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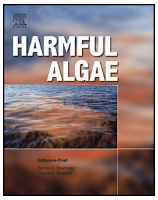

Abundance and distribution of toxic *Alexandrium tamarense* resting cysts in the sediments of the Chukchi Sea and the eastern Bering Sea☆

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ABSTRACT

Abundance and distribution of the toxic dinoflagellate *Alexandrium tamarense* species complex resting cyst were investigated in the eastern Bering Sea and the Chukchi Sea for the first time. Sediment samples (top 0–3 cm depth) were collected from the continental shelf of the eastern Bering Sea (17 stations) and the Chukchi Sea (13 stations) together with a long core sample (top 0–21 cm depth) from one station in the Chukchi Sea during 2009–2012. The cysts were enumerated using the primuline staining method. Species identification of the cysts was carried out with multiplex PCR assay and the plate morphology of vegetative cells germinated from cysts in the both areas. *Alexandrium* cysts were widely detected in the both areas, ranging from not detected (<1 cysts cm^{-3}) to 835 cysts cm^{-3} wet sediment in the eastern Bering Sea and from not detected (<1 cysts cm^{-3}) to 10,600 cysts cm^{-3} in the Chukchi Sea, and all isolated cysts were genetically and morphologically identified as the North American clade *A. tamarense*. Their cysts were mainly distributed in the shallow continental shelf where the water depth was less than 100 m in both areas. The cysts were detected from the deep layer (18–21 cm depth of sediment core) of the long core sample. The present study confirmed the abundant existence of *A. tamarense* with wide range of distribution in these areas. This fact suggests that *A. tamarense* vegetative cells have appeared in the water column in the both areas. Furthermore, these abundant cyst depositions indicate that this species originally distributed in the Arctic and subarctic regions and well adapted to the environments in the marginal ice zone.

1. Introduction

Alexandrium tamarense (Lebour) Balech is a toxic marine dinoflagellate causing Paralytic Shellfish Poisoning (PSP). Because of the morphologically resembling species with *A. tamarense*, *A. catenella* and *A. fundyense*, they are recently grouped within *A. tamarense* species complex (Anderson et al., 2012). PSP occurrences by the toxic *A. tamarense* species complex were only known in Europe, North America and Japan in 1970s (Dale and Yentsch, 1978). Their distribution, however, expanded widely from the subtropical to the subarctic of the north hemisphere and into the temperate south hemisphere (Hallegraeff, 1993; Lilly et al., 2007), and it is suggested that recent ocean climate change affects the distributions of HAB species and their abundances (Hallegraeff, 2010).

The Chukchi Sea within the arctic region and the Bering Sea within the subarctic region belong to the marginal ice zone where is covered with sea ice in winter (from a few months to almost all year round), and the both areas have large continental shelves where water depth are less than 100 m. In recent years, sea ice reduction and increase of water temperature in summer have been reported in both areas (Stabeno et al., 2007; Markus et al., 2009), and some changes of physical environments and ecosystem in both areas have been discussed and reported, (Stabeno et al., 2001; Wassamann et al., 2011; Grebmeier, 2012). Plankton communities have also been influenced by this ocean climate change. For example, anomalous huge outbreaks of *Emiliania*

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huxleyi (Haptophyceae) bloom and abundant jellyfish were observed in the Bering Sea (Sukhanova and Flint, 1998; Brodeur et al., 1999). Changes of primary production and zooplankton community structure are also detected as sea ice reduction in the Chukchi Sea (Arrigo et al., 2008; Matsuno et al., 2011). Furthermore, the modeling study by Walsh et al. (2011) predicted the occurrences of the *Alexandrium tamarense* blooms in the northern part of the Bering Strait along with sea ice reduction. Thus, it has been suggested the occurrences and bloom formations of this species in both areas in the near future.

Despite the environmental changes, ecological studies on *Alexandrium tamarense* species complex and PSP occurrences are limited in the marginal ice zone including the eastern Bering Sea and the Chukchi Sea. Selina et al. (2006) reported the dense *A. tamarense* blooms in the coastal areas of the western Bering Sea and Kamchatka Peninsula (Russia), and their cysts isolated from these areas were shown to be *A. tamarense* eastern north American ribotype, using molecular biological analysis (Orlova et al., 2007). On the other hand, Horner (1984), and Okolodkov and Dodge (1996) did not report the existence of *A. tamarense* species complex in their phytoplankton monitoring survey in the Beaufort Sea and the whole areas of the Arctic Ocean including Chukchi Sea. Occurrences and blooms of this species were confirmed in the western Bering Sea, but its distribution remains almost unknown in the eastern Bering Sea and the Chukchi Sea. Nevertheless, shellfish poisoning and PSP illness for human in Alaska have often been reported since long ago (Hallegraeff, 1993). Lewitus et al. (2012) showed that high PSP toxin contaminations of shellfish have mainly occurred in the coastal areas of south east Alaska (Aleutian Islands, Aleutian Peninsula and Gulf of Alaska), and in the eastern Bering Sea with rather lower frequency than the south east Alaska. There are no reports of PSP incidents in the Chukchi Sea. These facts strongly suggest the existence of the toxic *A. tamarense* species complex in the Bering Sea, while its existence in the Chukchi Sea remains largely uncertain.

The research about the abundance and distribution of *Alexandrium tamarense* species complex in these marginal ice areas is an urgent subject. However, it is likely very difficult to carry out seasonal samplings in these areas to detect *A. tamarense* species complex because of the sea ice coverage during the winter season. So, we focused on the resting stage in the life cycle of this species. Many species of dinoflagellates including *A. tamarense* produce resting stage cells called 'cysts' depositing in the bottom sediments, and it is suggested that these cysts play an important role in bloom initiation as the seed population (Dale, 1977, 1983; Anderson and Wall, 1978; Ishikawa and Taniguchi, 1996). In addition, high cyst abundances in the bottom sediments have been reported from the areas where dense toxic *A. tamarense* species complex blooms have been observed, and these areas mainly locate in the temperate and subarctic areas (Turgon et al., 1990; Yamaguchi et al., 1995a, 2002; Shimada and Miyazono, 2005; Ishikawa et al., 2007; Yamamoto et al., 2009b; Horner et al., 2011). In the western Bering Sea, Orlova et al. (2004) found that *A. tamarense* cysts were dominant in the bottom sediments of the areas where *A. tamarense* bloomed. In this study, in order to estimate the potential of bloom formation in *A. tamarense* species complex in the eastern Bering Sea and the Chukchi Sea, we investigated distribution and abundances of the resting cysts in sediments.

2. Materials and methods

2.1. Sampling

Samplings in the eastern Bering Sea were carried out using the T/S *Oshoro-Maru* of the faculty of Fisheries, Hokkaido University in July 2009, and sediment samples were collected at 17 stations (water depths ranged from 45 to 135 m; Fig. 1). These stations include four stations located in the southern part of St. Lawrence Island and the other 13 stations located in the continental shelf of the south eastern Bering Sea. The sediment samples were collected with a gravity core sampler or a smith-McIntyre grab sampler, and top 3 cm cores were taken into plastic bottles, and stored in the cold-dark condition (1 °C) until analysis.

In the Chukchi Sea, the first samplings were conducted at five stations located in the continental shelf (water depth ranged 45–52 m; Fig. 1) from September to October 2010 with the R/V *Mirai*, Japan Agency for Marine-Earth Science and Technology. Then, the additional samplings were done at eight stations in the wider and deeper areas including the continental shelf and slope of the Chukchi Sea and the Arctic Basin (water depth ranged 30–3852 m; Fig. 1) from September to October 2012 with the R/V *Mirai*. The sediment samples were taken from all stations with the same manner in the Bering Sea. A long core sediment sample (Top 21 cm depth) was collected from one station in the continental shelf (St. 021, water depth of 49 m; Fig. 1) were collected with a gravity core sampler. The long core sediment sample was divided into seven parts with every three centimeter (top 0–3, 3–6, 6–9, 9–12, 12–15, 15–18 and 18–21 cm). All sediment samples were stored in the dark at 1 °C until use.

2.2. Cyst enumeration

The enumeration of the *Alexandrium tamrense* species complex cysts (Fig. 2A and B) was made by following the primuline-staining direct count method (Yamaguchi et al., 1995b). Aliquots of 5.0 g wet sediment samples were suspended in distilled water, sonicated for 60 s and sieved through plankton nets to obtain the size fraction between 20 and 150 μm. Size fractionated sediment samples were suspended in 10 mL distilled water with 15 mL centrifuge tubes. After the fixation with 1.0% glutaraldehyde for 30 min, these suspensions were centrifuged at 700 × *g* for 10 min. The pellets were suspended in 10 mL methanol with 15 mL centrifuge tubes and placed in a refrigerator for 2 days to remove algal fluorescent pigments. Then methanol suspended samples were centrifuged at 700 × *g* for 10 min, and pellets were suspended in 9 mL distilled water with 15 mL centrifuge tubes. One mL of primuline stock solution (2.0 mg mL^{-1}) was added to each tube (final concentration: 0.2 mg mL^{-1}) and left for 1 h in the dark. After the staining, the supernatants containing excess primuline were removed using centrifugation (700 × *g* for 10 min), and pellets suspended in 10 mL distilled water. After the same washing using centrifugation, pellets were finally suspended in distilled water to 5.0 mL total for microscopic observations. Each stained 0.1 mL-sample was placed uniformly on a plastic chamber slide (19 mm × 44 mm; Chamber Slide II, IWAKI) with dilution to distilled water with the addition of distilled water (0.9 mL) and observed with an inverted epifluorescence microscope (Eclipse TE200, Nikon) under the blue light excitation (450–490 nm). When it is difficult to find and identify *Alexandrium* cysts with thick sedimentation, a dissecting needle was used for the cysts sorting. The cysts were counted in triplicate for each sample, and the cyst densities per g wet sediment were determined from the average value of the cyst numbers. Then, the cyst densities per g wet sediment were converted to the cysts per cm^{-3} sediment using the specific gravity of each sediment sample. The specific gravity of sediment samples were obtained by following Kamiyama (1996). Each sediment sample (2–3 g wet weight) was suspended in correctly measured 10 mL distilled water with a plastic 15 mL graduated cylinder on a flat weighing machine, and the weight of added sediment samples and increased volumes were recorded. The apparent specific gravity of wet sediment was calculated by dividing added sediments (g) by increased volume

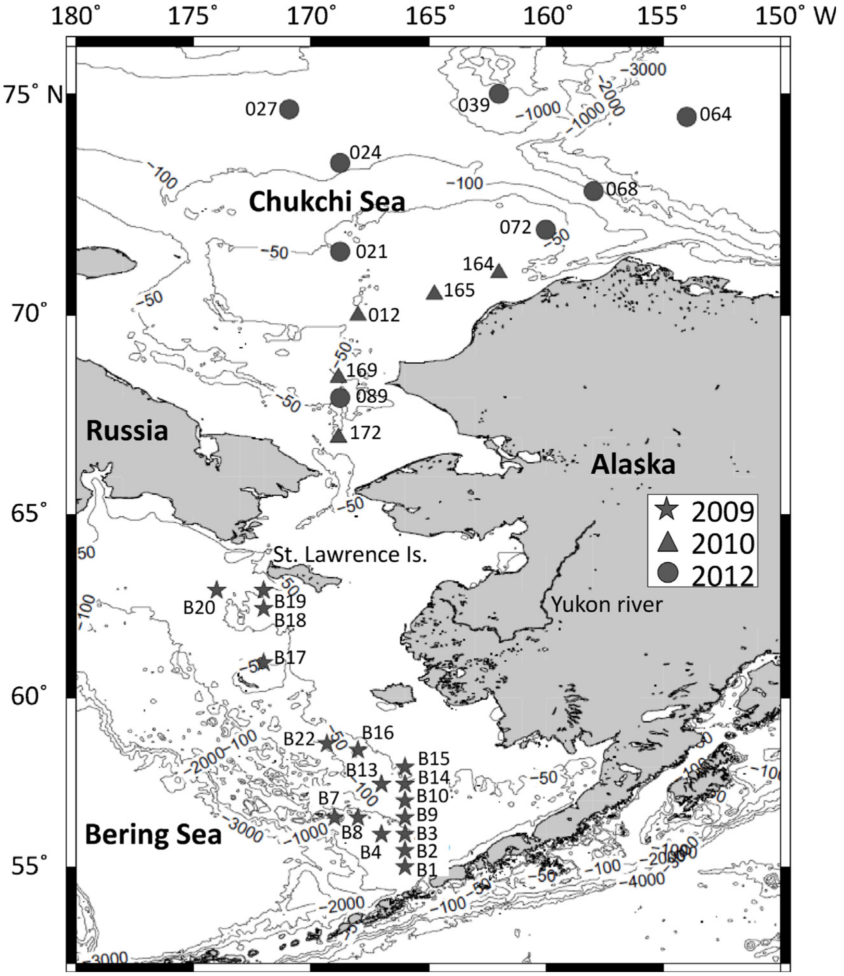

Fig. 1. Location of sampling stations in Chukchi Sea and the eastern Bering Sea. ★, ▲ and ● indicates sampling stations by 2009, 2010 and 2012 cruises, respectively.

(mL). After the measurement of the specific gravity, the samples (2–3 g wet weight) were used for the measurements of mud contents. The samples were sieved using a 63 μm nylon mesh sieve. Fractionated samples (<63 μm particles which are defined as mud including silt and clay, and >63 μm particles which are defined as sand) were weighed after the drying at 95 °C, and mud contents were calculated following the equation.

$$\text{Mud contnets}(\%) = 100 \times \frac{\text{Dry weight of } < 63\ \mu\text{m particles (g)}}{\text{Dry weight of } > 63\ \mu\text{m particles (g)} + \text{Dry weight of } < 63\,\mu\text{m particles (g)}}$$

2.3. Species identification of the cysts

Alexandrium tamarense species complex cysts were known to be unable to morphologically distinguish between *A. tamarense* and *A. catenella* (Fukuyo, 1985). Furthermore, these species had been identified based on the morphological characters of thecal plates of their vegetative cells (Balech, 1995), but new methods for the identification of these species have been recently proposed based on molecular techniques (Anderson et al., 2012). Therefore, the species identification were carried out for the cyst populations in the eastern Bering Sea and the Chukchi Sea, based on not only the cyst morphology but also the molecular diagnostic technology and the plate morphology of the germinated vegetative cells.

From the sediment sample of the maximum density in the eastern Bering Sea and the Chukchi Sea (St. B14 in the eastern Bering Sea and St. 165 in the Chukchi Sea; Figs. 1 and 3), 25 and 60 cysts of *Alexandrium tamarense* species complex were picked up with Pasteur pipettes respectively and applied to the multiplex PCR assay developed by Nagai (2011) for species identification. The live cysts were isolated using a capillary and inoculated into 20 μL of tris-EDTA (TE buffer) in a 1.5 mL sampling tube and homogenized for 15 s by using of a pellet pestle. TE buffer (30 μL) was added to the tubes, and the solution in each tube was boiled for 15 min to extract DNA. Six primer pairs mix (Atama-F3-R1, Acat-F3-R2, Atami-F1-R1, Afra-F1-R3, Affn-F1-R2 and Apseu-F2-R2) for detecting six *Alexandrium* species (*A. tamarense* North American clade, *A. catenella* temperate Asian clade, *A. tamiyavanichii*, *A. fraterculus*, *A. affine* and *A. pseudogoniaulax*) simultaneously were used for multiplex PCR assay. The PCR was carried out on a thermal cycler (PG-808, ASTEC) in a reaction mixture containing 5 μL of 2× type-it Microsatellite PCR Master Mix reaction mixture (Qiagen), 0.2 μL of 10 mM of each primer (0.2 μM final concentration), 1 μL template DNA, and ultra-pure-quality water added to obtain a volume of 10 μL. Conditions of the PCR cycle were as follows: 5 min at 95 °C followed by 30 cycles of 30 s at 95 °C, 90 s at 60 °C, and 30 s at 72 °C, and a final elongation step for 30 min at 60 °C. Species identification was done by electrophoresing PCR products on 1.5% agarose gel.

Germination experiments were conducted for species identification based on the morphology of thecal plates. Twenty *A. tamarense* species complex cysts were isolated in the same manner

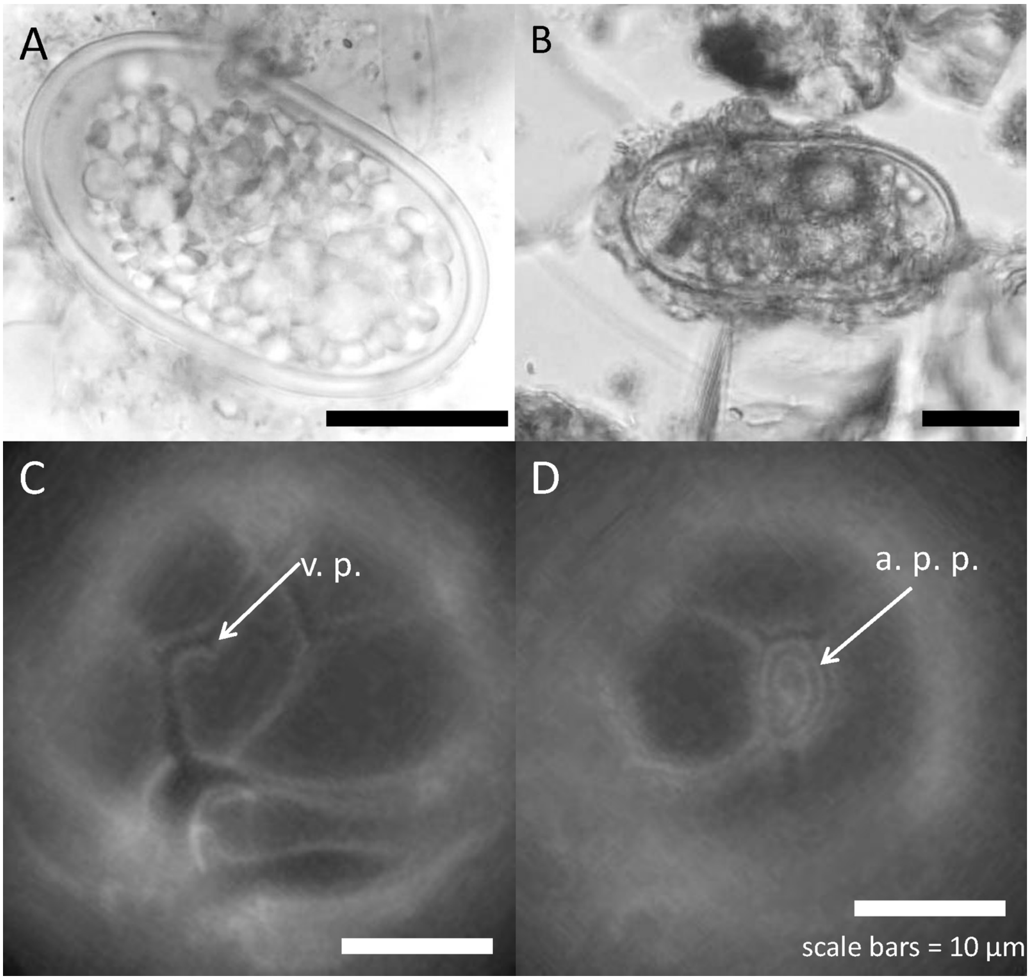

Fig. 2. Photomicrographs of naturally occurring *Alexandrium tamarense* species complex cysts in sediments samples in Chukchi Sea at 0–3 cm layer (a) and 18–21 cm layer (b), and of thecal plates of the *A. tamarense* species complex vegetative cell germinated from the sediment sample in the Chukchi Sea (c and d). After the formaldehyde fixation, this cell was stained by Calcofluor white and observed with an inverted epifluoresence microscope under UV light excitation. Scale bars = 10 μm. c. Ventral view. d. Apical view. White arrows in c and d indicate ventral pore (v.p.) and apical pore plate (a.p.p.), respectively.

of the multiplex PCR assay from the sediments of both areas, and each isolated cyst was inoculated into a well of the 24-well culturing plate with f/2 medium and incubated for germination at 5 °C under a 14 h light: 10 h dark photo-cycle, with light intensity of 50 μmol photons $m^{-2} s^{-1}$ to obtain geminating cells. The incubation was done for 2 weeks in March 2012. When the germination from the isolated cysts was observed, germinated cells were stained with fluorescent dye (Fluororescent Brightner28, SIGMA) after the fixation with 1% formaldehyde and observed with an inverted epifluorescence microscope (Eclipse TE200, Nikon) under UV light excitation (365 nm) following Fritz and Triemer (1985).

3. Results

3.1. Cyst abundance and spatial distribution

Fig. 3 presents the abundance and spatial distribution of *Alexandrium tamarense* species complex cyst in the Chukchi Sea and the eastern Bering Sea. Cyst densities in the eastern Bering Sea ranged from not detected (<1 cyst cm^{-3}) to 835 cysts cm^{-3}, and higher densities were observed along the shallow and coastal areas in the continental shelf of the south eastern Bering Sea (cyst densities ranged 159–835 cysts cm^{-3}; water depth ranged 54–84 m; Fig. 3B). On the contrary, cyst densities at stations in the south of the St. Lawrence Island and far from the coastal area were relatively low or not detected. In the Chukchi Sea, cyst densities ranged from not detected (<1 cyst cm^{-3}) to 1.06×10^4 cysts cm^{-3}. The cyst abundances were much higher in the continental shelf to the northern part of the Bering Strait than in deeper areas including the continental slope and the Arctic Basin (Fig. 3A).

3.2. Species identification

As the result of multiplex PCR assay, only *Alexandrium tamarense* belongs to the North American clade (Scholin et al., 1994) was detected from the picked up cysts in both areas. All germinated cells from the isolated cysts were rounded and solitary or rarely pairs, and had a small ventral pore on the first apical plate and the fishhook-shaped apical pore in the apical pore plate (Fig. 2C and D). From these morphological characters, germinated cells were identified as *A. tamarense* following Balech (1995).

3.3. Relationships between cyst abundances and mud contents, and water depth

Relationships between cyst abundances and mud (<63 μm sediment particles) contents in the eastern Bering Sea and the Chukchi Sea are presented in Fig. 4A and B, respectively, and Fig. 4C

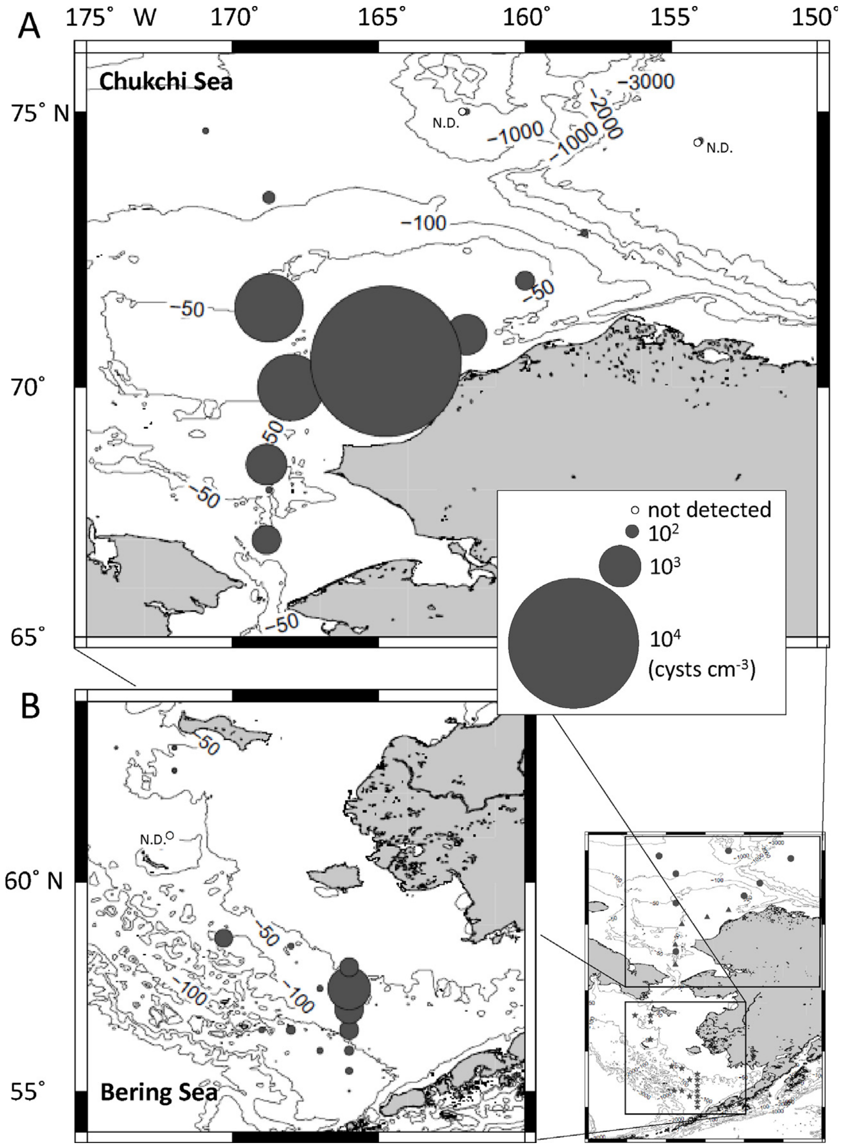

Fig. 3. Spational distributions of abundances in *Alexandrium tamarense* species complex resting cysts (cysts cm^{-3}) in the Chukchi Sea (a) and the eastern Bering Sea (b).

showed the relationships between cyst abundance and water depth in both areas. Mud contents at the stations where high cyst densities (>100 cysts cm^{-3}) were observed in the eastern Bering Sea and the Chukchi Sea ranged widely from 11.2% to 66.5% and from 2.4% to 85.6%, respectively. Thus, there were no significant correlation between cyst densities and mud contents. On the other hand, high cyst densities (more than 100 cysts cm^{-3}) were found in the stations where water depth was less than 100 m in both areas (ranging from 30 m to 84 m), and few cysts were detected at the stations with water depth of more than 150 m (cyst densities ranged less than 1 cyst cm^{-3}).

3.4. Vertical distribution

Vertical distribution of the *Alexandrium tamarense* species complex cysts is shown in Fig. 5. In all divided sediment samples from the 21 cm long core sample, *A. tamarense* species complex cysts were detected ranging from 73 to 2.82×10^3 cysts cm^{-3}. Vertical distribution revealed the first peak at the top 0 – 3 cm sediment sample (2.14×10^3 cysts cm^{-3}) and the second peak at the 9–12 cm depth sediment sample (2.82×10^3 cysts cm^{-3}).

4. Discussion

4.1. Existence of Alexandrium tamarense in the eastern Bering Sea and the Chukchi Sea and the risk of PSP occurrences

The present study confirmed the existence of *Alexandrium tamarense* species complex resting cysts in the eastern Bering Sea and the Chukchi Sea, and this cyst deposition was occupied by *A. tamarense* North American clade. Table 1 presents the comparison of cyst abundances in both areas to other areas where high cyst abundance and the dense blooms of *A. tamarense* species complex were reported. This table shows that the maximum density in the eastern Bering Sea (835 cysts cm^{-3}) is nearly the same as the cyst density in the Okhotsk Sea of the northern coast of Japan (1022 cysts cm^{-3}), and the highest cyst abundance in the Chukchi Sea (1.06×10^4 cysts cm^{-3}) was roughly the same as those in the Puget Sound, Gulf of Maine and the Japanese enclosed bay in the temperate region where large *A. tamarense* species complex blooms and high toxin contamination of bivalves have frequently occurred (cyst abundances of 4454–12,125 cysts cm^{-3}). These abundant cyst depositions strongly suggest that *A. tamarense* have

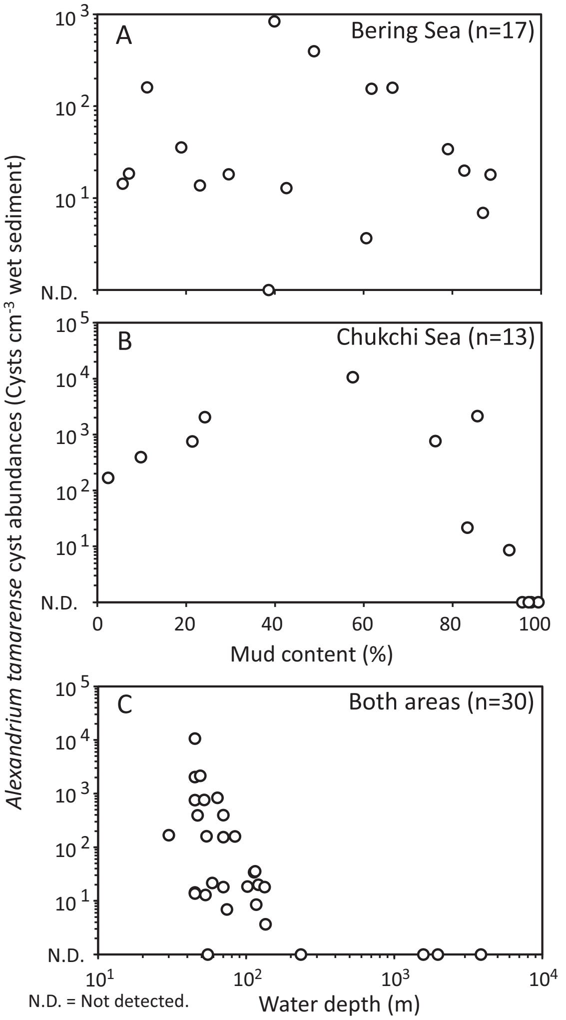

Fig. 4. Relationships between abundance of *Alexandrium tamarense* species complex resting cysts and the mud content of sediments at 13 stations in Chukchi Sea (a) and 17 stations in the eastern Bering Sea (b), and relationship between abundance of the resting cysts and water depth of 30 stations in both areas (c).

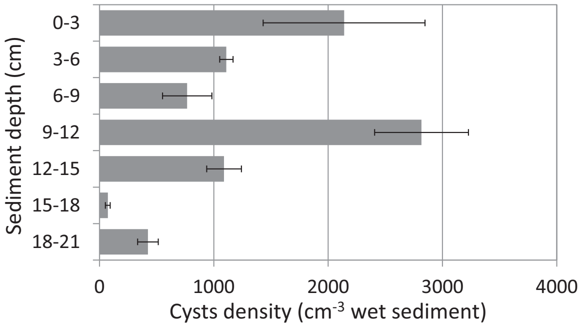

Fig. 5. Vertical distribution of *Alexandrium tamarense* species complex resting cysts in the core sediments collected from St. 021 in Chukchi Sea by the 2012 R/V *Mirai* cruise.

appeared in the water column with the same level as the other PSP occurrence areas in large continental shelves of the eastern Bering Sea and the Chukchi Sea, while the toxicity of vegetative cells has not been uncertain in these areas.

In the present study, we also revealed for the first time the distribution of *Alexandrium tamarense* cysts in the Chukchi Sea at the highest level in the world. Such abundant cyst deposition of this species hitherto has not been known in the large marginal ice zone including the Arctic and subarctic regions. This fact indicates that *A. tamarense* has adapted to the environments of the Arctic shallow areas including Chukchi Sea, although the ecology of the *A. tamarense* vegetative cells and/or PSP incidents still remain largely unknown. Careful researches should be conducted about these problems in the eastern Bering Sea and the Chukchi Sea in the future.

4.2. Relationship between the cyst distribution and mud contents

Distributions of dinoflagellate cysts were known to be strongly influenced by the hydrographic conditions in their sedimentary processes (Tyler et al., 1982), and similar distributions of cysts and mud contents were reported in many other areas (Anderson and Keafer, 1985; Yamaguchi et al., 1995a, 2002; Shimada and Miyazono, 2005; Horner et al., 2011). In this study, however, any significant relationships were not found between the *A. tamarense* cyst densities and mud contents in the eastern Bering Sea and the Chukchi Sea, and this is a quite interesting result as compared with the results in other areas. Some authors reported that cyst distributions did not always show the same tendency to the distributions of muddy sediments (Yamaguchi et al., 1995a; Ishikawa et al., 2007; Yamamoto et al., 2009b), and they also suggest that the distribution of vegetative cells sometimes directly decides the cyst distribution. In the same way, it seems that in the eastern Bering Sea and the Chukchi Sea, the cyst distribution pattern was more heavily influenced by the mechanisms of the cyst supply from vegetative cell populations in the water columns than hydrographic effects in the cyst sedimentary processes. In other words, the cyst distribution might directly reflect the distribution of vegetative cells in both areas. The shallow continental shelf (water depth ranged from 50 to 100 m) in the south eastern Bering

Table 1
Comparison of cyst abundances in the bottom sediment between this study and other sea areas where high cyst abundances and dense *Alexandrium tamarense* species complex blooms were reported.

Sea Area	Maximum cyst density	Reported species	Refference
St. Lawrence Estuary, Canada	1500 cysts cm^{-3}	*A. tamarense*	Turgeon et al., 1990
Hiroshima Bay, Japan	4454 cysts cm^{-3}	*A. tamarense*	Yamaguchi et al., 2002
Tokuyama Bay, Japan	8137 cysts cm^{-3}	*A. catenella*	
Bay of Fundy in Gulf of Maine, USA	2000 cysts cm^{-3}	*A. fundyense*	Anderson et al., 2005
Sea of Okhotsk, Japan	1022 cysts g^{-1}	*A. tamarense*	Shimada and Miyazono, 2005
Funka Bay, Japan	2568 cysts g^{-1}	*A. tamarense*	
Mikawa Bay, Japan	7311 cysts cm^{-3}	*A. tamarense*	Ishikawa et al., 2007
Osaka Bay, Japan	5683 cysts cm^{-3}	*A. tamarense*	Yamamoto et al., 2009b
Puget Sound, USA	12,125 cysts cm^{-3}	*A. catenella*	Horner et al., 2011
Gulf of Maine, USA	6715 cysts cm^{-3}	*A. fundyense*	McGillicuddy et al., 2011
Eastern Bering Sea	835 cysts cm^{-3}	*A. tamarense*	This study
Chukchi Sea	10,600 cysts cm^{-3}	*A. tamarense*	

Sea where high cyst densities were recorded is called as MSD (Middle Shelf Domain), and MSD is known to develop the strong stratification in summer (Coachman, 1986; Schumacher and Stabeno, 1998). Furthermore, Shimada et al. (2006) and Woodgate et al. (2010) reported that inflow of warm Pacific Summer Water (PSW) passed through the Bering Strait to the continental shelf of the Chukchi Sea has been recently increasing, and Walsh et al. (2011) concluded in his model simulation that the occurrences of *A. tamarense* will be frequent in the Chukchi Sea as increasing inflow of PSW. In these ways, some specific ocean environments would affect the growth of *A. tamarense*, and cyst abundances revealed in this study would reflect the recent bloom records in both areas. The investigations are urgently needed on the population dynamics of *A. tamarense* in the water columns together with the cyst distribution.

4.3. Relationship between the cyst distribution and marine environments

High cyst abundances in the shallow areas where the water depth was shallower than 100 m in the eastern Bering Sea and the Chukchi Sea clearly indicates that the water depth affects the cyst distribution in both areas. Light excitation has been reported to promote the incidents of some dinoflagellate cysts germination (Kremp, 2001), and the newly germinated cells of *Alexandrium tamarense* are thought to require the vertical movements from the bottom to the euphotic zone for the success of the proliferation. Accordingly, the light condition would be important for the cyst germination at the sea bottom and the growth of vegetative cells with enough light at the euphotic layer. Thus, it is considered that the abundant cyst depositions of *A. tamarense* occurred in the shallow areas of continental shelves, as the results of the course of life cycle including excystment, multiplication in the water column, and encystment in the same areas.

Abundant cyst depositions in the shallow continental shelf in the eastern Bering Sea and the Chukchi Sea also suggest the existences of *Alexandrium tamarense* in the coastal areas such as enclosed embayments, lagoons and estuaries, because the dense blooms and PSP incidents were mainly reported from the coastal areas (Franks and Anderson, 1992; Mėndez et al., 1996; Sorokin et al., 1996; Itakura et al., 2002; Fauchot et al., 2005; Genovesi et al., 2008; Yamamoto et al., 2009a). Therefore, *A. tamarense* appearances have probably occurred in some environments in the coastal areas of north Alaska (e.g. fjords, lagoons and estuary of Yukon River).

Few cysts were detected at the some shallow stations where water depth was less than 100 m in the eastern Bering Sea (St. B13, B16, B17, B18, B19 and B20) and the Chukchi Sea (St. 089). Most of these stations in the Bering Sea (St. 17, 18, 19 and 20) located at the south of the St. Lawrence Island, and this area forms the open water area during winter called 'polynya' (Smith et al., 1990). Moreover, in a such type of the station in the Chukchi Sea (St. 089), a novel oxygen deficient water was firstly detected at the bottom layer ($<3.0\ \mathrm{mg\ O_2\ L^{-1}}$) during the R/V *Mirai* cruise in 2012 (Kikuchi, personal communication). These local environments are also supposed to largely affect the cyst distribution. Consequently, it is needed to consider the various specific environments in the marginal ice zone and coastal areas, and to study *Alexandrium tamarense* ecology in the Arctic and subarctic regions.

4.4. Vertical distribution

The vertical distribution of the *Alexandrium tamarense* cyst in sediment has been reported to reflect the past bloom records in the sea (Yamaguchi et al., 1995a; Mizushima and Matsuoka, 2004; Miyazono and Nishina, 2007; Miyazono et al., 2012), and they tried to determine the first appearance and succeeding durations by knowing the sedimentation rate and the vertical distribution of cysts densities. Sedimentation rates were not investigated in this study, but McKay et al. (2008) reported that the mean sedimentation rate at a station in the continental shelf of the Chukchi Sea was 151 cm per 1000 years. Based on this datum and our findings that cysts were detected in all sediment samples which were divided from the top 21 cm long core sample, *A. tamarense* have been inhabiting in the Chukchi Sea from at least 120 years ago. Furthermore, the second peak at the 9–12 cm depth indicates the relatively succession duration for *A. tamarense* in about 60–80 years ago in this area. These inferences suggest that *A. tamarense* has originally distributed in the Arctic area regardless the recent drastic reduction of sea ice. Moreover, this specific distribution in the Chukchi Sea might be a key to explain the wide distribution of *A. tamarense* North American clade in the north hemisphere including the Atlantic and Pacific coastal areas. More detailed molecular phylogenetic analyses using the Arctic region strains of *A. tamarense* should be needed to know mechanisms of the range extension and globalization in *A. tamarense*.

5. Conclusion

In the present study, we confirmed wide distributions of the North American clade *Alexandrium tamarense* species complex by morphology and molecular-based techniques and also revealed high abundances of the resting cysts in sediments in the eastern Bering Sea and the Chukchi Sea, suggesting that this species is originally distributed to the Arctic regions, and may have caused dense bloom and PSP toxin contaminations in wild creatures in these regions from 120 years ago at least. Resting cysts are thought to be an adaptive survival strategy for overwintering in the marginal ice zone around the Arctic Ocean.

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