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The effects of light intensity on the growth of Japanese *Gambierdiscus* spp. (Dinophyceae)

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ABSTRACT

Marine toxic dinoflagellates of the genus *Gambierdiscus* are the causative agents of ciguatera fish poisoning (CFP), a form of seafood poisoning that is widespread in tropical, subtropical and temperate regions worldwide. The distributions of *Gambierdiscus australes*, *Gambierdiscus scabrosus* and two phylotypes of *Gambierdiscus* spp. type 2 and type 3 have been reported for the waters surrounding the main island of Japan. To explore the bloom dynamics and the vertical distribution of these Japanese species and phylotypes of *Gambierdiscus*, the effects of light intensity on their growth were tested, using a photoirradiation-culture system. The relationship between the observed growth rates and light intensity conditions for the four species/phylotypes were formulated at $R > 0.92$ ($p < 0.01$) using regression analysis and photosynthesis-light intensity (P-L) model. Based on this equation, the optimum light intensity (L_{max}) and the semi-optimum light intensity range ($L_{s\text{-}opt}$) that resulted in the maximum growth rate (μ_{max}) and ≥80% μ_{max} values of the four species/phylotypes, respectively, were as follows: (1) the L_{max} and $L_{s\text{-}opt}$ of *G. australes* were 208 μmol photons $m^{-2} s^{-1}$ and 91–422 μmol photons $m^{-2} s^{-1}$, respectively; (2) those of *G. scabrosus* were 252 and 120–421 μmol photons $m^{-2} s^{-1}$, respectively; (3) those of *Gambierdiscus* sp. type 2 were 192 and 75–430 μmol photons $m^{-2} s^{-1}$, respectively; and (4) those of *Gambierdiscus* sp. type 3 were ≥427 and 73–427 μmol photons $m^{-2} s^{-1}$, respectively. All four *Gambierdiscus* species/phylotypes required approximately 10 μmol photons $m^{-2} s^{-1}$ to maintain growth. The light intensities in coastal waters at a site in Tosa Bay were measured vertically at 1 m intervals once per season. The relationships between the observed light intensity and depth were formulated using Beer's Law. Based on these equations, the range of the attenuation coefficients at Tosa Bay site was determined to be 0.058–0.119 m^{-1}. The values 1700 μmol photons $m^{-2} s^{-1}$, 500 μmol photons $m^{-2} s^{-1}$, and 200 μmol photons $m^{-2} s^{-1}$ were substituted into the equations to estimate the vertical profiles of light intensity at sunny midday, cloudy midday and rainy midday, respectively. Based on the regression equations coupled with the empirically determined attenuation coefficients for each of the four seasons, the ranges of the projected depths of L_{max} and $L_{s\text{-}opt}$ for the four *Gambierdiscus* species/phylotypes under sunny midday conditions, cloudy midday conditions, and rainy midday conditions were 12–38 m and 12–54 m, 1–16 m and 1–33 m, and 0 m and 0–16 m, respectively. These results suggest that light intensity plays an important role in the bloom dynamics and vertical distribution of *Gambierdiscus* species/phylotypes in Japanese coastal waters.

1. Introduction

Ciguatera fish poisoning (CFP) is a worldwide marine seafood-borne illness (Friedman et al., 2008; Litaker et al., 2010). Worldwide, 50,000–500,000 cases are reported annually (Friedman et al., 2008; Litaker et al., 2010). In Japan, reports of CFP have increased not only in subtropical areas (Okinawa region) but also in temperate regions including the main islands of Japan, Honshu, Shikoku, and Kyushu regions, indicating a serious and growing threat to human health (Taniyama, 2008; Ishikawa and Kurashima, 2010; Oshiro et al., 2010, 2011; Toda et al., 2012; Yogi et al., 2013; Yoshimatsu et al., 2014). Between 1989 and 2010, approximately 80 CFP cases were reported in Japan (Oshiro et al., 2011; Toda et al., 2012).

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The causative agents of CFP appear to be benthic dinoflagellates of the genus *Gambierdiscus* (Adachi and Fukuyo, 1979), which exhibit ciguatoxin- and/or maitotoxin-like toxicities (Bagnis et al., 1980; Chinain et al., 1999; Parsons and Preskitt, 2007). Since the 1980s, researchers in Japan have reported that species of *Gambierdiscus,* such as *Gambierdiscus toxicus* are distributed throughout coastal waters (Hara and Horiguchi, 1982; Koike et al., 1991; Fukuyo et al., 2002). Kuno et al. (2010) reported that *Gambierdiscus* sp. type 1 and *Gambierdiscus* sp. type 2 are distributed in the subtropical region and the temperate regions, respectively (Kuno et al., 2010). Recently, Nishimura et al. (2013, 2014) confirmed the presence of not only *Gambierdiscus* sp. type 1 (i.e.,=*G. scabrosus*) and *Gambierdiscus* sp. type 2 but also *Gambierdiscus australes*, *Gambierdiscus* cf. *yasumotoi* (i.e.,=*Fukuyoa* cf. *yasumotoi* (Gómez et al., 2015), and *Gambierdiscus* sp. type 3 in the coastal waters of Japan by phylogenetic analyses (Nishimura et al., 2013, 2014). Nishimura et al. (2013) reported that the distribution of *Gambierdiscus* spp. in Japanese coastal areas was as follows: *G. australes* and *G. scabrosus* are distributed in the subtropical region and the temperate regions, whereas *G.* cf. *yasumotoi* (=*F.* cf. *yasumotoi*) is distributed only in the subtropical region. In addition, *Gambierdiscus* sp. type 2 is distributed in both the temperate regions and the subtropical region, while *Gambierdiscus* sp. type 3 is found only in the temperate regions. Ciguatoxin- and/or maitotoxin-like toxicities were detected in cultures of *G. australes*, *G. scabrosus*, and *Gambierdiscus* sp. type 3 by using a mouse bioassay (Nishimura et al., 2013). Considering this information, blooms of toxic *G. australes*, toxic *Gambierdiscus* sp. type 1 (i.e.,=*G. scabrosus*) and toxic *Gambierdiscus* sp. type 3 could be causative agents of CFP in subtropical and temperate areas of Japan (Nishimura et al., 2013). Thus, it is important to understand the bloom dynamics of toxic *Gambierdiscus* species/phylotypes and their growth characteristics in order to reduce CFP outbreaks in Japan.

In coastal waters, *Gambierdiscus* spp. form blooms that are influenced by various environmental factors, including temperature (Yasumoto, 1980; Parsons et al., 2010; Tester et al., 2010, 2013), salinity (Parsons and Preskitt, 2007; Parsons et al., 2010), and light (Parsons et al., 2012; Tester et al., 2013). Yoshimatsu et al. (2014) have established a suitable culture method for Japanese *Gambierdiscus* species and have thereby clarified the effects of temperature, salinity and temperature-salinity interactions on the growth of Japanese *Gambierdiscus* species/phylotypes. The effects of light intensity on the growth of Japanese *Gambierdiscus*, however, have not yet been clarified.

To examine the light-responsive growth of *Gambierdiscus* spp., it is necessary to use an appropriate light source that effectively simulates the natural photosynthetically active radiation (PAR) present in oceanic water. Furthermore, to elucidate the effects of light on the growth of *Gambierdiscus* spp. under various light intensities, a system that includes a photoirradiation device capable of adjusting light intensities accurately is required. Recently, Yamaguchi et al. (2014) have developed a photoirradiation culture system that utilizes white light-emitting diodes capable of more closely simulating the wavelength spectrum of light entering the oceanic water column compared with that of fluorescent tubes and halogen lamps. This system is capable of adjusting light intensities through two polarizing filters by varying the rotation angles of the filters (Yamaguchi et al., 2014). These authors have examined the growth responses of the toxic benthic dinoflagellate *Ostreopsis* sp. 1, which was isolated from Japanese coastal waters and contains palytoxin analogs, to various light intensities using this system and have clarified the light-responsive growth of this benthic dinoflagellate species (Yamaguchi et al., 2014).

The purpose of the present study was to clarify the effects of light intensity on the growth of Japanese *G. australes*, *G. scabrosus* and *Gambierdiscus* spp. types 2 and 3 using a photoirradiation culture system to understand their bloom dynamics and distribution in Japanese coastal waters.

2. Materials and methods

2.1. Cultures

For the culture experiments performed in this study, four clonal strains – *Gambierdiscus australes* S080911_1, *Gambierdiscus scabrosus* (=*Gambierdiscus* sp. type 1) KW070922_1, *Gambierdiscus* sp. type 2 M080828_2, and *Gambierdiscus* sp. type 3 WI11G – were used. The *G. australes*, *G. scabrosus*, and *Gambierdiscus* sp. type 2 strains were isolated off the coasts of Susaki (33° 23′38″ N, 133° 20′29″ E), Otsuki (32° 46′22″ N, 132° 37′31″ E) and Muroto (33° 14′47″ N, 134° 10′42″ E) in Shikoku, Japan, respectively (Nishimura et al., 2013). The *Gambierdiscus* sp. type 3 strain was isolated off the coast of Kushimoto (33° 27′10″ N, 135° 47′34″ E) in Honshu, Japan. All strains were isolated from shallow waters at depths of 0 to 3 m. Among them, *G. australes* S080911_1 exhibited the highest toxicity (670×10^{-4} MU 1000 cells^{-1}) and *G. scabrosus* KW070922_1 exhibited moderate toxicity (20×10^{-4} MU 1000 cells^{-1}) in the dichloromethane soluble fraction (DSF), which is expected to contain ciguatoxins (Nishimura et al., 2013). When the aqueous methanol soluble fraction (MSF), which is expected to contain maitotoxin, was tested, *G. australes* S080911_1, *G. scabrosus* KW070922_1 and *Gambierdiscus* sp. type 3 WI11G exhibited toxicity (67×10^{-4} MU 1000 cells^{-1} (Nishimura et al., 2013)). Stock cultures of these strains were maintained in polypropylene (PP)-capped, flat-bottomed 50 mL glass test tubes (25×150 mm) containing 25 mL of IMK/2, which is half-diluted IMK medium (Yoshimatsu et al., 2014). The stock cultures were maintained in a growth chamber (MLR-351, Sanyo Co., Ltd, Japan) at a temperature of 25 °C and under cool white fluorescent illumination of 90–100 μmol photons m^{-2} s^{-1} on a L:D cycle of 12:12 h. To prepare the medium, deep seawater with a salinity of 33 was used. This water was collected from areas offshore of Muroto, Kochi, Japan and was provided by the Deep Seawater Research Institute, Kochi Prefecture, Japan.

2.2. Effects of light intensity on the growth of Gambierdiscus species/phylotypes

Based on the culture method described by Yoshimatsu et al. (2014), stock cultures of clonal strains of *Gambierdiscus australes* S080911_1, *Gambierdiscus scabrosus* (=*Gambierdiscus* sp. type 1) KW070922_1, *Gambierdiscus* sp. type 2 M080828_2 and *Gambierdiscus* sp. type 3 WI11G were maintained in PP-capped 50 mL flat-bottomed glass test tubes (25×150 mm, Eiken) containing 25 mL of IMK/2 medium at 25 °C, and the culture method was performed in duplicate. Culture vessels were exposed to approximately 90–100 μmol photons m^{-2} s^{-1} of cool-white fluorescent illumination on a 12:12 h L:D cycle (light period 0600 to 1800 h). The cultures were incubated under completely dark conditions for one day in order to avoid effects of light exposure in pre-cultures on their growth under conditions of various light intensities, after which they were inoculated into the culture test tubes. The inoculum size was adjusted to 17% (v/v) of the culture medium. Cultures of the four strains were placed into photoirradiation adjustable devices (Yamaguchi et al., 2014) and were subsequently incubated at 25 °C while exposed to the following eleven light intensities (produced by the white LEDs on 12:12 h L:D cycles): 0.00, 10.1-11.4, 30.3-33.2, 50.1-51.8, 80.2-83.4, 121–125, 161–162, 200–202, 256–260, 320–335, and 421–430 μmol photons m^{-2} s^{-1}. During this incubation