Harmful Algae 63 (2017) 13–22

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

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

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ARTICLE INFO

Article history:
Received 22 May 2016
Received in revised form 6 January 2017
Accepted 6 January 2017
Available online 22 January 2017

Keywords:
Alexandrium fundyense
Alexandrium tamarense (north American clade)
Physiology
Chukchi Sea
Bering Sea
Cell growth
Cyst germination
Toxin profile
Water temperature
Salinity
Irradiance
Saxitoxin

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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http://dx.doi.org/10.1016/j.hal.2017.01.001
1568-9883/

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).

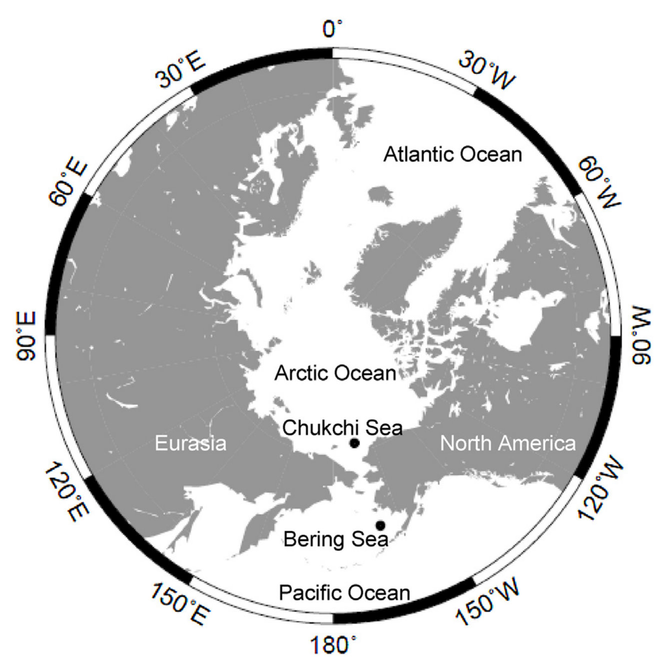

Fig. 1. Location of the study site of the eastern Bering Sea and Chukchi Sea shelves and sampling stations (●) for collection of sediments samples.