Distribution and abundance of resting cysts of the toxic *Alexandrium* spp. (Dinophyceae) in sediments of the western Seto Inland Sea, Japan

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**ABSTRACT:** Sediment samples were collected from 135 stations in the western part of the Seto Inland Sea (Iyo Nada, Suo Nada, Beppu Bay, Tokuyama Bay, Hiroshima Bay, Aki Nada, Hiuchi Nada and Bingo Nada) to determine the horizontal distribution and abundance of resting cysts of *Alexandrium* spp. (*A. tamarense* + *A. catenella*). Enumeration of the cysts was performed using the primuline-staining direct count method. Cysts of *Alexandrium* spp. were rarely found in Iyo Nada, Suo Nada and Beppu Bay, but were widely distributed in Tokuyama Bay, Hiroshima Bay, Aki Nada, Hiuchi Nada and Bingo Nada. Cyst concentrations ranged from not detected (ND) to 14, ND to 17, ND to 4, 93 to 8137, 8 to 4454, ND to 6, ND to 18 and 4–29 cysts/cm$^3$ wet sediment in Iyo Nada, Suo Nada, Beppu Bay, Tokuyama Bay, Hiroshima Bay, Aki Nada, Hiuchi Nada and Bingo Nada, respectively. The majority of cysts occurred in Tokuyama Bay and Hiroshima Bay, where higher densities were observed in the inner bay and along the coastal margin. Relatively higher cyst concentrations were observed at stations with a higher mud content. The abundance of *Alexandrium* spp. cysts in western Seto Inland Sea is lower than in the eastern Seto Inland Sea, except for Tokuyama Bay and Hiroshima Bay. However, because sporadic blooms of *Alexandrium* have been observed, continuing monitoring is necessary to prevent paralytic shellfish poisoning outbreaks in this area.

**KEY WORDS:** *Alexandrium catenella*, *Alexandrium tamarense*, cyst, paralytic shellfish poisoning, Seto Inland Sea.

**INTRODUCTION**

Annual shellfish production in the Seto Inland Sea is approximately 180 000 tons, with oyster and short-necked clam representing the major commercial shellfish resources. Production of cultured oysters, for example, accounts for approximately 70% of the total in Japan. Therefore, shellfish poisoning could be a major threat to the fishing industry in the Seto Inland Sea. It is of concern that paralytic shellfish poisoning (PSP) outbreaks are increasing in intensity and geographic distribution in south-west Japan. Toxic dinoflagellates *Alexandrium catenella* and *A. tamarense* are the most common causative organisms. Paralytic shellfish poisoning caused by *A. catenella* was first confirmed in the Seto Inland Sea in 1976. Since then, PSP incidents have recurred, especially along the coastal areas of the Kii Channel and Harima Nada.

Dinoflagellate cysts play an important role in species dispersal, bloom initiation and termination and survival under unfavorable conditions. Therefore, information on the distribution and abundance of cysts in natural sediments is essential for understanding the ecology and bloom dynamics of many toxic dinoflagellates. Cysts of these toxic dinoflagellates are also a significant source of shellfish intoxication because their toxin content can be higher than in vegetative cells.

For conducting shellfish toxin monitoring programs, it is important to determine accurately the abundance and distribution of cysts in shellfish farming areas. However, studies on the distribution of *Alexandrium* spp. (*A. catenella* and *A. tamarense*) cysts in western Japan have been fragmentary and limited to small embayments only. In order to understand the potential for PSP outbreaks in the area, our institute initiated a research program on this problem in 1993. We have developed a simple method that uses fluorochromes and epifluorescence microscopy for the rapid and precise...
20 g (wet weight) aliquots of the sediment samples were suspended in distilled water, sonicated and sieved through plankton netting to obtain a size fraction between 20 and 150 μm. The material remaining on the 20 μm netting was washed into a 15 mL polycarbonate centrifuge tube and the volume was brought to 10 mL with distilled water. Aliquots (5 mL) of sediment suspensions were pipetted into 15 mL centrifuge tubes and fixed with 1 mL of 5% glutaraldehyde solution. After fixation, the suspensions were centrifuged at 700 ¥g for 15 min and the supernatant was discarded. Cold methanol (5 mL) was added to the pellet and the tubes were placed in a refrigerator overnight. The methanol was replaced with 10 mL distilled water using centrifugation. Primuline stock solution (1 mL; 2 mg/mL) was added to each tube and left for 1 h in the dark. After staining, the supernatant was removed using centrifugation and the pellet was resuspended in distilled water and centrifuged again. Then, pellets were finally resuspended in 5 mL distilled water for microscopic observation using an epifluorescence inverted microscope under blue light excitation. For counting cysts, 0.1–1.0 mL stained sediment suspension was placed in a Sedgwick-Rafter chamber (RIGO). The number of cysts in the sediment suspension was determined at 100 ¥ magnification and counts were made in triplicate. The specific gravity of the sediment sample was determined according to the method of Kamiyama. Using this new method, the horizontal distribution and abundance of resting cysts of A. catenella and A. tamarense were previously investigated in the eastern part of the Seto Inland Sea, Hiroshima Bay and along Kyushu Island. The present paper details the results from the western part of the Seto Inland Sea.

MATERIALS AND METHODS

Sediment sampling

Sediment samples were collected with a gravity core-sampler (inner diameter 4 cm) or a Smith-McIntyre grab sampler (RIGO, Tokyo, Japan) from 36, 38, four, five, 16 and eight stations in Suo Nada, Iyo Nada, Beppu Bay, Aki Nada, Hiuchi Nada and Bingo Nada (October to November 1994), respectively, from eight stations in Tokuyama Bay (July 1997) and from 20 stations in Hiroshima Bay (July 2001; Fig. 1). The top 3 cm of sediment samples from the replicate samples were sliced off, mixed, placed in a plastic container and stored in the dark at 10°C.

Cyst counting

Enumeration of the cysts was performed using the primuline-staining direct count method. Five to
limits of the cyst count were 0.3–6.5 cysts/cm$^3$ wet sediment depending on the amount of the sediment used and the observed volume of the suspension. The mud content of the sediment samples was also determined using a stainless steel sieve with a pore size of 63 µm.

### Occurrence of vegetative cells

Occurrence of vegetative cells of *A. catenella* and *A. tamarense* in the area was summarized using data from annual reports and project reports on toxic plankton monitoring published from the prefectural fisheries experimental stations of Okayama, Hiroshima, Yamaguchi, Fukuoka, Oita and Ehime from 1982 to 1999.

### RESULTS AND DISCUSSION

#### Iyo Nada, Suo Nada and Beppu Bay

Figure 2 shows the horizontal distribution of the cysts of *Alexandrium* spp. (*A. catenella* and *A. tamarense*) in Suo Nada, Iyo Nada and Beppu Bay. The cysts were rarely found in the study area and were not detected at the majority of stations examined. However, relatively higher densities were observed in the north-western part of Iyo Nada and the eastern part and along the northern coastal margins of Suo Nada. As described below, a large number of cysts had accumulated in the sediment of Tokuyama Bay and Hiroshima Bay adjacent to this area. Thus, these high cyst abundance areas in Suo Nada were probably formed by the introduction of cysts from these bays by the current system in this area. Cyst concentrations in Iyo Nada, Suo Nada and Beppu Bay ranged from not detected (ND) to 14, ND to 17 and ND to 4 cysts/cm$^3$ wet sediment, respectively (Table 1).

In this area, a survey of cyst abundance was conducted in Suo Nada in 1987 (Japan Fisheries Agency, unpubl. data, 1988) and in 1996. The results indicated that cysts of *A. catenella* and *A. tamarense* were found at seven of 50 stations in 1987 and at nine of 15 stations examined in 1996. The cyst concentration was less than 6 cysts/cm$^3$ wet sediment in 1987 and ND to 11 cysts/cm$^3$ wet sediment in 1996. Higher cyst concentrations were found along the northern coastal margins of Suo Nada in both surveys. Thus, cyst abundance and the horizontal distribution have not changed over the past decade.

Results of the vegetative cell survey indicated that sporadic occurrences of *A. catenella* and *A. tamarense* cells have been observed since 1984 in Suo Nada, Iyo Nada and Beppu Bay. The cysts were rarely found in the study area and were not detected at the majority of stations examined. However, relatively higher densities were observed in the north-western part of Iyo Nada and the eastern part and along the northern coastal margins of Suo Nada. As described below, a large number of cysts had accumulated in the sediment of Tokuyama Bay and Hiroshima Bay adjacent to this area. Thus, these high cyst abundance areas in Suo Nada were probably formed by the introduction of cysts from these bays by the current system in this area. Cyst concentrations in Iyo Nada, Suo Nada and Beppu Bay ranged from not detected (ND) to 14, ND to 17 and ND to 4 cysts/cm$^3$ wet sediment, respectively (Table 1).

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Results of the vegetative cell survey indicated that sporadic occurrences of *A. catenella* and *A. tamarense* cells have been observed since 1984 in Suo Nada (Fig. 3). In the winter of 2000, a bloom of *A. catenella* (maximum cell density 542 cells/mL) first occurred in southern Suo Nada and caused PSP of oysters (H Tamori, pers. comm., 2000). In addition, red tides of *A. catenella* have occurred since 1986 in the Uwa Sea, which is located just south of Iyo Nada and Suo Nada. In the Uwa Sea,
widespread distribution of *Alexandrium* cysts has also been reported.\textsuperscript{13} Thus, continued monitoring is necessary to prevent PSP incidents occurring in this area.

**Tokuyama Bay**

Figure 4 shows the horizontal distribution of the cysts of *Alexandrium* spp. in Tokuyama Bay. The cysts were observed at all stations examined. Higher densities were observed at stations located in the inner part of the bay. Cyst concentrations ranged from 93 to 8137 cysts/cm\(^3\) wet sediment (Table 1). The mean cyst concentration in the bay was the highest among the study areas of the present investigation.

Horizontal and vertical cyst surveys were conducted in the bay in April, July and November 1981 (T Baba and T Ikeda, pers. comm., 2001). Their results showed that the highest cyst abundance was 2220 cysts/cm\(^3\) wet sediment in the innermost part of the bay. Cysts were observed from the surface to a sediment depth of 10 cm. Red tides of *A. catenella* occurred in the bay in 1979 and 1981. Thus, the high abundance of the cysts is probably attributable to these red tides.

Vegetative cells of *A. catenella* and *A. tamarense* have also been observed since the late 1970s and red tides of *A. catenella* have occurred in 1979, 1981 and 1997.\textsuperscript{17,18} In 1997, the maximum cell density of *A. catenella* was 43 000 cells/mL.\textsuperscript{18} A cyst survey of the present study was conducted approximately 1 month after this event. Thus, the extremely high cyst density in the inner bay is probably caused by the deposition of newly formed cysts during the red tide. Due to the high cyst density and occasional outbreaks of *A. catenella* red tide (Fig. 3), Tokuyama Bay is one of the important areas that needs to be monitored to minimize the effects of any potential outbreaks of PSP.

**Hiroshima Bay**

Figure 5 shows the horizontal distribution of cysts in Hiroshima Bay. Cysts of *Alexandrium* spp. were observed at all stations examined. Major cyst deposition areas were observed along the northern coastal margins of the bay. Cyst concentrations ranged from 8 to 4454 cysts/cm\(^3\) wet sediment (Table 1). Mean cyst concentration in the bay was highest next to Tokuyama Bay.

Hiroshima Bay has been affected by PSP outbreaks since 1992, with concentrations of *Alexandrium* spp. found to range from 16 to 1912 cysts/cm\(^3\) wet sediment in 1993.\textsuperscript{10} The horizontal cyst distribution of the present study was almost identical with previous results obtained in 1993, but the maximum density has doubled within the past 8 years. This is probably due to the repetitive occurrences of the vegetative cells of *A. tamarense* since 1992 (Fig. 3). Hiroshima Bay is characterized by the dominance of *A. tamarense*, whereas the dominant species in the other areas of western Seto Inland Sea is *A. catenella* (Fig. 3). The reason why *A. tamarense* dominates in Hiroshima Bay remains unknown. The present study indicates that Hiroshima Bay is one of the important areas that needs to be monitored for potential outbreaks of PSP.

**Aki Nada, Hiuchi Nada and Bingo Nada**

Figure 6 shows the horizontal distribution of cysts in Aki Nada, Hiuchi Nada and Bingo Nada. Compared with Suo Nada and Iyo Nada, cysts of *Alexan-
that cysts of *A. catenella* and *A. tamarense* were found at 16 of 50 stations examined. Cyst concentrations ranged from ND to 18 cysts/cm\(^3\) wet sediment and cysts were not detected in the central part of the area. Thus, the present study found that the cyst distribution has spread into almost all areas within the past decade, although abundance has not increased.

The results of the vegetative cell survey show that a small number of vegetative cells of both species, mainly *A. catenella*, have occurred since 1982 (Fig. 3). In addition, vegetative cells of *A. catenella* (maximum 3000 cells/L) and *A. tamarense* (maximum 1500 cells/L) were detected in south-eastern Hiuchi Nada in 1982. Thus, the potential for PSP incidents should be considered in this area.

**General considerations of cyst distribution in the western part of the Seto Inland Sea**

The mapping of toxic dinoflagellate cysts in marine sediments is a useful tool to indicate ‘hot spots’ where blooms have occurred or where blooms may occur in the future. For this purpose, it is important to determine the mechanism of the formation of seed beds where high numbers of cysts accumulate. Figure 7 shows the relationship between the concentration of cysts and the mud content of sediment samples (the percentage of drium spp. are widely distributed in these areas, although the density is low (at most 29 cysts/cm\(^3\)). Relatively higher densities were observed along the southern coastal margins of Hiuchi Nada and the eastern part of Bingo Nada. Cyst abundance in Aki Nada, Hiuchi Nada and Bingo Nada ranged from ND to 6, ND to 18 and 4–29 cysts/cm\(^3\) wet sediment, respectively (Table 1). This area has been characterized by repetitive occurrences of vegetative cells of *A. catenella* (Fig. 3).

In this area, a cyst survey was conducted in Hiuchi Nada and Bingo Nada in 1987 (Japan Fisheries Agency, unpubl. data, 1988). The results show...
particles less than 63 μm). There was a significant correlation between the logarithm of the concentration of cysts and mud content ($r = 0.50; n = 80; P < 0.0001$) and it is of note that many of the samples with cyst concentrations $> 10$ cysts/cm$^3$ are from sediments with a high mud content ($> 70\%$). Thus, higher cyst concentrations tend to be associated with muddy sediments, as reported previously.$^{10,11,15}$ In addition, the area with higher cyst densities in Hiroshima Bay corresponds to the area of $M_2$ (principal lunar semi-diurnal tide) tidal current amplitude less than 5 cm/s.$^{23}$ Moreover, the vertical circulation generated by the prevailing wind in a north–south direction may play an important role in increasing the abundance and dispersal of the cysts in the sediment of the bay.$^{23}$ Therefore, local hydrographic and sedimentary processes may account for the selective deposition of cysts in these areas.$^8,20,22$

For comparison with other areas of the Seto Inland Sea, ranges and means of the cyst abundance are summarized in Table 1. It is clear that the abundance of *Alexandrium* spp. cysts in the western Seto Inland Sea is lower than in the eastern Seto Inland Sea, except for Tokuyama Bay and Hiroshima Bay. However, it must be considered that there is a significant potential for future PSP outbreaks in previously unaffected areas. In addition, it is suggested that the longevity of buried cysts in the sediment is approximately 2–10 years (as a half-life).$^{24}$ Actually, cysts of *A. tamarense* from Hiroshima Bay sediment germinated after 7 years of storage (M Yamaguchi, unpubl. data, 2001). This
Fig. 7 Relationship between abundance of *Alexandrium* spp. resting cysts and the mud content of sediments in the Western Seto Inland Sea.

indicates that once cysts are deposited, the risk of PSP continues for a considerable time. Thus, careful monitoring (i.e. the occurrence of motile cells and assays of shellfish toxicity) are necessary to prevent and/or minimize the effects of PSP incidents in this area.

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