Densities of Dormant Cells of the Red Tide Flagellate *Heterosigma akashiwo* (Raphidophyceae) in Bottom Sediments of Northern Hiroshima Bay, Japan

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Abstract: The distribution of dormant cells of the red tide flagellate *Heterosigma akashiwo* (Raphidophyceae) was investigated by the extinction dilution method in sediments of northern Hiroshima Bay where red tides of *H. akashiwo* occur almost every year. Densities of the dormant cells in sediment samples ranged from 56 to 5,950 cells $\cdot$ cm⁻³ wet sediment in April 1987, and from 56 to 29,300 cells $\cdot$ cm⁻³ in March 1989. No significant relationship was observed between the number of dormant cells of *H. akashiwo* and mud content of sediment sample. High density stations of the dormant cells in sediments appeared to coincide with the areas with high concentrations of vegetative cells in water columns during the red tide season.

Key words: *Heterosigma akashiwo*, dormant cells, red tide, sediment

Introduction

Heterosigma akashiwo (Hada) Hada, Raphidophyceae, is known to be one of the most representative flagellates forming dense red tides. The red tides of *H. akashiwo* have been observed not only in coastal waters of Japan but also in Korea (Park *et al.*, 1989) and in the United States (Pratt, 1959). *H. akashiwo* red tides occasionally cause mortalities of caged fish such as yellowtail, sea bass, black sea bream, right-eyed flounder, etc. In Scottish and Irish coastal waters, unidentified alga "flagellate X" have killed farmed salmon and trout (Gowen, 1987). This "flagellate X" is now thought to be identical to *H. akashiwo* according to the available information on morphological features (Larsen and Moestrup, 1989). *H. akashiwo* had previously been confused with *Olisthodiscus luteus* Carter. Recently, Hara and Chihara (1987) showed that "bloom-forming" *O. luteus* is

identical to *H. akashiwo*.

Information on physiological characteristics of *H. akashiwo* had been accumulated (Iwasaki *et al.*, 1968; Iwasaki and Sasada, 1969; Honjo and Hanaoka, 1973; Tomas, 1978a, 1979, 1980a; Watanabe *et al.*, 1982; Yamochi, 1983). Some observations were made on *in situ* occurrences and population growth dynamics (Iizuka and Irie, 1968; Tomas, 1980b; Honjo and Tabata, 1985). However, investigations are scarce concerning life cycle of *H. akashiwo* including benthic resisting stage. Although Tomas (1978b) and Yamochi (1984, 1989a) reported the existence of benthic stage cells in *H. akashiwo*, morphology of the benthic stage cells in sea bottom sediments has not yet been identified. Since benthic stage cells, hereafter we call as dormant cells, presumably play a key role in initiating the red tides, to know densities of dormant cells in bottom sediments is considered to be important to understand the mechanisms for the red tide occurrences. In the present paper, the distribution of dormant cells of

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Fig. 1 Location of sediment sampling stations in northern Hiroshima Bay, the Seto Inland Sea.

H. akashiwo in sediments was investigated by the extinction dilution method (MPN method) in northern Hiroshima Bay, and its implications in the red tide occurrences were discussed.

Materials and Methods

Sediment samples were collected with a gravity core-sampler (inner diameter: 4 cm) (Kimata *et al.*, 1960) at seven stations located in northern Hiroshima Bay on 27 April 1987, and at ten stations on 29–30 March 1989 (Fig. 1). Top 1-cm depth of collected sediments (4–5 cores at each station) were taken into a plastic bottle, sealed tightly, and stored at 11°C in the dark afterwards.

The number of dormant cells of *H. akashiwo* in the sediment samples was enumerated by the extinction dilution method (Imai *et al.*, 1984, Imai, 1990). The procedure of the extinction dilution method is shown in Fig. 2. An appropriate amount (1–2 g wet weight) of sediment sample was suspended in sterile modified SWM-3 culture medium (Chen *et al.*, 1969; Itoh and Imai, 1987) at a concentration of $0.1 \text{ g} \cdot \text{ml}^{-1}$ in wet sediment

Fig. 2. Procedure of the extinction dilution method for enumeration of dormant cells of *H. akashiwo* in sediment samples.

weight. The culture medium contained GeO_2 ($1 \text{ mg} \cdot \text{l}^{-1}$) which is a specific inhibitor of diatom growth (Lewin, 1966). The suspension was sonicated for several seconds in order to segregate organic detritus. Serial 10-fold dilutions were made with the culture medium, and then 1-ml aliquots of diluted suspensions were inoculated into five replicate wells of disposable tissue culture cluster (48 wells, Costar). Incubation was carried out at a temperature of 22°C and illumination of 3,500 lux with 14 hL : 10 hD photo-cycle. The appearance of vegetative cells of *H. akashiwo* in each well was examined after the incubation period of 6–8 days using an inverted epifluorescence microscope. The well in which vegetative cells were identified was scored as positive. The most probable number (MPN) of dormant cells in the sediment sample ($\text{MPN} \cdot \text{g}^{-1}$ wet sediment) was then calculated according to the statistical table (e.g. Throndsen, 1978; Itoh and Imai, 1987). The density of dormant cells per cubic centimeter wet sediment was obtained by multiplying apparent specific gravity of wet sediment. Mud content of the sediment samples in March 1989 was measured using a $63 \mu\text{m}$ opening netting.

In the sediment samples of 1987, the number of dormant cells of *H. akashiwo* was enumerated after one month of storage period. In the samples of 1989, incubation for the enumeration by the extinction dilution method was started from the day of sampling. No vegetative cells of *H. akashiwo* were detected in water samples collected on 27 April 1987, and on 29–30 March 1989, with microscopical observations of 1-ml samples from the depths of 0, 5, and 10 m.

Results

Figure 3 shows the densities of dormant cells of *H. akashiwo* in bottom sediments of northern Hiroshima Bay enumerated by the extinction dilution method. In April 1987, the dormant cells ranged from 56 (St. 3) to $5,950 \text{ cells} \cdot \text{cm}^{-3}$ wet

sediment (St. 1). Stations with high density of the dormant cells ($>1,000 \text{ cells} \cdot \text{cm}^{-3}$) were found at Sts. 1, 2, 5 and 6. These stations are located near the coast of Hiroshima (the mouth of Ohta River) and Iwakuni (the mouth of Nishiki River). In March 1989, the densities of dormant cells of *H. akashiwo* ranged between 56 (St. 10) and $29,300 \text{ cells} \cdot \text{cm}^{-3}$ wet sediment (St. 5). The densities were much abundant at the mouth of Ohta River, $29,300 \text{ cells} \cdot \text{cm}^{-3}$ at St. 5, and $16,000 \text{ cells} \cdot \text{cm}^{-3}$ at st. 6. Amounts of dormant cells tended to be more numerous in March 1989 than in April 1987. Although there is a fluctuation in both years, the densities of dormant cells tended to be more abundant near the coast of Honshu.

Figure 4 shows the relationship between the number of dormant cells of *H. akashiwo* and mud content of the sediment samples collected in March 1989. Mud content varied from 76% (St. 1) to 94% (St. 4). No significant relationship was found between the densities of dormant cells and mud content of sediment samples.

Discussion

The densities of dormant cells of *H. akashiwo* in bottom sediments of northern Hiroshima Bay were $56\text{--}5,950 \text{ cells} \cdot \text{cm}^{-3}$ in April 1987, and $56\text{--}29,300 \text{ cells} \cdot \text{cm}^{-3}$ in March 1989. In Osaka Bay, Yamochi (1989b) enumerated the number of benthic cells (dormant cells) of *H. akashiwo* in bottom sediments of 21 stations by similar method, and obtained the data of $5\text{--}270 \text{ cells} \cdot \text{g}^{-1}$ wet sediment in June 1986, and $27\text{--}5,400 \text{ cells} \cdot \text{g}^{-1}$ in October 1987. The distribution of dormant cells of *H. akashiwo* was also measured in Suo-Nada, western Seto Inland Sea, and the number ranged between 20 and $790 \text{ cells} \cdot \text{g}^{-1}$ wet sediment in autumn of 1987 (K. Terada, personal communication). Compared the data of northern Hiroshima Bay with Osaka Bay and Suo-Nada, the densities of dormant cells in April 1987 are about in similar range. On the other hand, in March 1989, the maximum number in northern Hiroshima Bay was

Fig. 3. Densities of dormant cells of *H. akashiwo* in bottom sediments of northern Hiroshima Bay, enumerated by the extinction dilution method. Numerals indicate the number of the dormant cells per cubic centimeter wet sediment.

Fig. 4. Relationship between the number of dormant cells of *H. akashiwo* and mud content of the sediment samples.

one order of magnitude higher value than that in Osaka Bay and Suo-Nada.

High density stations of dormant cells of *H. akashiwo* were found in the coastal area of Honshu such as the mouth of Ohta River. High density stations in bottom sediments appeared to coincide with the areas with high concentrations of vegetative cells in water columns during the red tide season. And no relationship was observed between the density of dormant cells and mud content of sediment sample. In the case of the toxic dinoflagellate *Alexandrium excavatum* (*Gonyaulax excavata*), however, White and Lewis (1982) reported that dense seed bed areas were found in a zone of fine brown sediment in Bay of Fundy, Canada. Thus, it is an important problem to understand the effects of hydrographic and sedimentary processes on dense seed bed formation in *H. akashiwo*.

In recent years, it has been well documented that

the presence of cysts (dormant cells) in bottom sediments encourages the survival of species and initiation of blooms in rather many dinoflagellates including toxic species (Wall, 1971; Anderson *et al.*, 1983; Sako *et al.*, 1985). In raphidophycean flagellates, the morphology was identified in the cysts of *Chattonella* (*C. antiqua* and *C. marina*) (Imai and Itoh, 1986, 1988) and *Fibrocapsa japonica* (Yoshimatsu, 1987) from bottom sediments of the Seto Inland Sea. The studies concerning the ecophysiological aspects of the cysts of *Chattonella* (Imai *et al.*, 1986, 1989; Imai and Itoh, 1987; Imai, 1990) strongly suggested the importance of cysts in initiating the summer red tides in the Seto Inland Sea. Yamochi (1984, 1989a, b) also suggested the significant role of "benthic cells" in bloom formation of *H. akashiwo* in Osaka Bay.

The present study revealed the existence of abundant dormant cells of *H. akashiwo* in bottom sediments of northern Hiroshima Bay, where *H. akashiwo* red tides occur almost every year. Studies are in progress concerning the seasonal changes in germinability of dormant cells in sediments and vegetative cell concentrations in water columns of northern Hiroshima Bay. We have recently succeeded in identifying the morphology of the cyst of *H. akashiwo* from the bottom sediments of northern Hiroshima Bay (Imai *et al.*, in preparation). The detailed results of the observations will be published elsewhere.

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