Regional Studies in Marine Science 7 (2016) 111–117

Contents lists available at ScienceDirect

Regional Studies in Marine Science

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

First record of two warm-water HAB species *Chattonella marina* (Raphidophyceae) and *Cochlodinium polykrikoides* (Dinophyceae) on the west coast of Hokkaido, northern Japan in summer 2014

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HIGHLIGHTS

- *Chattonella marina* and *Cochlodinium polykrikoides* were detected for the first time in northern Japan.
- The two species sporadically appeared in the warm condition (>20 °C).
- These species were supposed to be dispersed with warm current or were transported artificially.
- Monitoring of unpreserved seawater is useful to detect the spread of HAB species.

ARTICLE INFO

Article history:
Received 31 March 2016
Received in revised form
24 May 2016
Accepted 24 May 2016
Available online 9 June 2016

Keywords:
Chattonella marina
Cochlodinium polykrikoides
Tsushima Warm Current
Hokkaido
Climate change

ABSTRACT

The warm-water red tide flagellates *Chattonella marina* (Raphidophyceae) and *Cochlodinium polykrikoides* (Dinophyceae) were observed for the first time on the west coast of Hokkaido in summer 2014 by daily monitoring of the surface seawater. The two species sporadically appeared in the warm condition (>20 °C) during a period when the Tsushima Warm Current predominated off Hokkaido from July to September. The HAB species are hypothesized to have dispersed naturally with the Tsushima Warm Current or were transported artificially. Our results suggest that occurrences of naked flagellates such as *C. marina* and *C. polykrikoides* had not been found in the past monitoring systems aiming to detect armored toxic dinoflagellates using fixed seawater samples collected along the coast of northern Japan. For subarctic sea areas affected by climate change, it is concluded that monitoring of freshly sampled, unpreserved seawater samples is important for detecting the spread of warm water HAB species in areas affected by warm currents such as Hokkaido, in order to mitigate potential damage by HABs.

1. Introduction

Harmful algal blooms (HABs) are increasing in frequency and global distribution in recent years (e.g. Hallegraeff, 1993, 2004), and it is of concern that warm-water HAB species are able to invade high-latitude areas with climate change (e.g. Dale et al., 2006). The Sea of Japan, a marginal sea of the northwestern Pacific, is known for its remarkable warming during the last century (e.g. Gamo, 1999; Tanaka, 2002). Hence, warm-water HAB species frequently recorded along the coast of Kyushu and San-in region potentially can expand their distribution northwards with warming. *Chattonella marina* is notorious as a fish-killing raphidophyte forming red tide, causing large scale fish mortalities from temperate and subtropical water around the world (e.g. Edvardsen and Imai, 2006). *Cochlodinium polykrikoides* is also notorious as an ichthyotoxic, shellfish-killing dinoflagellate reported especially along the coast of southern Korea (e.g. Kim et al., 2004). HABs of these two species have been causing serious damages to fisheries along southwestern Japan (e.g. Matsuoka and Iwataki, 2004; Imai, 2012). The northern limit of occurrence of *C. marina* previously has been reported as Maizuru Bay, Kyoto Prefecture (Tanaka et al., 1977), and for *C. polykrikoides* as Kunda Bay, Kyoto Prefecture (Nagai et al., 2009) along the Sea of Japan coast of Honshu. These species have not been recorded in Hokkaido so far. However, it can be assumed

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http://dx.doi.org/10.1016/j.rsma.2016.05.010
2352-4855/

that vegetative cells of HAB species have been carried northward with the Tsushima Warm Current (TWC), a branch of the Kuroshio that flows along the Japanese coast (e.g. Hase et al., 1999), and reached the coast of Hokkaido in summer when the sea surface temperature (SST) rises above 20 °C. Substantial daily monitoring of HAB species have been carried out using unpreserved seawater samples at the coastal station of the Hokkaido Central Fisheries Research Institute (HCFRI) from spring 2013, when a bloom of the giant diatom *Coscinodiscus wailesii* occurred along the Sea of Japan coast of Hokkaido (Shimada et al., 2013). The monitorings have been also conducted aiming to detect living cells of HAB species including fragile species such as *C. marina* and *C. polykrikoides* which are difficult to identify in fixed seawater samples (e.g. Natsuike et al., 2012). In the present paper, the first detection of the red tide organisms *C. marina* and *C. polykrikoides* was reported from the water of Hokkaido in summer 2014.

2. Materials and methods

2.1. HAB monitoring at the coastal station

Almost daily monitoring (5 days a week without Saturday and Sunday) of HAB species was started at the coastal station of HCFRI (Fig. 1, 43°12′11.4″N, 140°46′33.4″E) from the late February 2013. A bucket of seawater was collected from the sea surface at 09:00 every week day and was used for monitoring HAB species after measuring SST and specific gravity using a bar thermometer and a hydrometer. The freshly collected, unpreserved seawater sample (500 mL) was concentrated to ca. 5 mL using a nylon net (20 μm mesh, before July 13, 2014) or Nuclepore membrane filter (2 μm pore size, after July 14, 2014), and 1/5–3/5 part of the concentrate was observed using an inverted microscope (Diaphot TMD, Nikon, Tokyo). Any HAB species were identified and counted, and the target species of the monitoring are the red tide / toxic species listed in Ohmura et al. (2012). The following sources were used to identify HAB species, Hallegraeff and Hara (2004), and Taylor et al. (2004). Salinity of the water sample was estimated to 3 significant digits from standard specific gravity at 0 °C (σ_0) using the conversion formula among σ_0, chlorinity and salinity Knudsen, 1901. Relationships between occurrences of *Chattonella marina* / *Cochlodinium polykrikoides* and environmental conditions were examined utilizing SST and current vector data at 4 m depth from the Japan Sea data assimilation experiment (JADE2, http://jade2.dc.affrc.go.jp/jade2/, last visited May 16/ 2016).

2.2. Sediment sampling and treatment

To examine the potential for presence of HAB species, samples of bottom sediments was collected using a TFO gravity corer at two stations in the Port of Otaru in May 25, 2015 (Fig. 1, Ironai: 43°12′16.9″N, 141°00′22.3″E, Katsunai: 43°11′28.2 N, 141°01′10.0″E). Sea surface temperature was measured using a bucket and a bar thermometer at each station. Microscopic observations of the surface sediment (<3 cm, ca. 4–8 g) were performed within five days after the sampling, aiming to detect the cysts of *C. marina*. The sediment samples were sieved through a plankton net to obtain the size fraction between 20 and 150 μm and suspended in filtered seawater to 10 mL for the observations, then subsamples (0.5 mL) of the 10 mL samples were used for the observations. After the observations, the remaining samples (9.5 mL) were incubated for ten days at 22.5 °C under a 12 h: 12 h light: dark cycle with ca. 85 μmol photons m^2 s^{-1} provided with white fluorescent illumination, and subsamples (3 mL) of the 9.5 mL samples were observed again under the microscope to detect any germinated vegetative cells of HAB species.

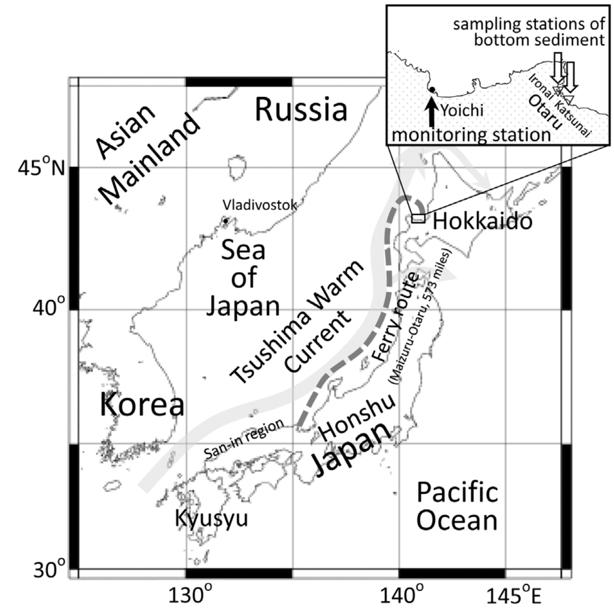

Fig. 1. Map showing the monitoring station at Yoichi (43°12′11.4″N, 140°46′33.4″E), Hokkaido, the monitoring stations of bottom sediment in Otaru (Ironai: 43°12′16.9″N, 141°00′22.3″E, Katsunai: 43°11′28.2 N, 141°01′10.0″E) and the schematic path of the Tsushima Warm Current and the ferry route between Maizuru and Otaru.

Fig. 2. Photomicrographs of the warm water harmful algal species detected at the monitoring station in Yoichi. 1: *Chattonella marina*, 2: *Cochlodinium polykrikoides* (scale bar: 10 μm).

3. Results

3.1. Occurrence of *Chattonella marina* and *Cochlodinium polykrikoides* at the coastal station

The dates and cell densities of the occurrences of *Chattonella marina* and *Cochlodinium polykrikoides* at the coastal station are given in Table 1, and these micrographs of the species are shown in Fig. 2. Vegetative cells of *C. marina* were identified based on the following characters: 30–70 μm long × 20–30 μm wide, oblong to obovoid shape with two subequal flagella (Hallegraeff and Hara, 2004). *Cochlodinium polykrikoides* were identified with the following characters: the cells form short chains consisting of 2–8 cells, the individual cells have a girdle making 1.8–1.9 turns around the cells (Taylor et al., 2004).

Based on the results obtained from early summer to autumn (June–October in 2013, 2014 and 2015), HAB species occurrences are described as follows. The harmful red tide raphidophyte *C. marina* appeared for the first time on July 3, 2014, with a cell density of 5 cells L^{-1}, increased with the cell density of 20 cells L^{-1} on July 8. In the next year 2015, *C. marina* cells were found again on July 17, 2015, with a cell density of 3 cells L^{-1}, occurring sporadically until September 1 with the maximum cell densities of

Table 1
Dates of occurrences and cell densities of the two harmful algal species detected in the present study.

HAB species	Date of occurrence (month/day) (cell density, cells L^{-1})									
	Year 2013	2014				2015				
Raphidophyceae										
Chattonella marina	Not detected	7/3 (5)	7/8 (20)			7/17 (3)	7/20 (10)	7/21 (10)	7/28 (3)	7/30 (3)
						8/2 (7)	8/19 (3)	8/31 (5)	9/1 (3)	
Dinophyceae										
Cochlodinium polykrikoides	Not detected	8/29 (30)	9/17 (10)	9/18 (1520)	9/24 (40)	Not detected				
		9/25 (880)	9/26 (7)	9/29 (4)						

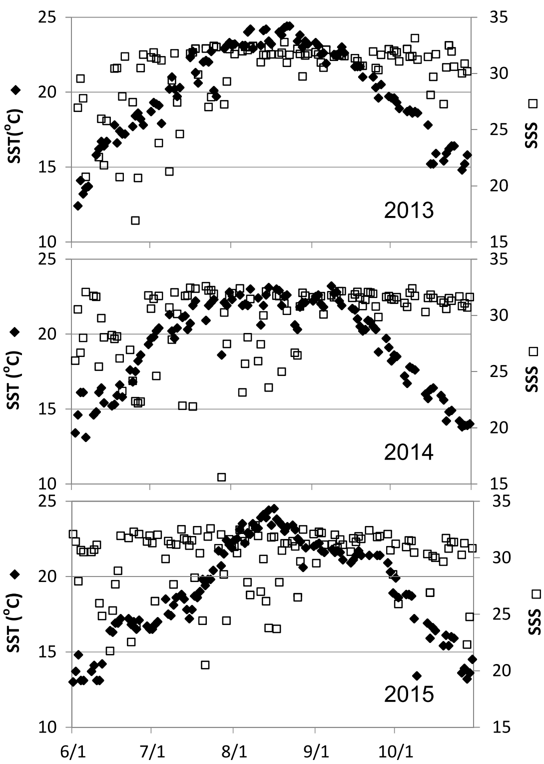

Fig. 3. Sea surface temperature (SST) and salinity (SSS) in time series, during the period of June–October of 2013 (top), 2014 (center) and 2015 (bottom) at the monitoring station at Yoichi.

10 cells L^{-1} on July 20 and 21. On the other hand, the harmful red tide dinoflagellate *C. polykrikoides* was found for the first time on August 29, 2014, increased to the cell density of 1520 and 880 cells L^{-1} on September 18 and 25, decreased on September 29 with cell density 4 cells L^{-1}, and thereafter was not detected. Due to the cell densities of *C. marina* were possibly underestimated before July 13, 2014 due to the use of a nylon net (20 μm mesh) to concentrate the cells of naked flagellates such as *C. marina* as they might pass through the net.

3.2. Seasonal changes of water temperature and salinity at the coastal station

Fig. 3 shows sea surface temperature (SST) and salinity (SSS) during the survey at the coastal station in 2013, 2014 and 2015. The SST rose above 20 °C in early July, maximum temperatures were recorded during mid August–early September, and decreased below 20 °C in late September each year. The SSS changed almost stably with the value of 30–33 during the period of higher SST above 20 °C. Lower SSS below 30 sometimes occurred in early June–late July 2013 and early June–late August 2014 and 2015.

3.3. Seasonal changes of water temperature and current vector around the Sea of Japan

Regarding monthly changes in the horizontal distribution of water temperature at 4 m depth from JADE2, it is noticed that the area of higher temperature (>20 °C) rapidly spread northward during June–July each year (Fig. 4). Similarly, as to the horizontal distribution of current vector at 4 m depth, it is explained that the TWC dominated during June–September every year, including the branch of TWC flowing along the Japanese coast and another branch that separates eastward from the east coast of the Korean Peninsula (Fig. 5).

3.4. Presence or absence of cysts of Chattonella marina in sediment samples

Cysts and vegetative cells of *C. marina* were not detected from the sediment samples collected at the two stations in the Port of Otaru and their resuspended samples after ten day incubation. Sea surface temperature at the two sampling stations were measured as 13.7 °C (Ironai) and 13.8 °C (Katsunai).

4. Discussion

4.1. Notorious HAB species Chattonella marina and Cochlodinium polykrikoides coming up to the coast of northern Japan

The occurrence of *Chattonella marina* and *Cochlodinium polykrikoides* is of great concern to local fisheries since HAB events of warm-water species including these two species have led to extensive economic damage to fisheries, especially aquaculture in coastal waters of the world. The maps in Figs. 6 and 7 show the occurrences of the two species based on past reports (Orlova et al., 2002; Matsuoka and Iwataki, 2004; Nagai et al., 2009; Orlova and Morozova, 2009; Imai, 2012; Shimane Prefecture, "Shimane Pref houdou akasio 2014 2137", http://www3.pref.shimane.jp/houdou/files/026E1542-58C3-4267-B6C6-F5724723CAD3.pdf., last visited May 16/ 2016). The monitoring station of the present study is approximately 500 miles away from the previously recorded area of occurrences of the two species.

Red tides due to *C. marina* have caused serious damages every summer along the southwestern coast of Japan (e.g. Imai, 2012), and have also been recorded in far eastern Russia (Orlova et al., 2002). The first occurrence of *C. marina* was

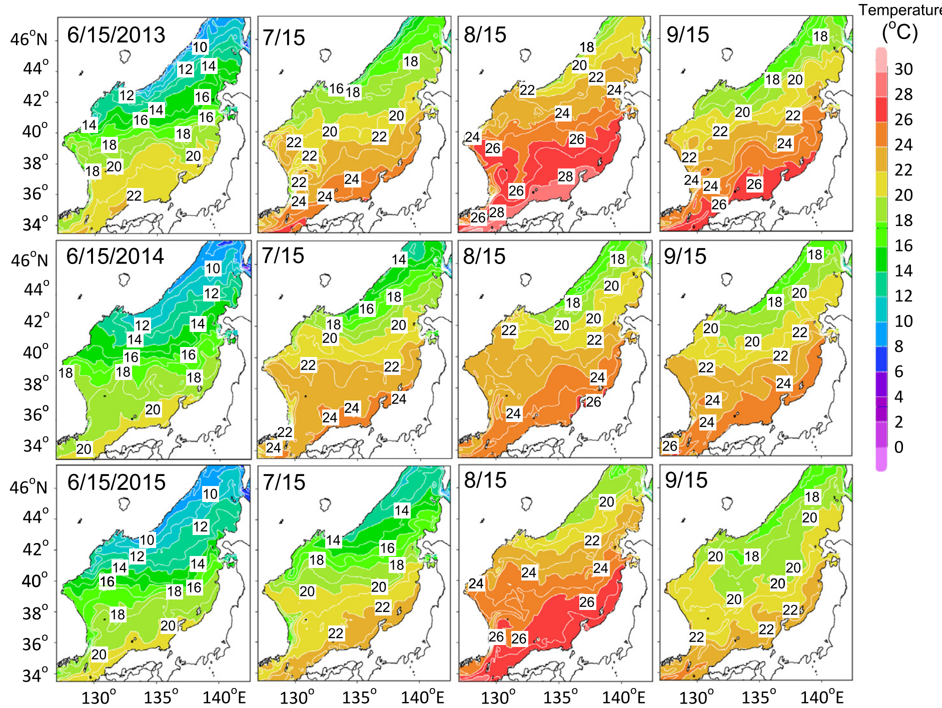

Fig. 4. Horizontal distribution of water temperature at 4 m depth in the Sea of Japan in June–September of 2013 (top), 2014 (center) and 2015 (bottom), modified from graphic-derived data from http://jade2.dc.affrc.go.jp/jade2/ (last visited May 16/ 2016).

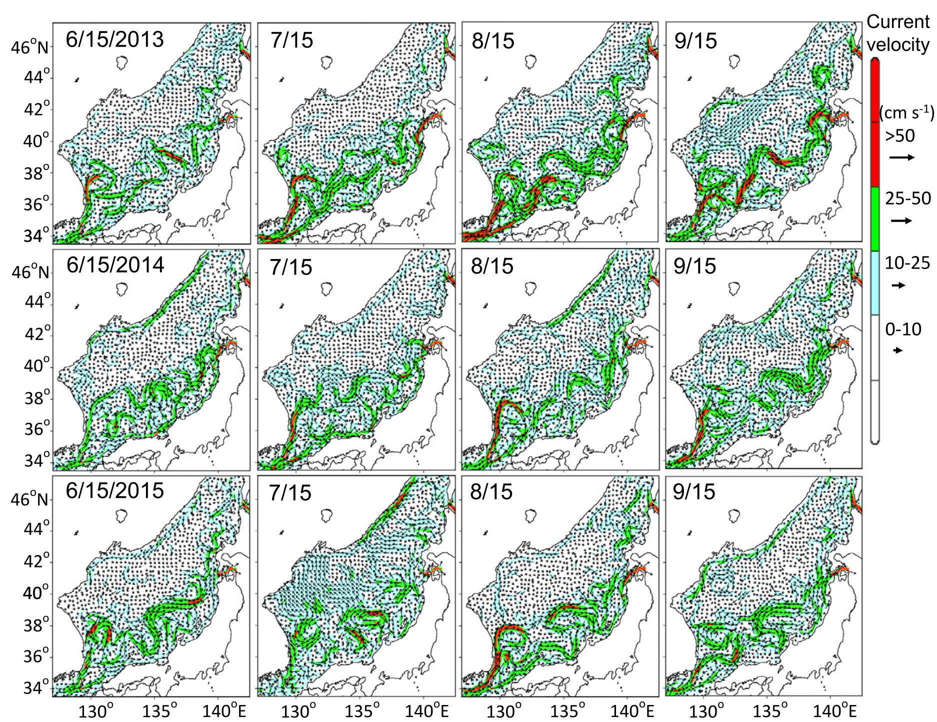

Fig. 5. Horizontal distribution of current vector at 4 m depth in the Sea of Japan in June–September of 2013 (top), 2013 (center) and 2014 (bottom), modified from graphic-derived data from http://jade2.dc.affrc.go.jp/jade2/ (last visited May 16/ 2016).

recorded in the present study in early July 2014 when red tides of *C. marina* were observed in western Kyushu of Omura Bay and Sasebo Bay, Nagasaki Prefecture (Nagasaki prefecture, "Nagasaki h26akasiohoukoku", http://www.marinelabo.nagasaki.nagasaki.jp/news/akasio/h26akasiohoukoku-1%20668.pdf, last visited May 16/ 2016). Nevertheless the occurrences of *C. marina* at the coasts between Kyushu and Hokkaido was not reported at that time in routine monitorings of HABs by local governments such as Shimane, Tottori, Hyogo, Kyoto Prefectures, etc. (e.g. Shimane prefecture, "Shimane Suigi nenpou 2014", http://www.pref.shimane.lg.jp/industry/suisan/shinkou/suigi/publish/jigyouhou/, last visited May 16/ 2016). Therefore the location source of *C. marina* has not been identified. However *C. marina* has been reported in the oceanic area of the Tsushima Strait (Imada and Honjo, 2001). Thus, the monitorings should be continued to detect the transportation of *C. marina* with the TWC. The monitoring of *Chattonella* cysts is

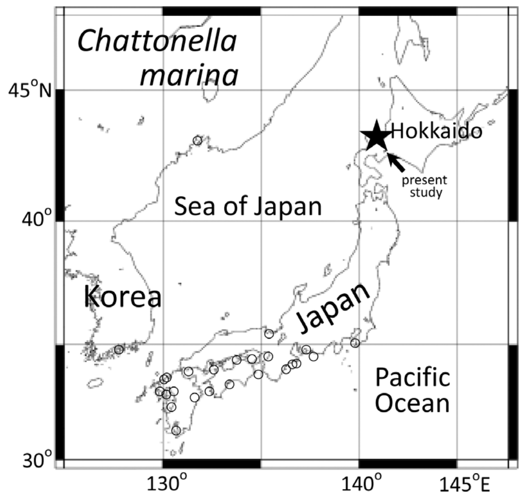

Fig. 6. The past occurrences of *Chattonella marina* (including *C. antiqua/ovata*) along the coasts of the Sea of Japan and Pacific Ocean, based on Imai (2012) and Orlova et al. (2002).

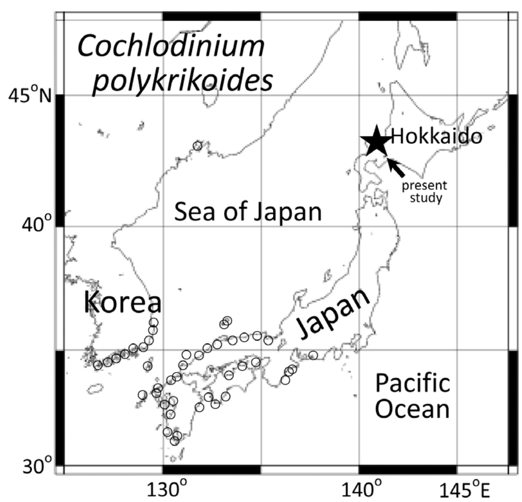

Fig. 7. The past occurrences of *Cochlodinium polykrikoides* along the coasts of the Sea of Japan and Pacific Ocean, based on Matsuoka and Iwataki (2004), Nagai et al. (2009), Orlova and Morozova (2009) and Shimane Prefecture, http://www3.pref.shimane.jp/houdou/files/026E1542-58C3-4267-B6C6-F5724723CAD3.pdf (last visited May 16/ 2016).

also essential, since *C. marina* is a warm-water species which has a maximum growth rate at 25 °C (Yamaguchi et al., 1991), and it has cyst stage for overwintering in the life cycle (Imai and Yamaguchi, 2012).

The red tide dinoflagellate *C. polykrikoides* is known as a warm-water HAB species which has a maximum growth rate at a temperature range of 21–26 °C (Kim et al., 2004), and clearly showed the expansion of its habitat in recent years (Kudela and Gobler, 2012). The red tides of *C. polykrikoides* have caused serious damage to fisheries along the coast of South Korea almost every summer (e.g. Kim et al., 2007), and have been transported to the Japanese coasts of San-in region with the TWC (Miyahara et al., 2005; Onitsuka et al., 2010). In 2013, 2014 and 2015, the red tides were reported along the coast of South Korea, especially in 2014, occurring continuously with the peaks of occurrences in September (National Research Institute of Fisheries and Environment of Inland Sea, 2013, 2014, 2015), and eventually appeared along the coasts of San-in region in middle September 2014 (e.g. Shimane Prefecture, "Shimane Pref houdou akasio 2014 2137", http://www3.pref.shimane.jp/houdou/files/026E1542-58C3-4267-B6C6-F5724723CAD3.pdf, last visited May 16/ 2016). At that time, the branch current flowing along the east coast of the Korean Peninsula flowed eastward adjacent to the northern front of TWC (Fig. 5), and the peak of cell densities of *C. polykrikoides* was also observed in September in the present study. Therefore, this coincidence indicates that the occurrences of *C. polykrikoides* in the present study are caused by a similar mechanism as the cases in San-in region (Miyahara et al., 2005; Onitsuka et al., 2010). Li et al. (2014) recently reported the cyst formation of *C. polykrikoides*, therefore it is also important that the monitoring should be carried out paying attention to the cysts.

4.2. Natural dispersal of the HAB species with the Tsushima Warm Current

Occurrences of various kinds of subtropical animals including nekton (e.g. yellowtail *Seriola quinqueradiata*, common blanket octopus *Tremoctopus violaceus* and greater argonaut *Argonauta argo*) and zooplankton (e.g. giant jellyfish *Nemopilema nomurai* and salp *Thetys vagina*) have been reported along the Sea of Japan coast of Hokkaido where the TWC predominates (Tabeta, 1969; Shimada, 2004; Ino et al., 2008; Uye, 2008; Shiga and Ito, 2011). Thus it is quite possible that the warm-water HAB species dispersed naturally with the TWC and subsequently it is strongly needed to monitor in Hokkaido the occurrences of new HAB species already reported from the TWC region (e.g. *Heterocapsa circularisquama* Kondo et al., 2012 and *Karenia mikimotoi* Imada and Honjo, 2001). It is also important to detect the source of the HAB species using molecular biological methods such as microsatellite analysis reported by Nagai et al. (2009), and to simulate the physical processes of the transportation of the HAB species from southern areas to the coast of Hokkaido using ocean modeling. The red tide raphidophyte *Heterosigma akashiwo* has been reported from the coast of Hokkaido and Kamchatka Peninsula (Hara, 1990; Edvardsen and Imai, 2006; Natsuike et al., 2015). However, it was not detected in the present study. Since *H. akashiwo* prefers eutrophic conditions in enclosed bays (e.g. Imai et al., 2014), we might not have detected the species as the monitoring station faces toward the open sea in the oligotrophic TWC region.

4.3. Anthropogenic transportation and potential naturalization of the HAB species

Regarding anthropogenic dispersal of the HAB species, transportation by ship is considered to be possible. Kitamae-bune trading ships were commonly used for tradings along the north coast of Japan as well as Hokkaido during the 17th–19th centuries (e.g. Flershem, 1964; Matsuki, 2007), and the large ferry boats (ca. 16,000 ton) have been operating every day from Maizuru to Otaru since the 1970s (Fig. 1). Furthermore, there is a regular route for cargo liners between Otaru and Vladivostok (Navis Shipping, "Navis Shipping schedule", http://navis-marine.ru/schedule/, last visited May 16/ 2016), from the coast of Amurskii Bay, far eastern Russia where *Chattonella* and *Cochlodinium* have been reported (Orlova et al., 2002; Orlova and Morozova, 2009). Since the anthropogenic introduction of HAB species via ballast water has been pointed out (e.g. Hallegraeff and Gollasch, 2006), there is a possibility that the HAB species have been transported to Hokkaido with ballast water.

Although cysts of *Chattonella marina* could not be found in the present study, the detection of vegetative cells of *C. marina* implies the progress of introduction and naturalization of the organism, considering the cyst formation for overwintering (Imai and Yamaguchi, 2012). There is little risk for missing detection of the cysts due to germination in the present study, since the temperature range for germination of *Chattonella* cyst was

15–30 °C (Imai, 1990) while the SST at the sediment sampling sites were relatively lower as 13.7–13.8 °C in the present study. It is important to monitor the occurrence of warm-water HAB species from water and sediment samples in international ports such as Otaru.

4.4. Necessity of HAB monitoring using unpreserved seawater

The warm-water HAB species were detected by the monitorings of this study by using of unpreserved seawater. The official HAB monitorings have generally been conducted to detect armored dinoflagellates, causative organisms of shellfish poisoning using fixed seawater samples (with glutaraldehyde etc.) from the coast of northern Japan. Since unarmored flagellates such as *Chattonella* are too fragile to identify in fixed seawater samples (e.g. Natsuike et al., 2012), a number of fragile HAB species may have not been detected in the past monitoring systems. Accordingly, the official monitorings should be carried out using unpreserved seawater samples in the coasts of northern Japan.

4.5. Monitoring systems of HABs in the subarctic region facing the climate change

In view of regional warming, how can we modify the monitoring system of HABs in the subarctic region in the future? Since the occurrences of warm-water HAB species are observed during a limited period in summer because of the cold season (SST $<$ 10 °C) for half the year, it is not likely that the harmful red tides occur in the subarctic waters. However, it is reported that the SST warming has been observed in the Sea of Japan off Hokkaido in winter with an increase of 0.77 °C per 100 years (Japan Meteorological Agency, "Sea surface temperature around Japan", http://www.data.jma.go.jp/gmd/kaiyou/english/long_term_sst_japan/sea_surface_temperature_around_japan.html). This phenomenon indicates the warming of annual minimum SST (5–6 °C) in the area and the lengthening of the higher temperature season (SST $>$ 10 °C). Most HAB species are able to survive, and it might be supposed that the warm-water HAB species can overwinter and colonize the Sea of Japan off Hokkaido in the future. Consequently, there is a potential for extensive dispersal of *Chattonella* or *Cochlodinium* species along the coast of Hokkaido in the Sea of Japan and thus increase the potential occurrence of harmful red tides in this area in the future. Red tides have the potential to cause significant damage to the fixed net fisheries and shellfish fisheries in the region. Impacts to large-scale fish farms are less likely as this form of aquaculture is not common around Hokkaido. Concerning the potential spread of harmful red tide species in the future, we conclude that monitorings of unpreserved seawater samples for coastal stations should be started in the subarctic region such as Hokkaido in summer when the SST exceeds 20 °C.

Acknowledgments

We are grateful to the members of HCFRI for keeping the almost daily monitoring at the coastal station.

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