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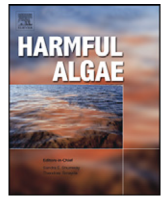

Viability of *Alexandrium tamarense* cysts in the sediment of Funka Bay, Hokkaido, Japan: Over a hundred year survival times for cysts

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ABSTRACT

The abundance of *Alexandrium tamarense* cyst was investigated vertically in a sediment core in Funka Bay, Hokkaido, Japan. Germination experiments were conducted to estimate the germination ability of the cysts at different depth layers of the sediment. Molecular identification with loop-mediated isothermal amplification (LAMP) of *A. tamarense* cysts and core dating with ^{210}Pb methods (CRS model) were performed. The results indicated *A. tamarense* has presented since at least 100 years in Funka Bay and intensive bloom started occurring in the early 1960s and has continued until the late 1980s. Cysts from shallow layers of the sediment core displayed greater viabilities than those from deeper layers. However, successful germination of *A. tamarense* cysts was observed even at the deepest layer, indicating the long-term survival ability (approximately 100 years), and this is the longest record for survival of resting cysts on this species at present. The abundance of viable cysts deposited during the past intensive bloom period was approximately 1.4 times higher than that of recently deposited cysts, suggesting that the cysts in the sub-surface dense layers could potentially function as seed populations in Funka Bay. Thus, dredging the sediment in Funka Bay may involve the risk for initiation of toxic blooms.

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1. Introduction

Funka Bay is an important area for scallop aquaculture in Japan. The annual production of Japanese scallop, *Mizuhopecten* (*Patinopecten*) *yessoensis*, is 100–120 metric tons in Funka Bay, accounting for one-third of total production in Hokkaido (Miyazono, 2006). Nevertheless, contamination of bivalves with paralytic shellfish poisons (PSP), caused by blooms of *Alexandrium tamarense*, is a serious economic hardship to the scallop industry around the bay. Numerous cysts of *Alexandrium* spp. are present in the sediment; these cysts take an important role as the seed stocks for bloom occurrence of *Alexandrium* spp. (Shimada and Miyazono, 2005). However, *A. tamarense* is the major causative plankton for PSP outbreaks in Funka Bay, although *Alexandrium catenella* is responsible for a PSP event in autumn 1988 (Hayashi, 1989; Noguchi et al., 1990), and this dinoflagellate species has not been found in any recent PSP event. Monitoring data of paralytic shellfish poisoning in scallops in Funka Bay revealed that PSP levels were high in the 1980s, but low in the 1990s and that this fluctuation coincided with the appearance of *A. tamarense* (Kudo et al., 2005). Vertical profiles of cyst abundance in the sediment showed the presence of dense subsurface layers of cysts, suggesting the past multi-decadal variation of *Alexandrium* spp. blooms (Miyazono and Nishina, 2007). If these cysts are viable, these dense layers in the subsurface may have caused frequent PSP outbreaks in Funka Bay.

Survival periods of various marine dinoflagellate resting stages have been reported ranging from several months to 100 years (Wall, 1971; Dale, 1983; Blackburn et al., 1989; Ribeiro et al., 2011). In the case of *A. tamarense* cysts, the survival period ranges from 1 year (Anderson and Wall, 1978) to 13 years (Nagai et al., 2007) for the sediment preserved in the laboratory. On the other hand, the period for the fresh sediment ranges from 3 years (McQuoid et al., 2002) to longer than 8 years (Mizushima and Matsuoka, 2004). These findings suggest that the survival period of *A. tamarense* cysts varies depending on the storage condition was adequate or not in nature or under laboratory conditions (i.e., anoxic conditions, water temperatures and light intensity). To understand the survival period of cysts it is necessary to conduct cysts analysis and core dating simultaneously for the same sediment core (Keafer et al., 1992). Sedimentation rate is reported using the ^{210}Pb method in Funka Bay (Matsumoto and Togashi, 1980; Honda et al., 2002, 2003). Based on the sedimentation rate in the bay, a 30 cm-long sediment core may allow us to track back

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approximately 100 years. In this study, we investigated the vertical profile of *A. tamarense* cyst abundance in Funka Bay and conducted germination experiments to estimate the germination ability of cysts originating from a different time period of sedimentation. Our results suggested longer survival periods than those previously reported in marine sediments. The germination success of cysts originating from different sediment depths indicated that some of the subsurface dense cysts may contribute to *A. tamarense* blooms in Funka Bay as a seed stock.

2. Materials and methods

2.1. Hydrography of the study area

Funka Bay is located in the southwest Hokkaido, Japan (Fig. 1) and is semi-enclosed bay. The longitudinal axis of the bay is aligned from north-west to south-east. It lies between 42°00′ and 42°35′ N and 140°18′ and 141°00′ E, with a mean and maximum depth of 38 m and 97 m, respectively. The bay has 2315 km^2 surface areas and is connected to the western North Pacific Ocean through a 30-km-wide shallow channel in its eastern side. Water in the bay is replaced twice a year by the inflow of Tsugaru Warm water from autumn to winter and Oyashio water or subarctic oceanic water from spring to summer (Ohtani, 1971; Ohtani and Kido, 1980). Tsugaru Warm water is originated from Tsugaru Warm Current, flowing into the North Pacific Ocean from the Japan Sea through the Tsugaru Strait, which is one branch of Tsushima Warm Current. Oyashio is a subarctic cold current that circulate counterclockwise in the northern part of the North Pacific Ocean. Sea surface temperature in the bay varies from less than 5 °C in March to more than 20 °C in August–September (Shimada et al., 2000). Salinity is relatively stable, ranging from 31 to 34 psu (Shimada et al., 2000; Odate and Imai, 2003). Bottom water, isolated from surface water, is formed from spring to autumn at the range of 0–5 °C (Ohtani, 1971). Dissolved oxygen is high in winter (>6.0 mg L^{-1}) and low from summer to autumn (<3.0 mg L^{-1}) (Okumura et al., 2011).

2.2. Sediment sampling

The sediment core sample was collected from the deepest point (Stn. A: 42°20′ N, 140°31′ E) in Funka Bay (Fig. 1). A 32 cm-long core was collected using a multiple corer (ASHURA: GS-type corer, inside diameter of core 8.2 cm) in February 2009. The core was sliced every 0.5 cm from 0 to 1 cm, every 1.0 cm from 1 to 10 cm, and every 2.0 cm from 10 to 32 cm, resulting in 22 samples between 0 and 32 cm. To avoid contamination of the sliced core, the outer ring of sediment next to the core liner was separated to remove any material that could have been smeared by the coring tube. Only the inner portion of each sample was subsampled for cyst experiments and sediment dating immediately after the core collection. The subsample for cyst experiments was divided into two plastic tubes. One set of the subsamples was used for estimations of cyst abundance and the batch culture experiments, and another set was used for the experiment to estimate germination success of the incubated cysts. The subsamples were stored at 5 °C in the dark on-board and 3 °C in the dark in laboratory. Exposure to daylight during on-board processes was minimized within 1 h. While evidence of bioturbation was observed in some offshore sediment cores, well-preserved laminated sediments near the study area indicated little bioturbation (Miyazono and Nishina, 2007).

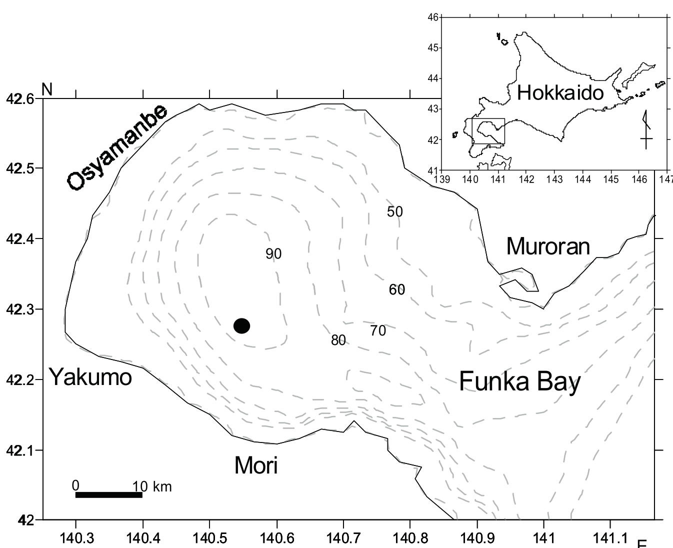

Fig. 1. Sampling location. The core sample was collected at Stn. A.

2.3. Core dating

Sediment samples were dried at 60 °C for over 24 h and then ground with a mortar and pestle. A well-mixed sediment powder was placed in a styrene cube (28 cm^3, approximately 20 g of sediment) for 3 weeks in order to attain radioactive equilibrium. Gamma activity was measured using an ultra-low background Ge semiconductor detector and a multichannel analyzer (MCA-7700, SEIKO EG&G). Using the constant rate of supply (CRS) model, core dating was calculated based on excess ^{210}Pb (Appleby and Oldfield, 1978).

2.4. Abundance of cysts in the sediment

A portion of the sediment sample from each depth interval was used for counting cysts. Counting was performed by the primuline-staining method (Yamaguchi et al., 1995). Aliquots of sediment samples (1 g wet weight) were suspended in distilled water, sonicated, and sieved through a nylon mesh to obtain a size fraction between 20 and 150 μm. The material retained on the 20-μm mesh was then washed, fixed by 5% formaldehyde contained sea water, and stained with primuline. Microscopic observations were made using an epifluorescence inverted microscope (IC-70 with IX-FLA, Olympus, Tokyo, Japan) under blue light excitation. Cyst counts were performed in triplicate, and the results indicate average of triplicate counts ±SD in cysts g^{-1}dry sediment.

2.5. Viability of *A. tamarense* cysts from each sediment layer

The experiment was conducted in middle April 2009. A portion of each sediment sample was used in the batch culture experiment to confirm excystment in all samples of 0–32 cm layers. Aliquots of sediment samples (2 g wet weight) were obtained, sonicated, sieved through a nylon mesh to obtain 10 mL size fractionation between 20 and 150 μm, and finally suspended into 300 mL capacity flasks (Corning) with 150 mL of a modified *f*/2 culture medium (Nagai et al., 2004). The suspensions were incubated at 12.5 °C under 100 μmol photons m^{-2} s^{-1} using a cool fluorescent light with a 12-h light:12-h dark cycle for 2–4 weeks. In order to confirm the excystment of *A. tamarense* cysts from each sediment layer, the morphology of ca. 20 *A. tamarense* vegetative cells from each supernatant of the batch cultures was examined under an inverted microscope immediately after concentration of the supernatant using 20-μm nylon mesh.

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