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The physiological adaptations and toxin profiles of the toxic *Alexandrium fundyense* on the eastern Bering Sea and Chukchi Sea shelves

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ABSTRACT

Abundant cyst distributions of the toxic dinoflagellate *Alexandrium fundyense* (previous *A. tamarense* north American clade) were recently observed on the north Chukchi Sea shelf and on the eastern Bering Sea shelf, suggesting that *A. fundyense* is both highly adapted to the local environments in the high latitude areas and might cause toxin contamination of plankton feeders. However, little is known about the physiological characteristics and toxin profiles of *A. fundyense* in these areas, which are characterized by low water temperatures, weak sunlight, and more or less permanent ice cover during winter. To clarify the physiological characteristics of *A. fundyense*, the effects of water temperature and light intensity on the vegetative growth and toxin profiles of this species were examined using *A. fundyense* strains isolated from one sediment sample collected from each area. Using the same sediments samples, seasonal changes of the cyst germination in different water temperatures were investigated. Vegetative cells grew at temperatures as low as 5 °C and survived at 1 °C under relatively low light intensity. They also grew at moderate water temperatures (10–15 °C). Their cysts could germinate at low temperatures (1 °C) and have an endogenous dormancy period from late summer to early spring, and warmer water temperatures (5–15 °C) increased germination success. These physiological characteristics suggest that *A. fundyense* in the Chukchi Sea and eastern Bering Sea is adapted to the environments of high latitude areas. In addition, the results suggest that in the study areas *A. fundyense* has the potential to germinate and grow when water temperatures increase. Cellular toxin amounts of *A. fundyense* strains from the eastern Bering Sea and Chukchi Sea were ranged from 7.2 to 38.2 fmol cell^{-1}. These toxin amounts are comparable with *A. fundyense* strains isolated from other areas where PSP toxin contamination of bivalves occurs. The dominant toxin of the strains isolated from the Chukchi Sea was saxitoxin, while most *A. fundyense* strains from the eastern Bering Sea are dominated by the C2 toxin. Toxin profiles similar to those detected in Chukchi Sea have not been reported by any previous research. The dominance of a highly toxic PST variant in Chukchi *A. fundyense* suggests that presence of the species at low cell concentrations may cause toxin contamination of predators. This study revealed that abundant *A. fundyense* cysts deposited on the eastern Bering Sea and Chukchi Sea shelves potentially germinate and grow with PSP toxin contents in the local environments. In conclusion, a high risk of PSP occurrences exists on the eastern Bering Sea and Chukchi Sea shelves.

1. Introduction

The toxic dinoflagellate *Alexandrium fundyense* Balech is known to be distributed mainly in temperate to subarctic coastal waters (Steidinger and Tangen, 1997; Lilly et al., 2007). However, occurrences of this species have recently been reported from colder regions around the Arctic region. Distributions of their

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resting cysts and vegetative cells have been revealed from the coastal areas of the Kamchatka Peninsula to the Bering Strait in the western Bering Sea (Selina et al., 2006; Orlova and Morozova, 2013). The paralytic shellfish poisoning (PSP) toxin contamination by *A. fundyense* has been reported on scallops from the west coast of Greenland (Baggesen et al., 2012) and on mussels from the coast of Iceland (Burrell et al., 2013). These evidences suggest that *A. fundyense* has the potential to form toxic blooms not only in the temperate to subarctic areas where their toxic bloom occurrences were originally known, but also in the Arctic region including the Bering Sea where their occurrences were recently reported. The occurrence of *A. fundyense* was originally reported from the coastal area near Point Barrow in the Chukchi Sea of the Arctic Ocean during summer (Bursa, 1963). After that, however, their occurrences were not observed in the Arctic Ocean, including the Chukchi Sea (Horner, 1984; Okolodkov and Dodge, 1996). The occurrences of *A. fundyense* vegetative cells are also unknown in the eastern Bering Sea. However, abundant depositions of *A. tamarense* (north American clade, which clade is recently grouped in *A. fundyense*; John et al., 2014) resting cysts were found in the bottom sediments surfaces of the vast continental shelves of the eastern Bering Sea and Chukchi Sea (Gu et al., 2013; Natsuike et al., 2013). The findings suggest that *A. fundyense* cysts deposited on sediments potentially germinate, and the germinated vegetative cells appear in the water column containing PSP toxins on the eastern Bering Sea and Chukchi Sea shelves. In addition, *A. fundyense* shows regional differences in physiological characteristics, especially concerning the effects of temperature and light intensity on growth and germination (Miyazono, 2002; Yamamoto and Tarutani, 1997). Therefore, *A. fundyense* strains around the Arctic region are suspected to be adapted to colder temperature and lower light intensity. Furthermore, Anderson et al. (1994) suggests that the toxin profiles of *A. fundyense* are different between local populations, and that toxicities are higher in higher latitudes than lower latitudes in northeastern Canada. Oshima et al. (1982) also report different toxin profiles for *A. fundyense* strains collected from different areas in north Japan.

Both the eastern Bering Sea and Chukchi Sea have vast continental shelves and are covered with sea ice during the winter season; Sea ice coverage occurs roughly from January to April in the eastern Being Sea and from December to June in the Chukchi Sea shelf. Recently, warming climate changes and the increase of water temperature were reported from both shelves (Grebmeier, 2012; Hunt et al., 2011). On the eastern Bering Sea shelf, the climate regime shift from a cold water period to a warm water period has been reported to occur repeatedly in a span of several years from at least 1970′s (Hunt et al., 2011). The climate shift to warming caused increase of water temperature comparing the cold water period during summer in the eastern Bering Sea shelf where the water depth ranged from approximately 50–100 m (Hunt et al., 2011; Ohashi et al., 2013). For example, Hunt et al. (2011) and Ohashi et al. (2013) reported the recent clime regime shift from the warm water period during 2001–2005 to the cold water period during 2007–2009. During these periods, the maximum water temperatures of surface and bottom layers during the summers of the warm period were ranged from 12 to 15 °C and 3–5 °C, while those temperatures during summers of the cold period ranged 8–12 °C and 0–2 °C (Hunt et al., 2011; Ohashi et al., 2013). On the Chukchi Sea shelf, recent global warming was suspected to cause the drastic sea ice reduction (Shimada et al., 2006; Woodgate et al., 2010). The sea ice reduction during summer introduced the inflow of the Pacific summer waters from the Bering Sea, and thus water temperature has recently increased during summer on the Chukchi Sea shelf (Shimada et al., 2006). In these ways, it could be hypothesized that climate warming might promote cyst germination and subsequent growth in the water column and thereby favor bloom development and toxicities in Arctic waters.

In these ways, the abundant *A. fundyense* cysts on the eastern Bering Sea and Chukchi Sea can be considered to germinate and appear with PSP toxins in the colder and lower light conditions that are characteristic of the Arctic region. Furthermore, the recent warming climate changes in both areas may be more conducive to their germination and vegetative growth. However, their physiological characteristics in both areas are hardly investigated. This study is aimed to investigate the characteristics of the germination of resting cysts and growth of vegetative cells, as well as the toxin profiles of *A. fundyense* strains isolated from the bottom sediments in the eastern Bering Sea and Chukchi Sea shelves.

2. Materials and methods

2.1. Field sampling

Sampling on the eastern Bering Sea shelf was carried out at one station (57°30′N, 166°W; Fig. 1) using the T/S *Oshoro-Maru* in July 2012. Water depth, bottom water temperature at 55 m layer, and bottom salinity at 55 m layer were 64 m, 0.57 °C, and 31.6 at the sample collection. The sediment sample was collected with a Smith-McIntyre grab sampler, and the top 3 cm of a core was taken from the grab sample and placed into a plastic bottle. On the Chukchi Sea shelf, the sampling was conducted at one station (71°30′N, 168°45′W; Fig. 1) in September 2012 with the R/V *Mirai*. Water depth, bottom water temperature at 40 m layer, and bottom salinity at 40 m layer were 49 m, 4.1 °C, and 31.9 at the sample collection. One sediment sample was collected using a gravity core sampler, and the top 3 cm of the core was placed into a plastic bottle. Both sediment samples were stored in cold-dark conditions (1 °C) until analysis. Natsuike et al. (2013) report that abundant *A. fundyense* cysts distributed at both station of eastern Bering Sea and Chukchi Sea (835 and 2140 cysts cm^{-3}, respectively).

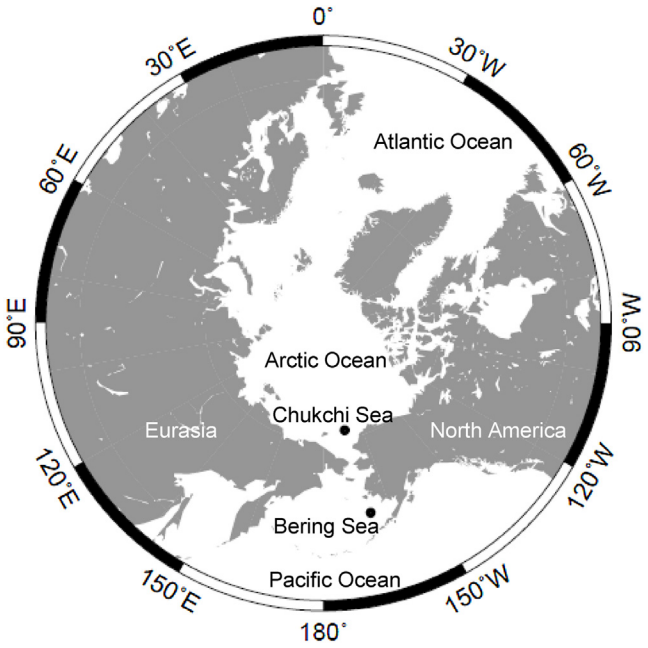

Fig. 1. Location of the study site of the eastern Bering Sea and Chukchi Sea shelves and sampling stations (●) for collection of sediments samples.

2.2. Germination assay of A. fundyense cysts

2.2.1. Pretreatment of germination assay

Seasonal changes of the potential germination rates of the *A. fundyense* cysts were investigated under different temperature conditions, with the sediment samples collected from the eastern Bering Sea and Chukchi Sea, using the most probable number (MPN) method (Imai et al., 1984). The sediment samples collected by field sampling (see above) were used for the MPN assay. Both samples were stored at 1 °C in the dark until use. Aliquots of 4.0 g of the sediments (wet weight) were suspended in 10-mL sterile seawater and sonicated for 60 s. Suspended samples were prepared by sieving with 150 and 20 μm nylon nets, and the obtained sediments were thoroughly washed with sterile seawater to remove excess particles larger than 150 μm and smaller than 20 μm. The residues on the 20 μm net were collected into 50-mL centrifuge tubes and diluted to 40 mL with f/2 medium (Brand et al., 1981) including germanium dioxide (GeO_2; final concentrations reached 100 μg L^{-1}).

2.2.2. Incubation for cysts germination

These dilutions were called 10^{-1} dilutions. Aliquots of 4.0 mL10^{-1} dilutions were diluted to 40 mL f/2 medium including GeO_2 (10^{-2} dilutions). This 10-fold dilutions were continued twice to make 10^{-3} and 10^{-4} dilutions. Five aliquots (1.0 mL) of each dilution series were dispensed into wells of tissue culture microplates. The sterile seawater and cultural medium for the procedures were stored at 1 °C until use, These sample procedures were operated on ice in a cold room (4–8 °C) with light intensity of less than 10 μmol photons m^{-2} s^{-1}. The dilution series from both Bering Sea and Chukchi Sea samples were incubated in 1, 5, 10, and 15 °C under a 14 h light: 10 h dark photo-cycle, with the light intensity of 20 μmol photons m^{-2} s^{-1} for 20 days.

2.2.3. Estimation of germination number using MPN method

Appearances of *A. fundyense* vegetative cells were observed for each well, using an inverted microscope (Eclipse Te200, Nikon Co.), after 10 and 20 days of incubation. Wells with observed *A. fundyense* vegetative cells were scored positive, and, based on the matrix of positive and negative wells, MPN of the *A. fundyense* germinated cells from the cysts was estimated according to statistical tables (Throndsen, 1978). These experiments to estimate the germination numbers of *A. fundyense* cysts at different temperature were conducted every month from March 2013 to March 2014. To confirm that the cyst density in the original sediment sample did not change during the experimental period, direct cysts counts with Primuline staining method (Yamaguchi et al., 1995) was conducted using an epifluorescence microscope (Eclipse TE200, Nikon Co.). The method and result of the direct cyst counts are described in Supplementary information.

2.3. Isolation of the A. fundyense strains

Isolation of *A. fundyense* strains from the sediment samples collected from the eastern Bering Sea and Chukchi Sea in 2012 was conducted in March 2013. Aliquots of 3.0 g wet sediment samples (the whole samples were c.a. 80–100 g wet weight) were suspended in 30-mL of sterile seawater in a 50-mL centrifuge tube. After 60-s sonication, suspended samples were sieved through nylon nets to obtain the size fraction between 20 and 150 μm. Size fractionated samples on the 20-μm net were washed repeatedly with a squirt bottle filled with sterile seawater. These samples were suspended in 30 mL of sterile seawater and added to 0.3 mL of AM9 antibiotics (Provasoli et al., 1959) to remove the bacteria. The sterile seawater for these procedures were stored at 1 °C until use, and the procedures were operated on ice in a cold room (4 °C) with light intensity of less than 10 μmol photons m^{-2} s^{-1}. The suspended samples with the antibiotics were stored at 4 °C for 24 h in the dark. The suspended samples were diluted 100-fold with f/2 medium (Guillard, 1975), as well as germanium dioxide (final concentrations reached 100 μg L^{-1}) to prevent the growth of diatoms. Aliquots of the 1.0-mL diluted samples were dispensed to each well of a 48-well micro plate. These samples in the microplates were incubated for germination at 5 °C under a 14 h light: 10 h dark photo-cycle, with a light intensity of 50 μmol photons m^{-2} s^{-1}. After incubation for 2 weeks, one vegetative cell was isolated from each well and inoculated in a well of a 48-well microplate dispensed with 1 mL of autoclaved f/2 medium. When cells had grown to a density of approximately 1000 cells mL^{-1}, subsamples were taken and stained, subsamples were stained with calcofluor white M2R (Fritz and Triemer, 1985). The stained cells were observed with an inverted epifluorescence microscope under UV light excitation (365 nm) and identified as *A. fundyense* by the morphological features of thecal plates of the cells following Fukuyo (1985). By this method, 30 and 41 clone cultures of *A. fundyense* strains were isolated from the sediment samples in the eastern Bering Sea and Chukchi Sea respectively.

These clone cultures were maintained with f/2 medium at 5 °C under a 14 h light: 10 h dark photo-cycle, with a light intensity of 50 μmol photons m^{-2} s^{-1}. Clone cultures were then confirmed based on whether they were axenic or not, using the DAPI staining method (Porter and Feig, 1980). All strains were used for analysis of toxin profiles, and two axenic cultures from each site were used for the growth experiments concerning the effects of temperature, salinity, and light intensity.

2.4. Growth experiments concerning the effects of temperature, salinity, and light intensity

Four axenic clonal cultures of *A. fundyense* isolated from the eastern Bering Sea (BE-2 and BE-9 strains) and Chukchi Sea (CH-5 and CH-20 strains) were used for the growth experiments. Seawater collected from Funka Bay (salinity was 33). Using the seawater and distilled water, the salinity-different f/2 media (10, 15, 20, 25, 30) were prepared (Yamaguchi et al., 1991). To prepare the f/2 media which salinity was 35, some collected seawater was concentrated with heating (95 °C) until the salinity reached 50. Then, collected seawater were slightly mixed with concentrated seawater until the salinity reached 35 (Kawamura et al., 1998). After filter sterilization of the medium, 4 mL of medium were dispensed into 8 mL plastic culture tubes (Evergreen scientific). Aliquots of 0.1 mL *A. fundyense* axenic clonal cultures were inoculated into the medium at final cell concentrations of 1000 cells mL^{-1}, and these tubes were incubated at 1, 5, 10, 15, 20 and 25 °C under a 14 h light: 10 h dark photo-cycle, with light intensity of 120 μmol photons m^{-2} s^{-1}. Using the same tube culturing method, the effect of light intensity was examined at 15 °C under a 14 h light: 10 h dark photo-cycle, with varying light intensity (30, 50, 70, 90, 120, and 150 μmol photons m^{-2} s^{-1}). The salinity of the medium was controlled at 30 and enriched with f/2 nutrients.

All culture experiments were performed in triplicate, and all tubes were incubated until maximum growth yield was recorded. The cell growth was monitored by measuring the *in vivo* Chl *a* fluorescence using a fluorophotometer (10AU005, Turner Designs Co.) every other day. From the average growth yields of the triplicate cultures, specific growth rates were calculated. Based on the formula modified by Lederman and Tett (1981), the light compensation point (I_0, μmol photons m^{-2} s^{-1}), half saturation intensity (K_s, μmol photons m^{-2} s^{-1}), and theoretical maximum growth rate (μ_{max}, day^{-1}) of each strain was approximated from

the result of the growth rate at each light intensity using the non-linear least-squares method.

2.5. Analysis of the profiles of paralytic shellfish toxins in the cultured A. fundyense strains by HPLC-FLD

All 71 strains of *A. fundyense* isolated from the bottom sediments of the eastern Bering Sea and Chukchi Sea were cultured in 50 mL f/2 medium in 100 mL Erlenmeyer flasks. After obtaining sufficient growth (>1000 cells mL^{-1}), 12 mL subsamples of the cultures were placed into centrifuge tubes. The subsamples were centrifuged ($600 \times g$, 3 min), and the pellets of algal cells were diluted two-fold using 0.2 mol L^{-1} acetic acid. After cell-disintegration with sonication, the liquids were filtered with ultrafiltration (Ultrafree, UFC30GV00, Millipore) and then used for the HPLC-FLD analysis. This cell collection for analysis of the toxin profile was conducted in 2013.

Paralytic shellfish poisoning toxin content was analyzed using HPLC-FLD (L-7000 series, Hitachi) by the method of Oshima (1995), with some modification. The HPLC-FLD analysis was performed on a reversed-phase column (Inertsil C8-3, GL Science; 4.6 mm i.d. × 250 mm, φ5 μm). The mobile phase was adjusted to pH 7.1 for GTX and STX, and to pH 6.8 for C toxins. The flow rate of the mobile phase was fixed at 0.7 mL/min and those of the oxidizing reagent and acidifying reagent were fixed at 0.35 mL/min and 0.2 mL/min, respectively. All chemicals and solvents used were of HPLC or analytical grade. The standards of GTX1, GTX2, GTX3, GTX4, decarbamoyl (dc) GTX2, dcGTX3, C1, C2, and neoSTX were provided by the Food Safety and Consumer Affairs Bureau in the Ministry of Agriculture, Forestry and Fisheries of Japan. The standard material of decarbamoyl saxitoxin (dcSTX) was kindly supplied by Professor Y. Oshima of Tohoku University. STX used as a standard material was purified in our laboratory and kindly quantified by Professor M. Asakawa of Hirohima University.

Cell numbers provided for the toxin profiles were not counted, and thus the cellular toxin amounts of each *A. fundyense* strain could not be obtained. Therefore, additional analysis to determine the cellular toxin amounts were conducted in 2016 (analysis of toxin profiles were conducted in 2013 soon after isolating *A. fundyense* strains). Four *A. fundyense* strains isolated from the bottom sediments of the eastern Bering Sea (BE-12 and BE-22) and Chukchi Sea (CH-33 and CH34) were maintained in 50 mL f/2 medium in 100 mL Erlenmeyer flasks at 15 °C under a 14 h light: 10 h dark photo-cycle, with a light intensity of 50 μmol photons $m^{-2}\,s^{-1}$. After obtaining sufficient growth (>1000 cells mL^{-1}), thier toxin contents were measured in the same manner as described above, and the cell concentrations were counted using a hemacytometer under a microscope. Cellular toxin contents of each strain (fmol $cell^{-1}$ and fg STX equivalent $cell^{-1}$) from the eastern Bering Sea and Chukchi Sea were calculated.

3. Results

3.1. Cyst germination

The seasonal changes of the germination success represented by cell numbers (MPN g^{-1}) at different temperatures (1, 5, 10, and 15 °C) are shown in Figs. 2 and 3. Numbers of germinated cysts changed with seasons and temperature. In the sample from the eastern Bering Sea, germination numbers were higher during February to May, but were not detected from June to February at 5 °C and from September to November at 10 and 15 °C. On the other hand, the germination numbers in the sample from the Chukchi Sea were high from February to August, and low from September to January (Fig. 2). In the sample of the Chukchi Sea, geminated cells were not detected from October to January at 5 °C, from October to December at 10 °C, and November at 15 °C. Cyst germination at 1 °C from both areas was only observed during April and May. When the incubation temperature was increased, germination numbers in the samples from both areas increased during early spring to summer (Fig. 2). During the germination experiments, the cyst densities of each sediment sample did not show significant seasonal changes, ranging from 370 ± 53 cysts g^{-1} wet weight for the sample collected from the eastern Bering Sea and 970 ± 120 cysts g^{-1} wet weight for that collected from the Chukchi Sea (Fig. S1).

3.2. Effects of water temperature, salinity, and irradiance on the growth of A. fundyense

Effects of the water temperature and salinity on the growth of *A. fundyense* are indicated in Fig. 4. All strains isolated from the sediment samples collected from the eastern Bering Sea shelf (BE-2 and BE-9) and Chukchi Sea shelf (CH-5 and CH-20) grew at water temperatures ranging from 5 to 20 °C, but not at 1 and 25 °C. Half of the initial populations survived at 1 °C for 2 weeks of incubation, whereas no strains could survive at 25 °C for longer than 2 days. Their growth rates reached a maximum at 15 °C. All strains grew at salinities ranging from 10 to 35 psu. Their growth rates were highest at salinities between 20 and 25 psu, and were relatively higher at 20–35. Their growths were prevented at low salinities and low temperatures (less than 10 °C and 15 psu).

Table 1 shows the calculated values of theoretical maximum growth, half-saturated light intensity, and the light compensation point from the approximations of each strain. The growth rates of all the strains nearly reaches a maximum at more than 120 μmol photons $m^{-2}\,s^{-1}$, and the light compensation points were approximately 20 μmol photons $m^{-2}\,s^{-1}$.

3.3. Toxin profiles

Fig. 5 shows the toxin profiles of the *A. fundyense* strains isolated from the cysts in sediment samples collected from the eastern Bering Sea and Chukchi Sea, and average toxin profiles in each area are indicated in Table 2. All strains contained some PSP toxins, and their profiles were completely different between the eastern Bering Sea and Chukchi Sea. The most dominant toxin was C2 (72 ± 11 mol%) in all 30 strains from the eastern Bering Sea shelf, and the second and third dominant toxins were GTX3 (11 ± 6.2 mol %) and GTX4 (9.7 ± 6.6 mol%), respectively. In contrast, with almost all 40 strains from the Chukchi Sea, the most dominant toxin was STX (78 ± 11 mol%) except for one strain (CH-34). The second and third dominant toxins were C2 (8.1 ± 3.6 mol%) and GTX3 (5.6 ± 5.6 mol%), respectively. In one strain (CH-34) from the Chukchi Sea, the dominant toxins were, in order, GTX3 (38 mol%), C2 (22 mol%), and STX (15 mol%). GTX1, GTX2, dcGTX3, and C1 were also detected in some strains, but their average contents from both areas were very low (<1 mol%). The cellular toxin contents of the Bering Sea (BE-12 and BE-22) and Chukchi Sea (CH-33 and CH-34) strains were ranged from 7.2 to 38.2 fmol $cell^{-1}$. Toxin profiles of these strains from Bering Sea were comparable with the previous results, but toxin profiles of the Chukchi Sea strains were different from the

Table 1
The parameters of the *Alexandrium fundyense* strains with the interaction between growth rates and irradiance, calculated from the algal assay following the formula by Lederman and Tett (1981).

	BE-2	BE-9	CH-5	CH-20
μ_{max} (day^{-1})	0.51	0.46	0.49	0.45
I_0 (μmol photons $m^{-2}\,s^{-1}$)	20	22	20	24
K_S (μmol photons $m^{-2}\,s^{-1}$)	52	48	64	44

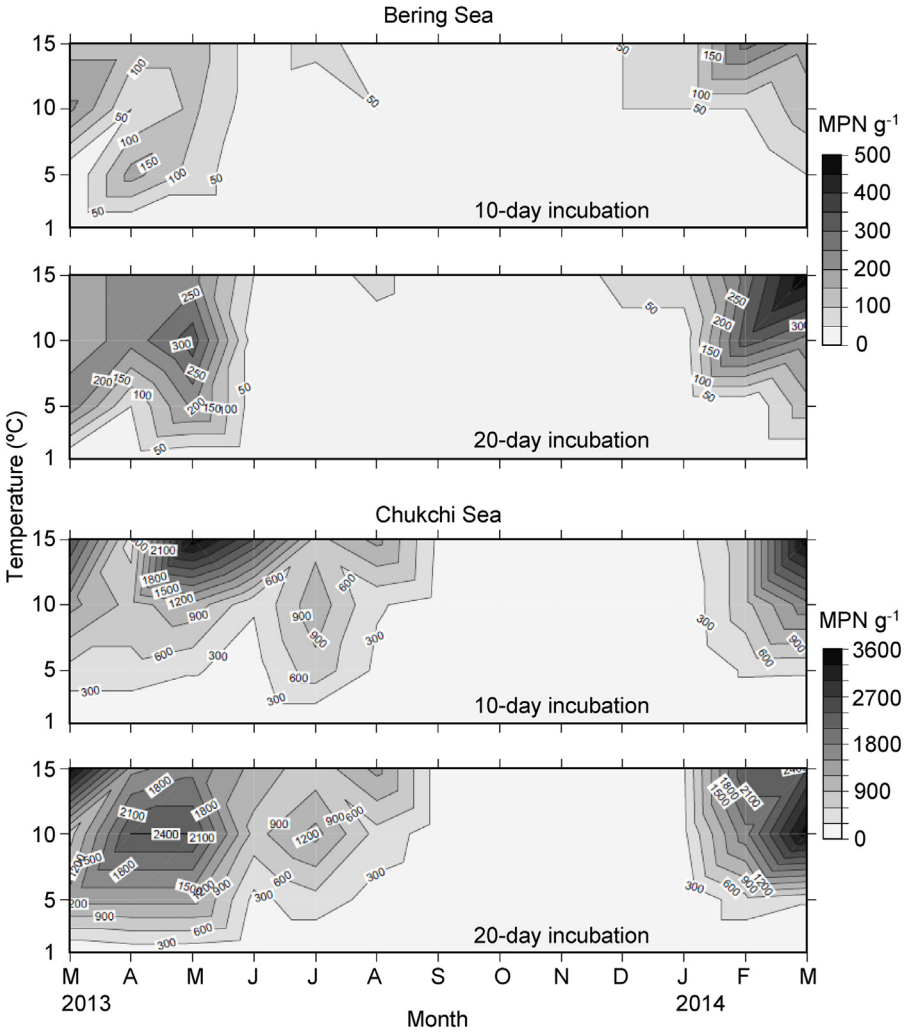

Fig. 2. Seasonal changes in the germination success rates (MPN g^{-1} wet weight) of *Alexandrium fundyense/pacificum* cysts at different water temperatures (1, 5, 10, and 15 °C) in surface sediment samples (top 0–3 cm) from the eastern Bering Sea and Chukchi Sea shelves.

previous results (Table S1); C2 were most dominant (43.8–94.7%) in all strains, and STX were not detected. These toxin profiles were similar to those of previous Bering Sea strains (Fig. 5).

4. Discussion

4.1. Germination characteristics of A. fundyense cysts

The cysts of A. *fundyense* are known to have endogenous dormant periods of ca. 1 to 12 months after formation of resting cysts (Anderson et al., 2012), and the dormancy of *A. fundyense* is usually thought to help them survive cold winter (Anderson, 1980; Anderson and Keafer, 1987). Our data also suggest that cysts from eastern Bering Sea and Chukchi Sea remain dormant in autumn and early winter (Fig. 2) during low temperature and light periods, which are less favorable for growth of *A. fundyense*. In late winter germination commences, which helps the species to inoculate a seed population which can immediately exploit favorable growth conditions in early spring, especially in Bering Sea. Thus, the endogenous dormancy of *A. fundyense* cysts are considered to contribute to overwintering in the eastern Bering Sea and Chukchi Sea. The cyst forming dinoflagellate *Scrippsiella hangoei* are common in Baltic Sea in the subarctic region and appear during winter to spring when the water temperature ranged 0–10 °C. Their cysts have the endogenous dormancy period during summer to autumn and the germination temperature window between 0 and 9 °C (Kremp and Anderson, 2000). Mandatory dormancy of this species is considered to be the primary factor regulating the timing of germination, and temperature also controls the timing of germination for their vegetative growth (Kremp and Anderson, 2000). In this study, the endogenous dormancy and the germination temperature of *A. fundyense* cysts are also thought to contribute to the suitable timing of germination for their suitable growth in water column in the Bering Sea and Chukchi Sea. On the other hand, the observed dormancy periods from the Chukchi Sea were few-month shorter than those from the eastern Bering Sea; the cysts from the Chukchi Sea could germinate during February to August, while the cysts from the eastern Bering Sea could germinate during February to May (Fig. 2). In the Chukchi Sea, the duration of sea ice cover is few-months longer than that in the eastern Bering Sea. Therefore, the longer possible germination periods of the Chukchi cysts during summer is considered to adapt to the colder environments in the arctic region where sea ice covered in almost half of a year (Fig. 2).

This study revealed that numbers of *A. fundyense* germinated cells increased with increase of incubation temperature (Fig. 3). Similar relationships between germination rates and water temperature have been reported by Anderson (1998),Perez et al. (1998), and Itakura and Yamaguchi (2001). When the incubation temperature was increased, the germination rates of *A. fundyense*

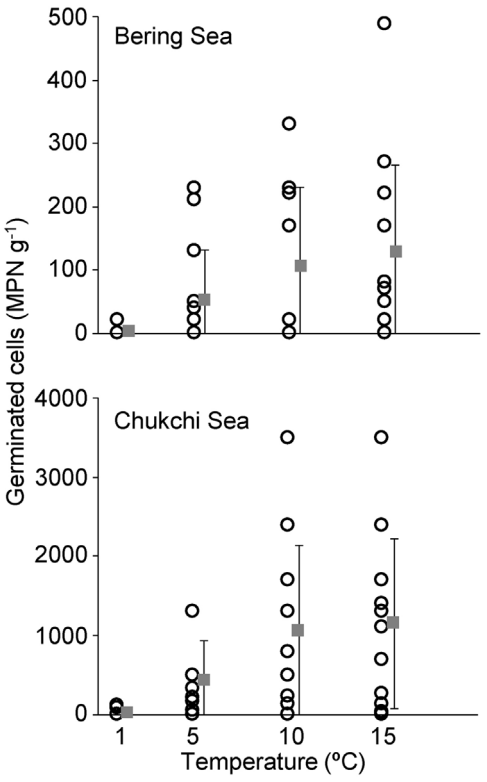

Fig. 3. Relationships between incubation temperature (°C) and germination success rates (MPN g^{-1} wet weight) of the *Alexandrium fundyense* cysts after 20-day incubation. Closed squares and error bars indicate the average and standard deviation at each temperature.

cysts increased, peaking between 10 and 15 °C, in both the sample from eastern Bering Sea and Chukchi Sea shelves (Fig. 3). Furthermore, this study has also revealed that *A. fundyense* cysts on the eastern Bering Sea shelf and Chukchi Sea could germinate at low numbers as cold as 1 °C in a few months of spring. On the Bering Sea shelf, Natsuike et al. (2013) found abundant deposition of *A. fundyense* cysts in the middle shelf domain, where water depths range from 50 to 100 m. Bottom temperatures in this area stayed below 2 °C during the summers of the cold period, and increased to 2–5 °C during the warm period after the climate shift (Ohashi et al., 2013). On the Chukchi Sea shelf, the bottom temperature during summer reached more than 6 °C when inflow of the Pacific summer water was vigorous, due to the recent sea ice reduction, while it reached less than 5 °C when inflow of the Pacific summer water was weak (Matsuno et al., 2011). Therefore, warming trends on the Bering Sea shelf by climate regime shift and on the Chukchi Sea shelf by warming could significantly promote the germination of *A. fundyense* cysts. Thus the abundant germinated cells are considered to contribute to increase of *A. fundyense* occurrences in both areas. On the other hand, *A. fundyense* cysts could germinate at low ratios in colder bottom temperatures to maintain their populations in both areas.

4.2. Growth characteristics of A. fundyense vegetative cells

Strains of *A. fundyense* isolated from the eastern Bering Sea and Chukchi Sea grew at 5 °C, and could survive at 1 °C for almost 2 weeks (Fig. 4). The theoretical optimum temperature for the growth of these strains was calculated at 13.4 °C. Yamamoto and Tarutani (1997) reported that *A. fundyense* strains isolated from Hiroshima Bay in temperate Japan could not grow at 5 °C and reached an optimum between 15 and 20 °C. The strains studied

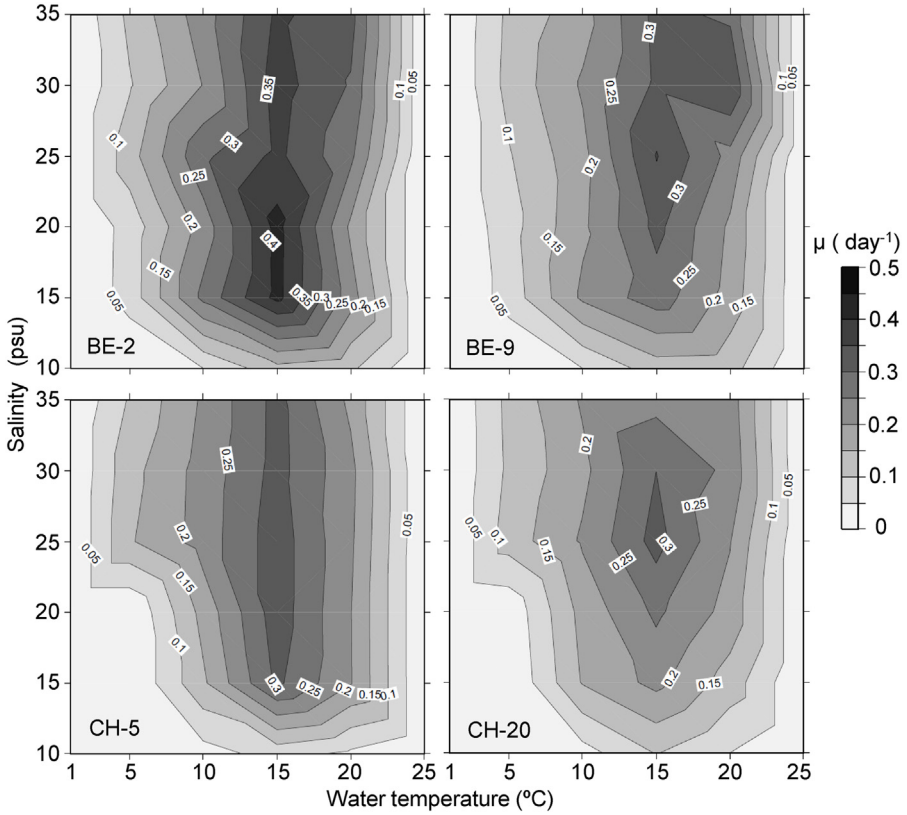

Fig. 4. Growth rates (day^{-1}) of *Alexandrium fundyense* strains (BE-2, BE-9, CH-5, and CH-20) isolated from the eastern Bering Sea and Chukchi Sea shelves as functions of salinity and water temperature.

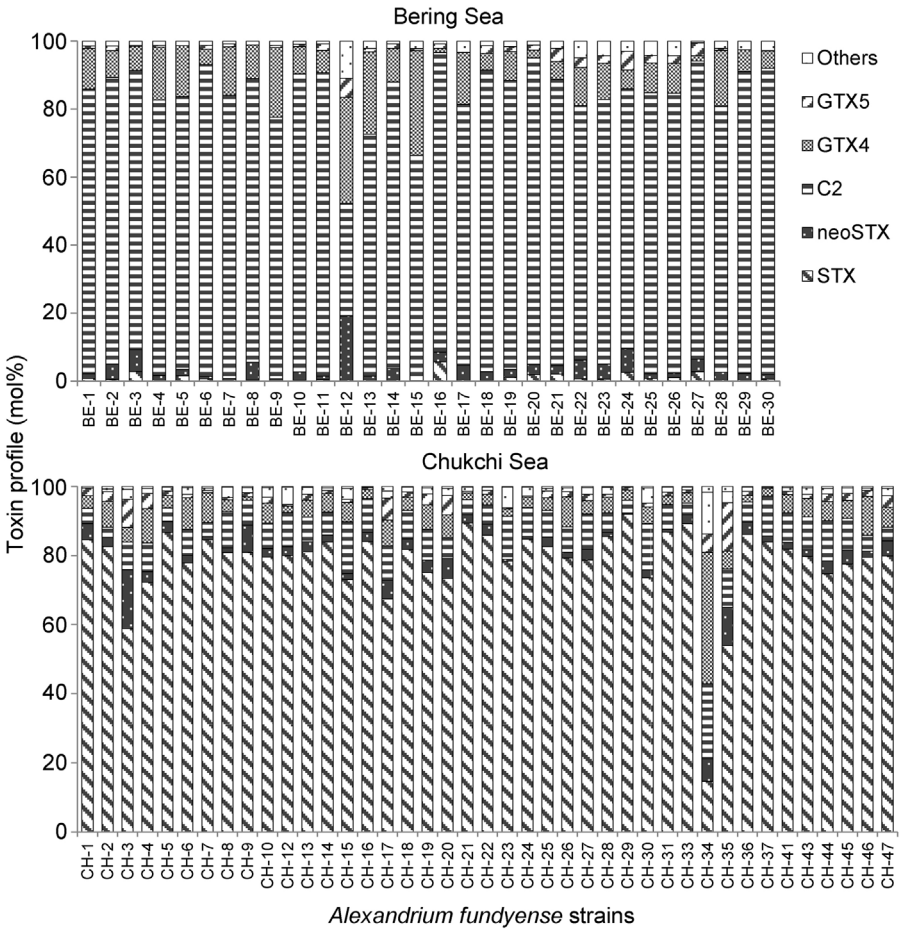

Fig. 5. Profiles of paralytic shellfish toxins (mol%) in the cultured strains of *Alexandrium fundyense* established and isolated from the cysts in the sediments collected from eastern Bering Sea and Chukchi Sea shelves.

here had higher growth rates at 5 °C than reported from *A. fundyense* strains isolated from Mikawa Bay in temperate Japan and the St. Lawrence Estuary in subarctic Canada. Their growth optimum was lower than 20 °C (Watras and Chisholm, 1982; Yamamoto et al., 1995). Based on their growth characteristics at different temperatures, the strains from the eastern Bering Sea and Chukchi Sea appear to be adapted to colder conditions, such as would be found in seasonal sea ice zones. Miyazono (2002) also reports that an *A. fundyense* strain isolated from the Funka Bay in colder regions of temperate Japan has the growth ability at temperatures from 3 and 5 °C, reaching its temperature optimum between 10 and 15 °C. These features were comparable to the strains from the eastern Bering Sea and Chukchi Sea.

Table 2
Average toxin profiles with standard deviation (±SD) of paralytic shellfish poison in the cultured *Alexandrium fundyense* strains isolated and established from the cysts in sediments collected from the eastern Bering Sea and Chukchi Sea shelves. N means number of culture strains.

	Bering Sea (n = 30)	Chukchi Sea (n = 41)
GTX4	9.71 (±6.64)	2.18 (±2.56)
GTX1	0.679 (±0.418)	0.198 (±0.257)
GTX3	11.0 (±6.23)	5.65 (±5.65)
GTX2	0.360 (±0.276)	0.173 (±0.150)
dcGTX3	0.506 (±0.224)	0.049 (±0.017)
GTX5	1.36 (±1.29)	2.05 (±2.03)
C1	0.447 (±0.940)	0.004 (±0.024)
C2	72.2 (±10.9)	8.14 (±3.57)
neoSTX	2.83 (±2.70)	3.35 (±2.97)
STX	0.851 (±1.14)	78.2 (±12.4)

On the eastern Bering Sea shelf, climate change has affected summer water temperatures (McFarlane et al., 2000; Hunt et al., 2011). Maximum surface water temperature ranged 10–14 °C during the summers of a warm period in late 1990s to the middle 2000s and from 6 to 10 °C during the summers of a cold water period (Ohashi et al., 2013; Stabeno et al., 2001). Salinity fluctuated between 31 and 32.5 during these warm and cold periods (Stabeno et al., 2001). The present study reveals that *A. fundyense* is able to grow under the temperature and salinity conditions that were observed during the summers of the warm period, but also during cold periods on the eastern Bering Sea shelf. This implies that they could increase in their abundance more rapidly during summer of a warm water period than that during summer of a cold water period. Therefore, the toxic *A. fundyense* occurrences would be expected to increase during summer under any future warming climate in the eastern Bering Sea (Hunt et al., 2011).

It has recently been reported that warming has promoted the reduction of sea ice and the increase of warm Pacific summer water inflows from the Bering Strait to the Chukchi Sea shelf (Shimada et al., 2006; Woodgate et al., 2010). This warm Pacific summer water inflow has raised the maximum water temperature of the surface layer to 10–12 °C during summer, compared to 0–6 °C when the inflow of this water was lower (Matsuno et al., 2011). This study demonstrates that higher water temperatures due to warming would be favorable for the growth of *A. fundyense* in the Chukchi Sea, and also that the decrease in sea ice caused by the recent warming trend could cause the increase of bloom occurrences of *A. fundyense* on the Chukchi Sea shelf. Surface salinity of the typical water of the Chukchi Sea is known to be relatively lower than that

of the Pacific summer water due to sea ice melting. However, according to the results of this study (Fig. 4), the salinity (26–33) there is suitable for the growth of *A. fundyense*. In fact, lower than full salinities seem to favor growth of some strains. Therefore, salinity is not expected to affect their growth in the Chukchi Sea.

Light compensation points of *A. fundyense* strains were reported at 76, 45, and 35 μmol photons $m^{-2} s^{-1}$, isolated from the Hiroshima Bay of Japan, Mikawa Bay of Japan, and the east coast of the USA (Langdon, 1987; Yamamoto et al., 1995; Yamamoto and Tarutani, 1997). These values were higher than those observed in the present study (20–24 μmol photons $m^{-2} s^{-1}$, Table 1). On the other hand, Miyazono (2002) reports somewhat lower light compensation points for the *A. fundyense* strains isolated from Funka Bay, Hokkaido, Japan (3.5–4.5 μmol photons $m^{-2} s^{-1}$), and suggests an adaptation of *A. fundyense* to the low light conditions in higher latitudes. The lower compensation points of *A. fundyense* strains in the present study also suggest that *A. fundyense* strains in both the eastern Bering Sea and Chukchi Sea are adapted to the lower light intensities found in high latitude areas.

4.3. Toxin profile of A. fundyense

The toxin profiles of the strains from the eastern Bering Sea and Chukchi Sea were different depending on the sampling site; dominant toxin of the strains from the eastern Bering Sea was C2, while that from the Chukchi Sea was STX (Table 2 and Fig. 5). Characteristics of the *A. fundyense* toxin profiles sometimes reflect the geographic distributions in some areas, such as the British Colombia in Canada (Cembella et al., 1987), the west coast of the Atlantic Ocean (Anderson, 1990) and North America and Canada (Anderson et al., 1994). Likewise, *A. fundyense* toxin profiles in the Pacific Ocean is primarily C2 (Cembella et al., 1987; Kim et al., 1993; Orlova et al., 2007; Sakamoto and Kotani, 1998; Shimada et al., 2011), suggesting that the C2 dominant group forms a same geographic population in the Pacific Ocean. In this study, the most dominant toxin of all strains from the eastern Bering Sea was also C2 (Table 2 and Fig. 5). The similar trends of the toxin profiles in the west and east coasts of the Pacific Ocean also indicate that *A. fundyense* is distributed with biogeophysical continuity in the area.

In contrast, STX was found to be the most dominant toxin of most strains from the Chukchi Sea (Table 2 and Fig. 5), and this feature is completely different from the other areas, including the Pacific (Cembella et al., 1987; Kim et al., 1993; Orlova et al., 2007; Sakamoto and Kotani, 1998; Shimada et al., 2011) and Atlantic Oceans (Anderson et al., 1994; Parkhill and Cembella, 1999; Touzet et al., 2008; Collins et al., 2009; Baggesen et al., 2012). This fact implies that *A. fundyense* from the Chukchi Sea forms a distinct population on the Chukchi Sea shelf, although *A. fundyense* strains from the Chukchi Sea were genetically confirmed the *A. fundyense* (*A. tamarense* North American clade) by Gu et al. (2013) and Natsuike et al. (2013). Furthermore, toxicity of STX is known to be much higher than C toxins. The toxicity of *A. fundyense* in the Chukchi Sea is probably much higher than those in the other areas. Therefore, *A. fundyense* on the Chukchi Sea shelf might cause shellfish poisoning at lower densities compared to other areas. On the other hand, one strain in forty-one strains in the Chukchi Sea (CH-34 strain as shown in Fig. 5) mainly contains GTX3 (38%), C2 (22%), and STX (15%). This toxin profile was similar to those previously collected on the Chukchi Sea shelf and in the Olytroskii Bay of the western Bering Sea (Orlova et al., 2007; Gu et al., 2013), and the *A. tamarense* from these areas were genetically confirmed as *A. fundyense*. This indicates a minor influence from the Bering Sea, potentially resulting in presence of several distinct intraspecific populations: One intraspecific variation was potentially the Chukchi Sea original group (STX dominant), and the other was highly related with the Bering Sea group (C2 dominant). However, Gu et al. (2013) isolated four *A. fundyense* strains from sediment samples collected on the Chukchi Sea shelf, reporting that C2 was the dominant toxin in all strains. These toxin compositions were completely different from those in this study, with one exception, while sampling location (69–72°N, 167°20′ – 169°45′W) and period (July 2010) for the isolation of *A. fundyense* strains were very close to those in the present study (71°30′N, 168°45′W, July 2012, respectively). These drastic changes in the toxin compositions of *A. fundyense* strains have been suggested to be due to the year-to-year changes of *A. fundyense* strains compositions on the shelf. In the Chukchi Sea, inflow of pacific summer water through the Bering Strait during summer has three different currents; one is from west coast of Russia, called Anadyr water, second is from eastern Bering Sea shelf, called Bering shelf water, and the last one is from Alaskan coastal areas, called Alaskan coastal water (Grebmeier, 2012). Their amount, direction or duration are different year to year depending on the sea ice coverage areas in the Chukchi Sea shelf. These currents may supply *A. fundyense* cells or cysts from the Bering Sea, which toxin profile is C2 dominant like Gu et al., 2013; and affect the local cyst populations in the shelf, although the occurrence of *A. fundyense* cells or cysts in these currents has not been observed yet. Otherwise, the present study found a slight difference in the endogenous period between the cysts from the eastern Bering Sea and the Chukchi Sea; our germination experiments revealed that a lower germination number was detected during June to November from the sediments samples collected in the eastern Bering Sea and during September to December in the Chukchi Sea (Fig. 2). Although Gu et al. (2013) did not explain when the germination experiments were conducted for the isolation of *A. fundyense* strains, the experimental period potentially affected the difference in strain composition between the present study and Gu et al. (2013).

Additional toxin analysis of some selected strains isolated from Bering Sea and Chukchi Sea revealed their cellular toxin amounts, ranging from 7.2 to 34.2 fmol $cell^{-1}$ and from 300 to 3340 fg STX eq. $cell^{-1}$ (Table S1). That toxin amounts are comparable not only to *A. fundyense* strains previously isolated from Chukchi Sea (9–41 fmol $cell^{-1}$; Gu et al., 2013) but also to *A. fundyense* strains isolated from other areas where toxin contaminations of bivalves were reported (1–1128 fmol $cell^{-1}$; e.g. Ichimi et al., 2002; Shimada et al., 2011). Natsuike et al. (2017) reported that *A. fundyense* occurred in the Chukchi Sea shelf with the highest cell density reaching 3.55×10^3 cells L^{-1}, and this highest cell density were enough high to cause PSP toxin contaminations of bivalves in the north Japan (Shimada et al., 2011). Therefore, *A. fundyense* cells in Chukchi Sea shelves are able to cause PSP toxin contaminations to other marine organisms with the observed cell density. Thus, a risk of PSP incidents exists at least in the Chukchi Sea shelf, and appearance of *A. fundyense* cells in the eastern Bering Sea shelf should be examined to evaluate a risk of PSP incidents. The toxin profiles of Chukchi Sea strains for analysis of cellular toxin amounts conducted in 2016 (Table S1), however, were different from the results of toxin profiles determined in 2013. Toxin profiles of *A. fundyense* strains are reported to be varied according to the nutrient conditions of cultural medium and their growth stages (Anderson et al., 1990). In this study, cultural conditions were not same during the period of these analyses. Thus, the differences of such cultural conditions potentially affected the toxin profiles of *A. fundyense* strains. Moreover, cell densities in the medium for toxin analysis also may affect the cellular toxin amounts (Shimada et al., 2011). Thus, although the present study confirmed that the cellular toxicity of *A. fundyense* strains isolated from the eastern Bering Sea and Chukchi Sea were comparable to those from other areas, the toxicity of natural *A. fundyense* populations are still unclear.

4.4. Conclusion

The present study revealed the physiological characteristics of *A. fundyense* isolated from the eastern Bering Sea and the Chukchi Sea shelves, located in the seasonal sea ice areas. Their cysts can germinate at low temperature and have an endogenous dormancy period from late summer to early spring, suggesting an adaptation to the germination during spring and early summer in high latitude areas. Their growth abilities at lower temperature and light intensity appear to be adaptations to the environments of cold areas in high latitudes. Cyst germination and cell growth of *A. fundyense* were highly promoted by the increase of surface and bottom water temperatures, respectively, suggesting that *A. fundyense* potentially increase with warming climate on the Bering Sea shelf and the Chukchi Sea shelves. Therefore, warming in both areas would contribute to the increase of toxic *A. fundyense* occurrences. The present study also revealed that the toxin compositions of *A. fundyense* were different between Bering Sea strains (C2 dominant) and Chukchi Sea strains (STX dominant), and their cellular toxicities were comparable to those of other *A. fundyense* strains from other areas where toxin contaminations of bivalves were reported. Therefore, a risk of PSP occurrences exists in the eastern Bering Sea and Chukchi Sea shelves.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.hal.2017.01.001.

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