⟨Note⟩

Historical Record of *Alexandrium* spp. (Dinophyceae) in Southern Coastal Area of Korea

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Abstract - **To investigate the historical record of *Alexandrium* spp. in southern coastal areas of Korea, two sediment cores were collected from Gamak Bay and Yeoja Bay. Germination experiments revealed that the ellipsoidal *Alexandrium* cysts isolated from Gamak Bay and Yeoja Bay are morphologically identical to a toxic dinoflagellate *A. tamarense*. The ellipsoidal *Alexandrium* cysts in Yeoja Bay appeared from 30 to 32 cm depth upwards (ca. 1980s), and their concentration increased around 10 to 12 cm depth (mid-1990s). Similarly, cyst concentration in Gamak Bay also increased from 40 to 44 cm depth (ca. 1990s). These results coincide with the reports of Paralytic Shellfish Poisoning caused by *A. tamarense* in 1980s and 1990s along the southeast coast of Korea.**

Key words : sediment core, *Alexandrium* cyst, germination experiment, Gamak Bay, Yeoja Bay

INTRODUCTION

Marine dinoflagellates are a major component in plankton communities and play an important role as primary producers in marine ecosystems. Among them, some dinoflagellates produce toxins that cause paralytic, neurotoxic, diarrhetic and hepatotoxic shellfish and ciguatera fish poisoning, especially in association with dinoflagellate blooms or red tides (Marret and Zonneveld 2003). For example, several species of the genus *Alexandrium* are well known producers of Paralytic Shellfish Poisoning (PSP) toxins in Korea and Japan coastal areas (Fukuyo 1985; Shin *et al.* 2008). The PSP causes negative environmental impacts and serious economic losses for aquaculture in many coastal areas of the world (Hallegraeff 1993). In Korea, the PSP incidents were frequently reported in 1980s and 1990s, due to the consumption of mussels, *Mytilus edulis* (Chang *et al.* 1987).

Many dinoflagellates including toxic species produce during their life cycle resting cysts that can be preserved in sedi-

ments (Head 1996). Encystment is mostly affected by the environmental factors such as water temperature, salinity, and nutrients (Marrent and Zonneveld 2003), suggesting that studies on distribution of dinoflagellate cysts can help our understanding of environmental characteristics in a given study area. In addition, cyst observations in sediments provide the information on historical occurrences of dinoflagellates in water column, since walls of dinoflagellate cyst made of organic and calcareous that can make them resistant to degradation (Marret and Zonneveld 2003). Here we introduce the historical occurrences of cysts of *Alexandrium* spp. which are well known as causative organisms for Paralytic Shellfish Poisoning (PSP) in southern coastal areas of Korea.

MATERIALS AND METHODS

1. Sampling and cyst analysis

Two sediment cores were collected from Yeoja Bay (Y1) and Gamak Bay (G1), Korea (Fig. 1). Core Y1 and G1 were obtained with a polycarbonate pipe of 10 cm in diameter by a scuba divers in May 2006. Y1 core was 100 cm in length

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whereas G1 was 84 cm. Samples for dinoflagellate cyst analyses from Y1 were undertaken on 1 cm and 2 cm intervals and sliced at 5 cm intervals from the top to 52 cm, and at 10 cm intervals to the bottom, while the core sample from G1 was
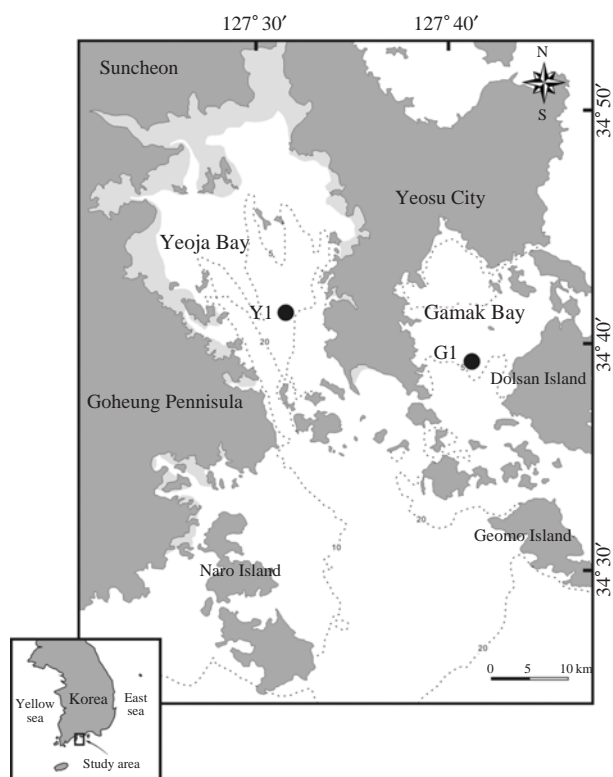

Fig. 1. Sampling stations in Yeoja Bay (Y1) and Gamak Bay (G1).

taken at 4 cm intervals. These subsamples were stored in dark and cool conditions at 4°C prior to further analysis.

The subsamples were processed according to the palynological method suggested by Matsuoka and Fukuyo (2000); approximately 2 g of each sample was placed into an acid-resistant 100 mL beaker and then treated with HCl and HF to remove calcium carbonate and silicate materials, respectively. The residues were rinsed with pure water, sonicated for about 30 sec, and sieved with 125 and 20 µm stainless steel screens of opening mesh size. The residues on 20 µm mesh were made up to 10 mL aliquots with adding of pure water. Identification of cysts of *Alexandrium* spp. was carried out on a 1 mL subsample of the 10 mL. In this study, cyst concentrations of *Alexandrium* spp. are shown as the sum of living and empty cysts and were calculated as number of cysts per grams of dry weight sediment.

According to Shin *et al.* (2010a, 2010b), the sedimentation rate in Y1 was 1.1 cm yr^{-1}, and that of G1 was 2.5 cm yr^{-1}. Consequently, the age of samples in Y1 was estimated to be 1915 AD at −100 cm, while that of G1 was estimated to be 1975 AD at −76 cm.

2. Observation of cysts of *Alexandrium* spp. and vegetative cells

Ellipsoidal cysts of *Alexandrium* spp. in subsamples were identified with the Primuline-staining direct count method
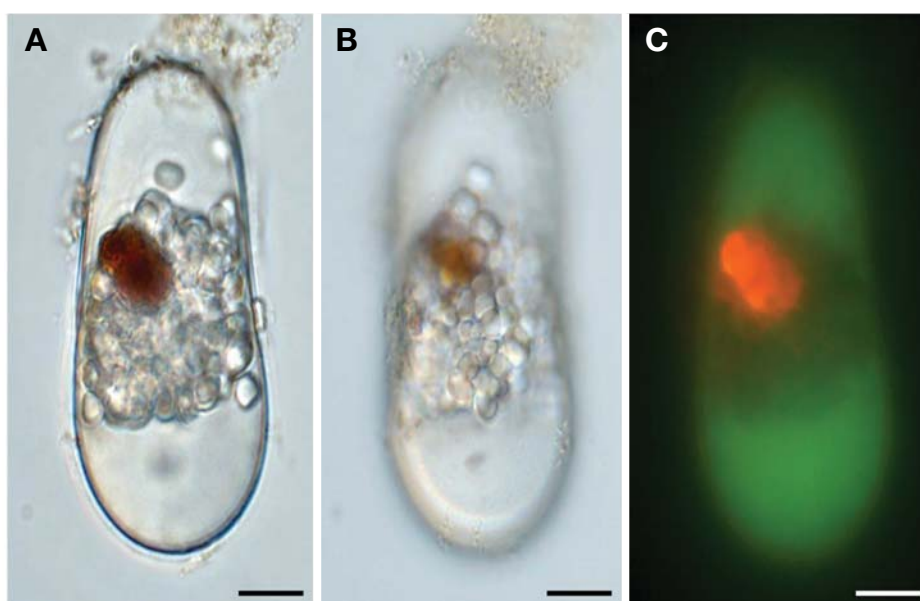

Fig. 2. Morphological features of ellipsoidal *Alexandrium* cysts under normal light (A, B) and UV light (C). Scale bars: 10 µm.

(Yamaguchi *et al.* 1995). The ellipsoidal cysts were isolated to observe the vegetative cells of *Alexandrium* spp. from surface sediments by micropipetting, using a capillary pipette. The isolated cysts were inoculated into the individual wells of 96 well tissue culture plates filled with the same filtered seawater and placed in a coolant bag during isolation. The sorted cysts were cultured at 15°C and ca. 12 µmol photons m^{-2} s^{-1} cool-white illumination under a 12L : 12D photocycle, and were daily checked for the appearance of vegetative cells. The morphological features of cysts and vegetative cells of *Alexandrium* spp. were recorded with a digital camera and scanning electron microscope (SEM).
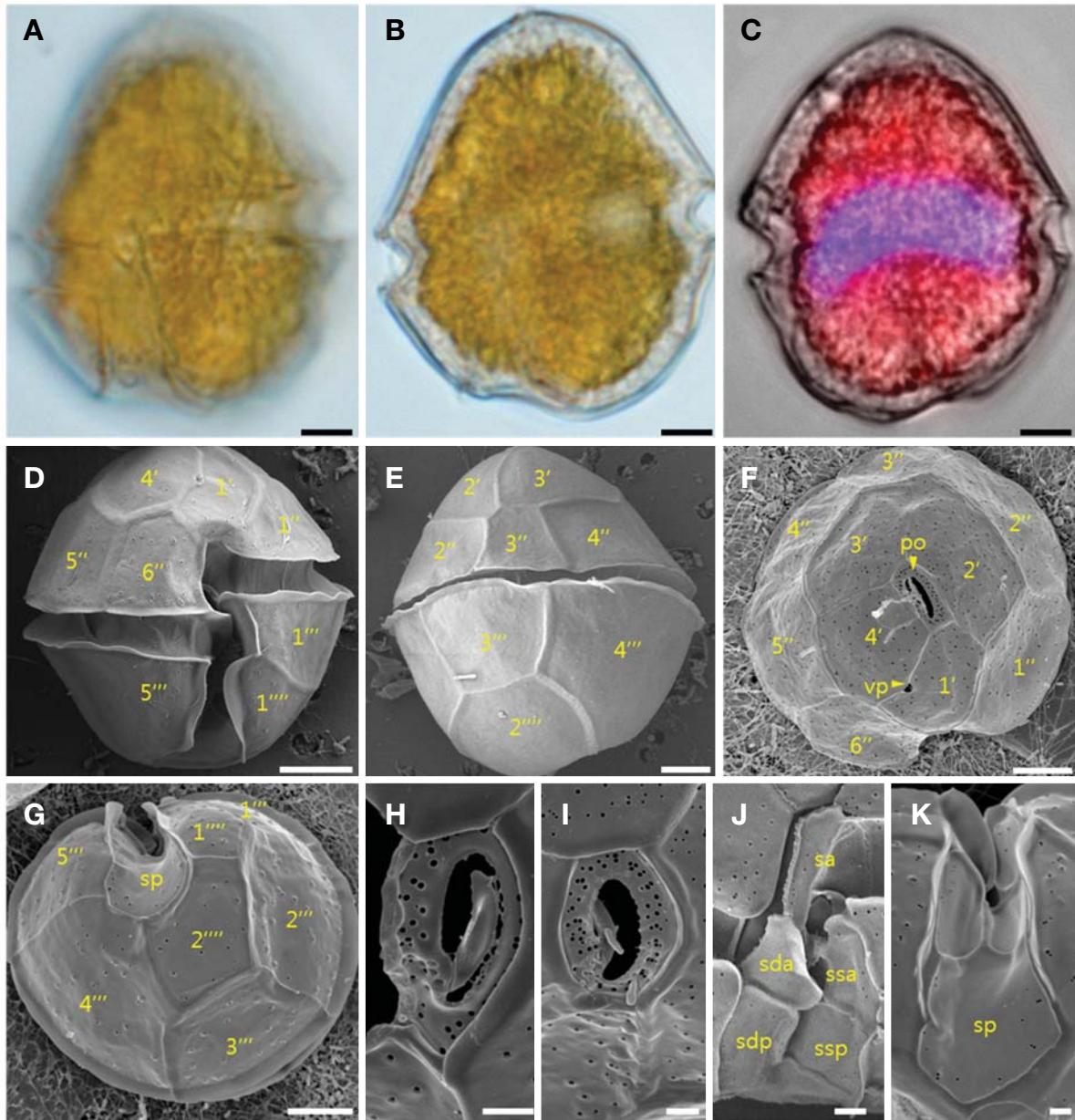

Fig. 3. Light and scanning electron microscopy (SEM) of vegetative cells of *Alexandrium tamarense* germinated from the ellipsoidal *Alexandrium* cysts. A-C: morphological features in light and UV light after staining with DAPI, D: ventral view, E: dorsal view, F: epitheca showing the apical pore plate and ventral pore, G: hypotheca showing the posterior sulcal plate, H and I: apical pore plates, J: sulcal plates, K: posterior sulcal plate. Abbreviation: po, apical pore plate; vp, ventral pore; sp, posterior sulcal plate; sa, anterior sulcal plate; sda, right anterior sulcal plate; ssa, left anterior sulcal plate; sdp, right posterior sulcal plate; ssp, left posterior sulcal plate. Scale bars: 5 (A-G) and 1 µm (H-K).
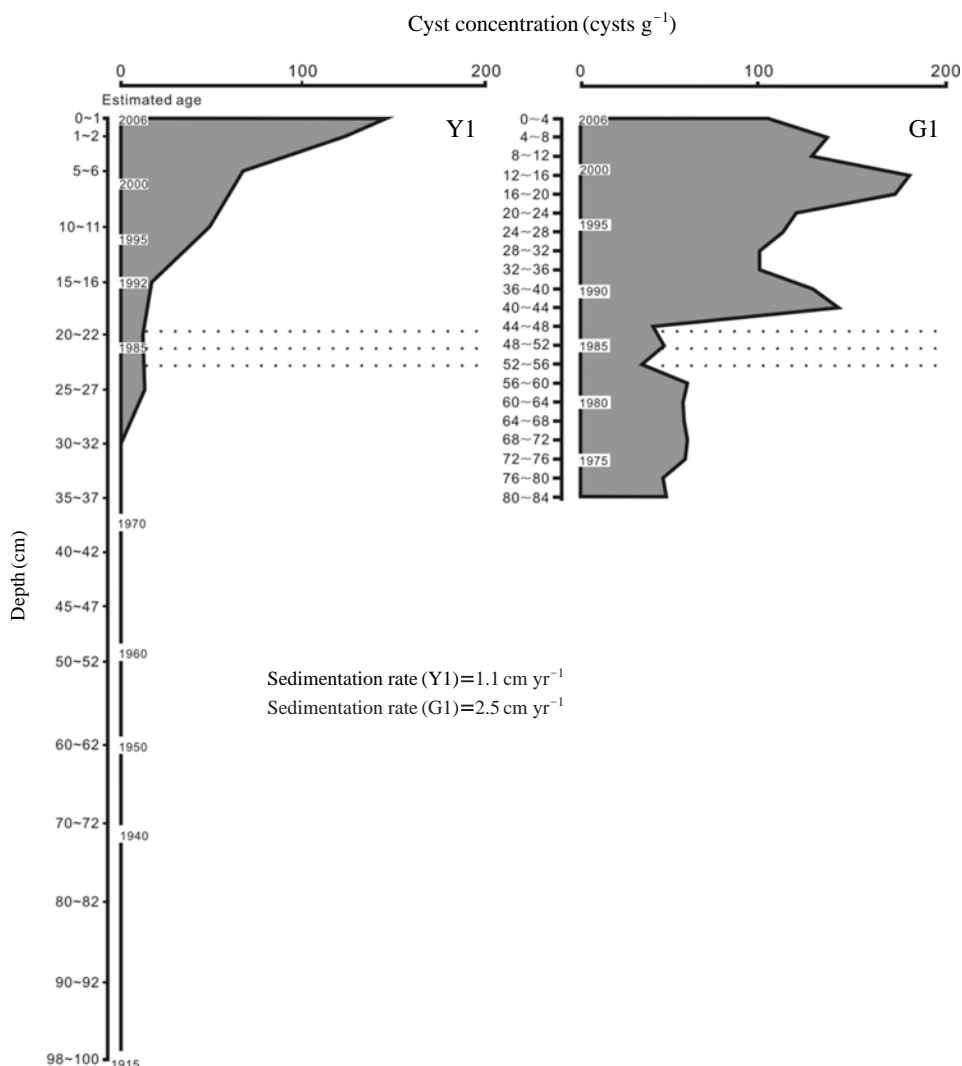
Cyst concentration (cysts g^{-1})

Fig. 4. Changes in concentrations of ellipsoidal *Alexandrium* cyst from Yeoja Bay (Y1) and Gamak Bay (G1).

RESULTS AND DISCUSSION

Most of the cysts of *Alexandrium* spp. observed in Yeoja Bay (Y1) and Gamak Bay (G1) were characterized by ellipsoidal and transparent wall (Fig. 2). In general, the ellipsoidal and transparent *Alexandrium* cysts are known to be produced by *A. catenella*, *A. tamarense* and *A. acatenella*, and these cysts do not shown morphological features that enable to differentiate each other (Yoshida *et al.* 2003). Consequently, germination experiments are required to identify the ellipsoidal and transparent *Alexandrium* cysts, and we have confirmed the vegetative cells from ellipsoidal cysts isolated in

Yeoja Bay and Gamak Bay (Fig. 3). The germinated cells were pentagonal in shape and the first apical pore (1′) was surrounded by 2′, 4′, 1″ and 6′ plates. According to Yoshida *et al.* (2003), most important character differentiating *A. tamarense* from *Alexandrium* spp. is the presence of the ventral pore. The ventral pore (vp) was clearly located in the 1′ plate and the posterior sulcal plate (sp) was also observed. Based on these morphological features, the germinated cells are identical to a toxic dinoflagellate *A. tamarense*, although we could not identify whether all the ellipsoidal cysts are definitely *A. tamarense*.

The ellipsoidal cysts of *Alexndrium* spp. in Yeoja Bay appeared from 30 and 32 cm depth upwards (ca. 1980s),

and their concentration increased around 10 to 12 cm depth (mid-1990s), and the concentration in Gamak Bay also increased in the 1990s (Fig. 4). In Korea, the first PSP caused by *A. tamarense* was reported at Busan in 1986 (Chang *et al.* 1987). In 1996, another PSP incident was reported at Geoje Island, the surrounding waters of which are connected with coastal waters of Busan and Jinhae bays to the northeast (Lee *et al.* 1997). Interestingly, our results are coincided with the reports of PSP outbreaks and historical occurrence of *A. tamarense* along the southeast coast of Korea, indicating that PSP outbreaks and increases of the ellipsoidal *Alexandrium* cysts occurred between the 1980s and 1990s in Korean coastal areas. In addition, *A. tamarense* in Japan was distributed mostly in the Hokkaido and Tohoku regions in the north, until the 1980s (Fukuyo 1985), and PSP caused by *A. tamarense* was first reported in 1992 (Asakawa *et al.* 1993). This suggests that the occurrences of *Alexandrum* cysts in Korean coastal areas may be related to the reports of *A. tamarense* in Japanese coastal areas.

Hallegraeff (1998) suggested that the toxic dinoflagellate *A. tamarense* is spread by ship ballast water. However since Yeoja Bay and Gamak Bay has not been used as a port of large cargo ships with ballast tanks, the possibility that ellipsoidal *Alexandrium* cysts were artificially introduced from other areas into Yeoja Bay and Gamak Bay by the ballast waters seems remote. The dinoflagellate cyst is non-motile stage in their life cycle (Marret and Zonneveld 2003). This indicates that the dinoflagellate cysts can be artificially introduced through the transport of bivalves, and that the presence of currents can lead to the transport of dinoflagellate cysts from where they were originally produce (Matsuoka and Fukuyo 2000). According to Cho (2000), the cysts of harmful bloom-forming dinoflagellates such as *A. tamarense* and/or *A. catenella* have been in Asian coastal areas for a few thousand years, and the Asian coastal areas such as Korea and Japan are characterized by the intrusion of Tsushima Warm Current, which is a branch of the Kuroshio Current. Possibly, the intrusion of Tsushima Warm Current may enhance the growth of *A. tamarense*, thus explaining why PSP outbreaks and increases of ellipsoidal *Alexandrium* cysts occurred between the 1980s and 1995 in Korean and Japanese coastal areas. However, we cannot identify clearly the source of introduction of *Alexandrium* spp. cysts in Yeoja Bay and Gamak Bay, due to the absence of scientific and applied research focused on the introduction of cysts and vegetative cells of *Alexandrium* spp. in Korean coastal areas.

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