

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 ($>20^{\circ}\text{C}$).
- 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.

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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 ($>20^{\circ}\text{C}$) 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.

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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 po-

tentially 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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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. Natsumi 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.

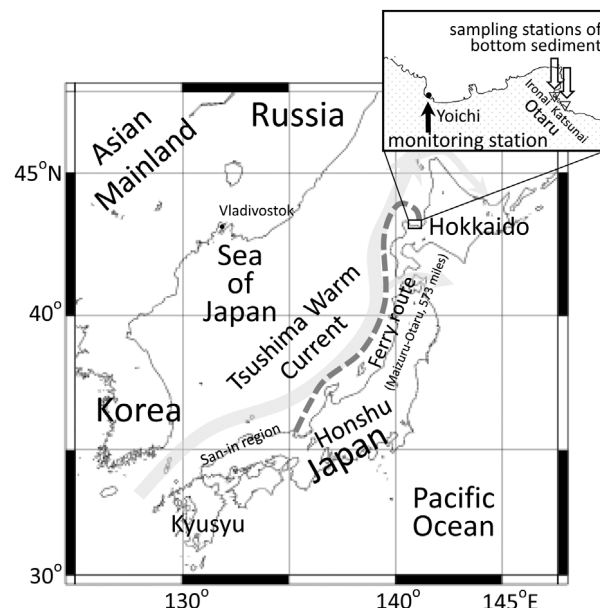


Fig. 1. Map showing the monitoring station at Yoichi (43°12'11.4"N, 140°46'33.4"E), Hokkaido, the monitoring stations of bottom sediment in Otaru (Ironai: 43°12'16.9"N, 141°00'22.3"E, Katsunai: 43°11'28.2"N, 141°01'10.0"E) and the schematic path of the Tsushima Warm Current and the ferry route between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

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