucampia zodiacus*, *Skeletonema* spp. and *Chaetoceros* spp. are shown in Fig. 2C–F. The maximum of total phytoplankton densities was observed in the surface water at Stn. H30 in August (3.2 × 10^4 cells ml^{-1}) (Fig. 2C). The major phytoplankton component was diatoms *Skeletonema* spp. and *Chaetoceros* spp. in the period examined (Fig. 2 E, F). In both layers at Stn. H30, *E. zodiacus* appeared from mid-December 2015 to late March 2016 (Fig. 2D).

Table 1 lists the maximum and minimum concentrations of dissolved trace metals in surface and bottom water at two stations of Harima-Nada in the investigation period. We examined correlation between the concentration of trace metals and the cell density of three dominant diatom species. There was a negative correlation between the cell density of *E. zodiacus* and the concentration of DFe, DMn, DZn (r = -0.52, -0.39, -0.45 in surface water, -0.52, -0.51, -0.48 in bottom water) at Stn. H30 from Oct 2015 to May 2016. Given the seasonal changes of trace metals concentration (data not shown), the results in this study suggest that these trace metals, especially iron, were consumed by *E. zodiacus* and may play an important role in forming harmful algal bloom affected to Nori in Harima-Nada. Further investigations are needed into these trace metal requirement for harmful algal species growth.

Acknowledgements

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