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Seasonal distribution of *Gambierdiscus* spp. in Wakasa Bay, the Sea of Japan, and antagonistic relationships with epiphytic pennate diatoms

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ABSTRACT

The occurrence of the ciguatera fish poisoning (CFP) causative *Gambierdiscus* spp. was confirmed in the Sea of Japan for the first time in 2009. This paper reports seasonal distribution of *Gambierdiscus* spp. and epiphytic diatoms in the Sea of Japan. Monitoring results suggested an antagonistic interaction in abundances between epiphytic diatoms and the dinoflagellate *Gambierdiscus* spp. Allelopathic effects of diatoms were considered to be involved in the competitive phenomenon. Therefore it is hypothesized that cell densities of epiphytic pennate diatoms on macroalgae are a novel determinant affecting the abundance of *Gambierdiscus* spp. other than sea water temperature, salinity and nutrients. Monitorings of the abundance of epiphytic diatoms would lead us to predict the occurrences of *Gambierdiscus* spp. blooms in the CFP area, and thereby the CFP risk assessments would be developed. Phylogenetic analyses indicated that *Gambierdiscus* spp. in the Sea of Japan belonged to *Gambierdiscus* sp. type 2 which was reported to be non-toxic. Nevertheless, based on morphological characteristics, at least two types of *Gambierdiscus* spp. were found in the Sea of Japan. It is needed to test the toxicity of the both types of *Gambierdiscus* recognized in the present study for evaluation of the probability of CFP outbreak risks in the Sea of Japan in the future.

1. Introduction

Ciguatera fish poisoning (CFP) is one of the most significant marine food-borne illness caused by the consumption of large-sized fish and reef fish in the tropical and subtropical sea, and affects 25,000–500,000 people annually around the world (Fleming et al., 1998; Lehane and Lewis, 2000; Yasumoto, 2005; Berdalet et al., 2017). The symptoms are characterized by gastrointestinal, neurological and cardiovascular disturbances, and the most characteristic symptom is the dysesthesia called “Dry Ice Sensation”. The major toxins of CFP are ciguatoxins (CTXs) and maitotoxins (MTXs). CTXs and MTXs are produced primarily by dinoflagellate species in the genus *Gambierdiscus* attached to macroalgae. Herbivorous fishes ingest *Gambierdiscus* cells attached to macroalgae. Furthermore, large-sized fish-eating fishes prey the herbivorous fishes and turn them to be poisonous. The consumption of strongly toxin-contaminated fishes via biological concentration causes poisoning and illness.

Gambierdiscus toxicus, which is the type species of the genus *Gambierdiscus*, was discovered in the Gambier Islands, French Polynesia in the late 1970’s (Adachi and Fukuyo, 1979). To date, *Gambierdiscus* species were found to be widely distributed throughout the tropical and subtropical seas in the world such as French Polynesia, the Caribbean Sea, Hawaiian Islands, Australia, and the Indian Ocean. In Japan, different phylotypes of *Gambierdiscus* sp. Type 1 (i.e., = *G. scabrosus*) (Kuno et al., 2010; Nishimura et al., 2013, 2014), *Gambierdiscus* sp. type 2 (Kuno et al., 2010; Nishimura et al., 2013), *Gambierdiscus* sp. type 3 (Nishimura et al., 2013), *G. australes* (Nishimura et al., 2013), and *G.* cf. *yasumotoi* (i.e., = *Fukuyoa* cf. *yasumotoi*) (Nishimura et al., 2013; Gómez et al., 2015) were reported based on the sequence analyses of D8-D10 region of the nuclear large subunit (LSU) ribosomal DNA (LSU rDNA D8-D10) and the nuclear small subunit (SSU) rDNA.

CFP has occurred in Nansei Islands in the subtropical region in Japan and the distribution of ‘*G. toxicus*’ has been investigated. In the main island of Japan, CFP has occurred sporadically in the temperate area of the Pacific coast (Oshiro et al., 2010; Toda et al., 2012). Moreover, the detections of ‘*G. toxicus*’ have been reported from the subtropical to temperate areas in the Pacific coasts (Hara and Horiguchi, 1982; Koike et al., 1991; Ishikawa and Kurashima, 2010). Although the existence of CFP has never been reported before in the Sea of Japan, the inhabitation of *Gambierdiscus* spp. was confirmed for the

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first time in Wakasa Bay in 2009 (Hatayama et al., 2011). The maximum cell density of *Gambierdiscus* spp. in the Sea of Japan at that time was almost the same level with that of Okinawa where CFP had actually occurred (Koike et al., 1991; Hatayama et al., 2011). Two *Gambierdiscus* spp. strains in the Sea of Japan were found to be *Gambierdiscus* sp. type 2, which was suggested to be non-toxic. On the other hand, *Gambierdiscus* sp. type 2 was reported to be dominant in the temperate area in the Pacific coast where CFP outbreaks had been reported (Nishimura et al., 2013). Nevertheless, in addition to *Gambierdiscus* sp. type 2, toxic species (*Gambierdiscus* sp. type 3, *G. scabrosus* and *G. australes*) were also found in the temperate area in the Pacific coast (Nishimura et al., 2013, 2014). Therefore, there is a possibility of the existence of toxic species in the Sea of Japan.

In the present study, the authors monitored *Gambierdiscus* spp. in order to assess the risk of CFP events in the Sea of Japan and investigated broad distribution in the coasts of the Sea of Japan and morphological and phylogenetic characteristics.

2. Materials & methods

2.1. Samplings and measurements of environmental factors

Collections of macroalgae samples were carried out by snorkeling in summer and by raking using a special instrument (Shoei Igaitoriki chu No.548-1) in winter, during the periods from March 2010 to March 2011 and from July to October 2011, respectively, in Niizaki, Ine town, Kyoto Prefecture (0.5–4.0 m depth, 35° 41.52′ N, 135° 18.28′E) (Fig. 1). Collected macroalgae samples were 6 species of Chlorophyceae, 22 species of Phaeophyceae, 23 species of Rhodophyceae and one species of *Liliopsida* during the entire investigation. In case of snorkeling, samples were placed into zipper plastic bags in the sea and put into polyethylene bottles on land, and in case of raking, samples were directly taken into 1L polyethylene bottles on land. Samples in polyethylene bottles were kept in a cool box on ice and brought back to the laboratory. In the field, the temperature and salinity of sea water were simultaneously measured using a water-temperature-salt-measuring-instrument (Kent, EIL5005) along with samplings. In October 2010, samples were similarly collected at three points of Noto Peninsula: Ogi (0–0.5 m depth, 37° 18.11′ N, 137° 14.21′E), Rokko Cape (0.5–1.5 m depth, 37° 31.43′ N, 137° 19.41′E) and Monzen (0–0.5 m depth, 37° 17.31′ N, 136° 43.42′E) in Ishikawa Prefecture, and at two points of Oshima Peninsula: Yoshioka fishery harbor (1.5–2.5 m depth, 41° 26.35′ N, 140° 14.17′E) and Yagoshi Point (2.0–4.0 m depth, 41° 31.26′ N, 140° 24.58′E) in Hokkaido for a broad distribution research (Fig. 1).

2.2. Sample treatments and cell observation/count

Algal sample treatments, observations and counts of epiphytic microalgal cells were described in the previous study by Ishikawa and Kurashima (2010). Briefly, after adding filtrated seawater up to 1L in the polyethylene bottles with samples, the bottles were sealed up. Subsequently the periphytic microalgae were detached by vigorously

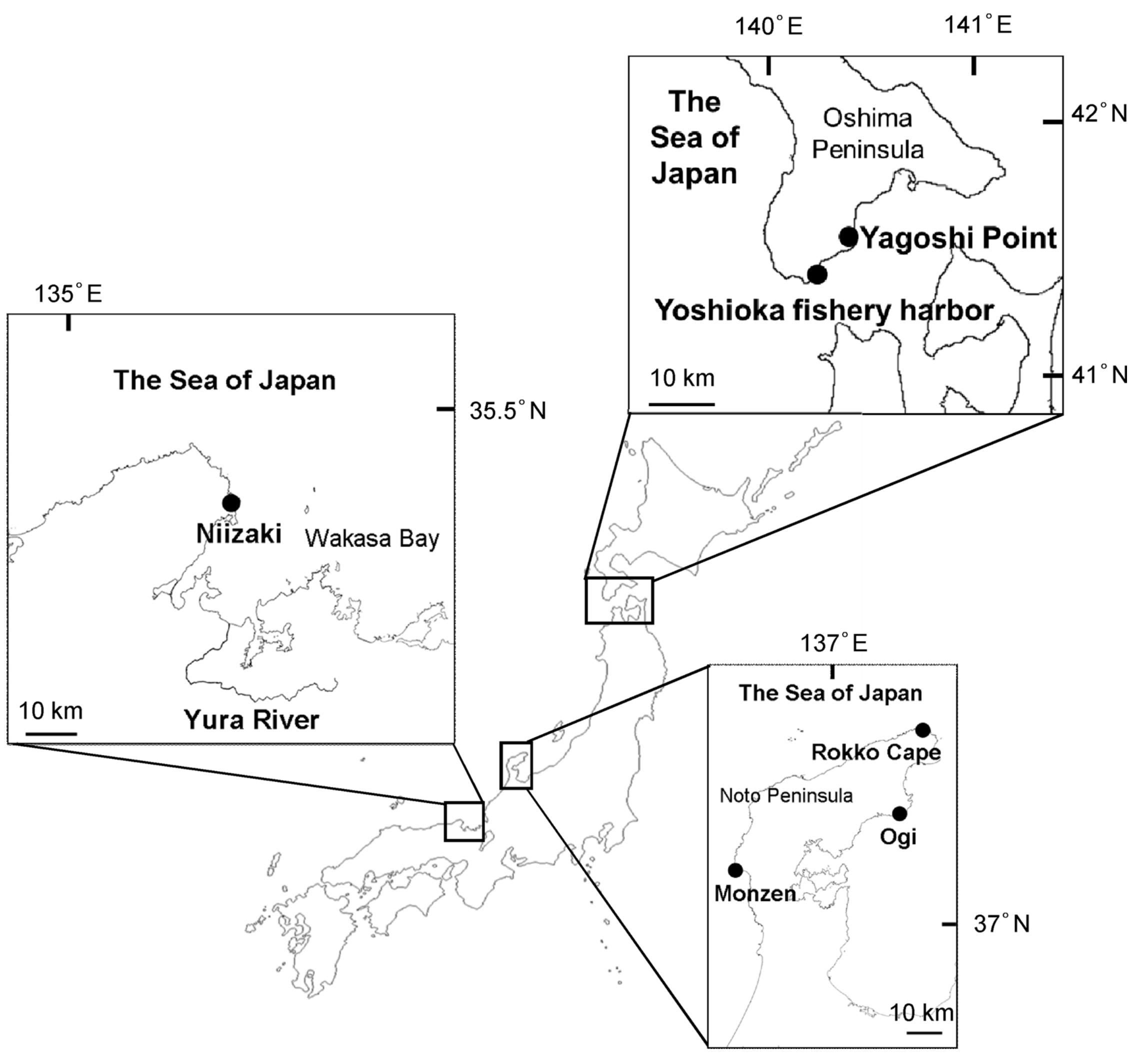

Fig. 1. Location of the sampling stations in the coasts of the Sea of Japan.