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Original article

In situ bottle experiments demonstrating feasibility of diatom resting stage cells in sediments to prevent red tides of noxious flagellates

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Introduction

It is empirically known that harmful algal blooms by noxious flagellates such as *Chattonella* spp. and *Karenia mikimotoi* have occurred when diatoms are scarce in water columns [1]. Diatoms produce resting stage cells under nutrient-depleted conditions, rapidly sink to sea bottom and disappear from the water columns [2,3]. Giving enough light to the abundant diatom resting stage cells is considered to enhance their germination [4]. Appeared vegetative cells are expected to rapidly proliferate in the photic layer and to overwhelm harmful flagellate populations by the exhaustion of inorganic nutrients (N, P) [4]. Simulating these situations, we conducted bottle experiments containing small amounts of bottom sediments and noxious flagellates of *C. antiqua* and *K. mikimotoi* in Saiki Bay of Oita Prefecture, Japan, in 2014. Here we report successful control of HAB species by diatoms.

Materials and methods

Sediments containing diatom resting stage cells were collected at Okimatsuura in Saiki Bay on 11 June 2014, with the density of 4.4×10^4 cells g^{-1} wet sediment. Dominant genera were *Navicula* (53%), *Skeletonema* (24%) and *Chaetoceros* (10%). Sediments stored in a refrigerator were added into 2 L volume transparent pet bottles at concentrations of 1 g L^{-1} and 0.1 g L^{-1}. *K. mikimotoi* was inoculated at a concentration of 20 cells mL^{-1} and *C. antiqua* 50 cells mL^{-1}. The 1/100 strength of the modified SWM3 medium [5,6] was added as nutrient source for phytoplankton in the bottles. Totally 2 L volume was attained with filtered seawater (GF/F Glass fiber filter). These bottles were hanged at surface (0 m) and the depth of 5 m on 25 September 2014, and samplings were made for cell enumeration of flagellates and diatoms, and for nutrient measurements. Experimental bottles were summarized in Table 1 with combined conditions of sediments and HAB species.

Table 1. Combination of conditions (HAB species and sediment concentrations) for experimental bottles

Bottle No.	Sediment density	HAB species
1	1 g L^{-1}	No addition
2	0.1 g L^{-1}	No addition
3	1 g L^{-1}	*Karenia mikimotoi*
4	0.1 g L^{-1}	*Karenia mikimotoi*
5	1 g L^{-1}	*Chattonella antiqua*
6	0.1 g L^{-1}	*Chattonella antiqua*

Results and discussion

Added concentrations of nutrients (1/100 SWM3) were 20 μM for NO_3-N and 1 μM for PO_4-P in the experimental bottles. Nutrient concentrations were decreased in all the experimental bottles during the incubation period (Fig. 1). Total phytoplankton biomass indicated by chlorophyll *a* increased from the day 4, and phytoplankton in the bottles put at surface water tended to grow faster than those suspended at 5 m depth

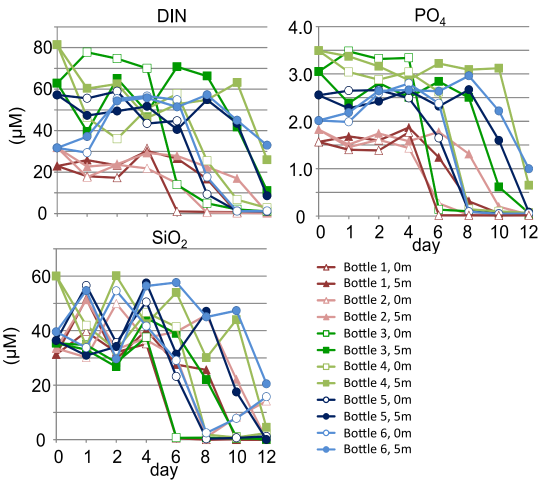

Fig. 1. Changes in nutrient concentrations in the experimental bottles during the bottle experiment.

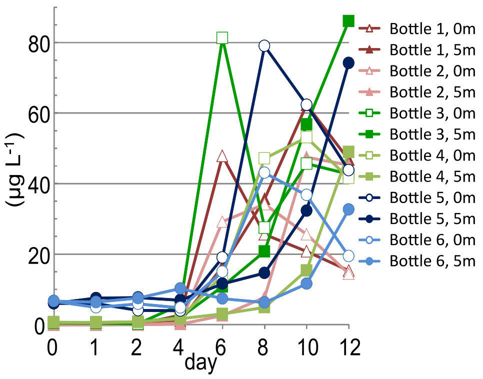

Fig. 2. Changes in chlorophyll a during the bottle experiment.

(Fig. 2). Figure 3 shows the growth and death of red tide species in the experimental bottles. In the red-tide dinoflagellate *K. mikimotoi*, cell densities initially increased from 20 cells mL^{-1} to about 70 cells mL^{-1} at most and then decreased after the increase of total phytoplankton biomass (substantially diatoms). The fish-killing raphidophyte *C. antiqua* showed consistent decrease in cell densities in all the experimental bottles.

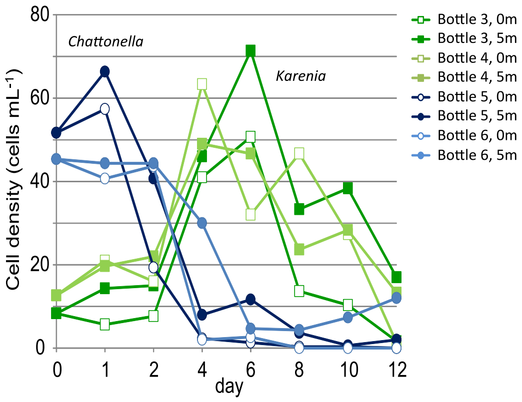

Fig. 3. Changes of *Karenia mikimotoi* in the bottles 3 and 4 and that of *Chattonella antiqua* in the bottles 5 and 6.

Diatoms grew well in all the bottles examined, though faster growths were observed in the bottles put at the surface (0 m) (Fig. 4). The most abundantly appeared diatoms were belonging to the genus *Skeletonema*. On the whole, proliferated diatoms through germination from the resting stage cells and growth during the incubation period overwhelmed *K. mikimotoi* and *C. antiqua*. In addition, no peculiar plankton appeared in the bottles, indicating the safety of using the bottom sediments. It is suggested that sediment-lift to euphotic zone is a feasible strategy to prevent red tides of harmful flagellates [4,6].

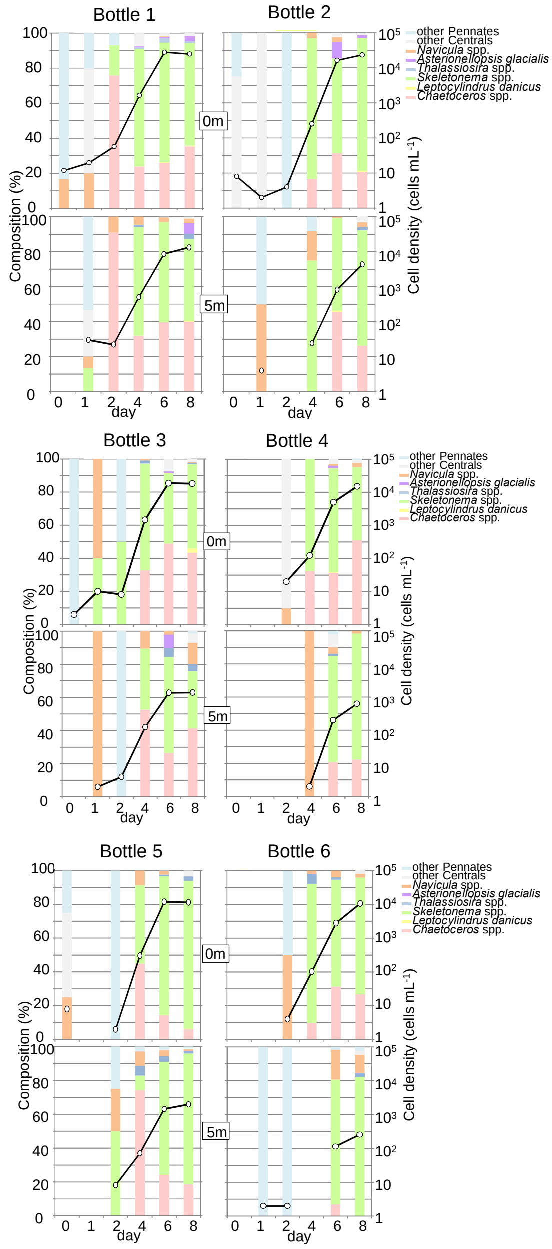

Fig. 4. Changes in cell densities and taxa composition histograms of diatoms in the experimental bottles 1–6 suspended at 0 m and 5 m depth at the point of the raft of Fisheries Research Department of Oita Agriculture, Forestry and Fisheries Study Instruction Center from 25 September to 7 October 2014.

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